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# Competing Behaviors of Thermoregulation and Ambush Foraging in the Timber Rattlesnake (*Crotalus horridus horridus*): A Mechanistic Assessment of Thermal Conduction

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Competing Behaviors of Thermoregulation and Ambush Foraging in the Timber Rattlesnake  
(*Crotalus horridus horridus*): A Mechanistic Assessment of Thermal Conduction

A dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy in Biology

by

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## ABSTRACT

The interaction between the biophysical environment and ectotherm morphology elicits behaviors designed to maintain internal body temperature ( $T_b$ ) within a range that promotes physiological functions. The short-term requirements of mass (energy requirements) and heat balance are subject to tradeoffs imposed by the organisms current physiological (heat and mass budgets) and environmental (biophysical, demographic, social, and predation) constraints and available resources. In temperate forests, extreme temperatures are common in summer even with intermittent sun exposure due to dense canopy cover. In Spring and Fall, temperatures can range from below freezing to 35 °C in 24 hrs. An ambush predator like the rattlesnake requires prolonged immobility (e.g., hours or days) to be an effective predator but thermoregulatory needs still take precedence. How then would a rattlesnake thermoregulate during periods of exposure to thermal extremes while remaining motionless for long periods? In this dissertation I explored the relationship between thermoregulation and ambush foraging using fixed videography, principles of heat transfer, and operative temperature models to address my general question. I found that in this study population *C. horridus* are rarely forced to make thermoregulatory movements. A combination of morphological features, thermal conduction, and positioning in the microhabitat are used to offset potentially extreme environmental temperatures throughout the active season. The results emphasize how adaptations for balancing tradeoffs between foraging and thermoregulation in a variable thermal environment help shape *C. horridus* life-history traits.

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I did a cost/benefit analysis for my time as a graduate student. Although the losses were far greater, there were still plenty of benefits. This chapter in my life has brought so many amazing people into my life. I wish I could thank every person individually and have one more chance to say thank you to others. First, I owe Steve a huge debt of gratitude for his patience, understanding, support, and patience...did I mention how patience he was? The rest of my committee (Mike, Marlis, and JD) for the constant words of encouragement and guidance. All of my labbies and extended labbies that date back through my time here. Each of you were pillars for me and I will always be indebted to you, specifically Jason, Chelsea, Max, Brenna, Ethan, Allie, Craig, Casey, and even Bannon. The Pebworths for always being available and including me and Eloise in their family. I want to thank Charlie Painter (Chazwad) for taking me under his wing during my formative years and showing me the old school herpetology ways and eventually becoming one of my best friends. You inspired me in so many ways. My desert family: Carp, Holycow, Chucksquatch, Timmy, the mutant Tarahumara, Douglasii, the Texans, and so many others I can't list. I want to especially thank my family for being so supportive even though they couldn't understand "why the hell I do what I do". And finally, my kids for sacrificing time with me and for being my first field crew.

## DEDICATION

I dedicate this to Collin, Eloise, Cody, Mom, and Dad. My kids deserved more time and my mom and dad always encouraged me. Thank you all for your unwavering support and love.

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## Dissertation Introduction

### Preface

My research interests are quite eclectic and have been shaped by my diverse experiences in biology. I have been fortunate to work with many taxa and be part of an array of opportunities involving several ecological disciplines. The mechanistic relationships between organism and environment have always fascinated me, as has my love of herpetofauna and specifically, rattlesnakes. Like many ecologists, I am interested in the interactions between an organism and its environment and how those interactions shape life history traits. Fortunately, one of my experiences introduced me to physiological-ecology and the associated tools for asking more detailed questions. Rattlesnakes are model organisms for exploring the mechanistic relationships between the organism and its environment. An ectotherm's relatively immediate physiological and behavioral responses to a variable environment can provide insight to important patterns that help shape life history traits. Here, I examine the two competing behaviors of thermoregulation and foraging in the Timber Rattlesnake (*Crotalus horridus*). The general consensus is the thermal environment acts as a source of constraint on ectotherm activities such as foraging. I explore whether ambush foraging behavior in the Timber Rattlesnake is thermally constrained by intense, intermittent solar exposure. Specifically, I examine the mechanisms and behaviors used to compensate for thermal extremes encountered by Timber Rattlesnakes in deciduous forests of the Ozark Highlands.



## Introduction

### *Heat Transfer Mechanisms*

In temperate climates, ectotherm daily and seasonal activities such as energy acquisition and social interactions are often constrained by physiology, the environment, and the available resources (Dunham et al., 1989). Short-term requirements are achieved through a series of interactions between the organism and the biophysical environment that elicit specific physiological and behavioral responses that in turn, shape life history traits. For ectotherms, these interactions are intended to maintain internal body temperature ( $T_b$ ) within a range that promotes physiological functions (Porter and Gates, 1969; Stevenson, 1985a; Huey, 1991; Sears et al., 2016) that allow the organism to perform other necessary activities such as foraging and mate searching. Consequently, changes to the biophysical environment or physical adjustments by the organism (e.g., shuttling or changes in posture/orientation) can significantly influence  $T_b$  (Porter and Gates, 1969; Tracy, 1982a; Bakken, 1992). However, frequent movements or adjustments are not conducive to ambush foraging. The operative environment often constrains the time allocated to competing daily functions such as foraging or social interactions (Dunham et al., 1989). For terrestrial ectotherms, daily activities are often constrained by the thermal environment (Grant and Dunham, 1988; Dunham et al., 1989; Beaupre, 1995). Rattlesnakes are low-energy organisms that exhibit traits ideal for studying mechanistic relationships with the environment such as low metabolic rates, ectothermy, and lack of an insulative layer (Huey et al., 1983; Cannatella and De Sa, 1993; Beaupre and Duvall, 1998; Beaupre and Douglas, 2009).

Heat transfer principles apply to all objects, living or non-living (Bakken, 1976). Radiation, convection, conduction, and evaporation are the heat transfer mechanisms that provide the energy transfer pathway between environmental factors (e.g., sunlight, thermal

radiation, air movement, etc.) and animal properties (e.g., conductance of tissue, absorptivity to radiation, body size, shape, and orientation, etc.; Porter and Gates, 1969). Porter and Gates (1969) derived the first heat budgets for various animals including snakes using a simple geometric model under steady-state conditions. A series of concentric cylinders were used to characterize a generalized organism and simplify the model of energy exchange between the environment and the animal based on the heat transfer theory and physical and physiological properties of the organism (Porter and Gates, 1969). Rattlesnakes are often found in a coiled position (i.e., a flattened cylinder) while foraging, thermoregulating, or resting. Thus, rattlesnakes naturally characterize a simplistic geometric shape without appendages, fur, or subcutaneous fat which facilitates thermal analyses (Porter and Gates, 1969). Additionally, the rattlesnake's relatively sedentary lifestyle and large body size in some species offers consistent access to individuals using radio telemetry. Consequently, rattlesnakes provide an ideal model for interpreting mechanistic links between physiology, ecology (Beaupre & Duvall 1998) and thermoregulation.

### ***Balancing Heat and Mass Budgets***

In temperate environments, ectotherm activity is often restricted to seasons when temperatures are generally conducive to physiological performance. During this limited timeframe, the organism must acquire and assimilate resources while maintaining a functionally acceptable range of body temperatures (Dunham et al., 1989). Ectotherms face numerous constraints as they interact with their environment to maintain balanced heat and mass (e.g., water, energy, and essential nutrients) budgets (Dunham et al., 1989). Ectotherms acquire energy primarily through two foraging techniques; active and sit-and-wait foraging. Active foraging

involves the organism moving through its environment actively searching for or pursuing food items. Sit-and-wait foragers are typically less-active organisms with low energy lifestyles often able to endure long periods between feedings.

In temperate climates geographic variation produces a variable thermal environment that translates to highly variable thermal microclimates (Beaupre, 1995). A terrestrial ambush predator like *C. horridus* must locate suitable and relatively stable thermal microclimates over a biologically relevant time-frame to successfully forage. If suitable microclimates are limited, individuals must adjust activity time, body posture and orientation, or conductance (Dunham et al., 1989). Because the thermal environment is often temporally and spatially so variable, temperature regulation often takes precedence over foraging requirements (Dunham et al., 1989).

Activities related to foraging and thermoregulation are considered constraints on the organism's behaviors intended to favor life histories that result in greater fitness (i.e., lifetime reproductive success) (Dunham et al., 1989). Thus, understanding the relative contribution of each mechanism or behavior used to balance individual daily time budgets is a critical aspect to understanding proximate causes of life history variation (Grant and Dunham, 1988).

### ***Thermal Microclimates***

The thermal environment strongly influences body temperature (Porter and Gates, 1969; Lillywhite, 1987), physiological rates (Huey, 1982; Stevenson, 1985c), and activity (Grant and Dunham, 1988) of terrestrial ectotherms (Beaupre, 1995). Ectotherm activities such as foraging, mate searching, and emergence are often thermally constrained in temperate climates (Shine and Madsen, 1996; Sears, 2005; Gunderson and Leal, 2016) when thermally suitable microclimates are limited (Beaupre, 1995). For some species, hibernation or brumation represents the most

extreme form of thermal constraint accounting for more than half of the life of an individual (Blouin-Demers et al., 2000a). Thus, the potential advantages of a longer active season (e.g., higher growth rates or earlier sexual maturity) assuming food is not limited, outweigh the risk associated with early emergence (e.g., exposure to extreme environmental temperatures) (Blouin-Demers et al., 2000a). But unpredictable and potentially lethal environmental temperatures in spring present a challenge for snakes emerging from hibernacula. Exposure to extreme low temperatures can be lethal or debilitate snakes and increase the risk of predation if caught outside the hibernaculum. A thermoregulatory strategy that would reduce the effects of extreme temperatures, provide concealment from predators, and extend surface activity time would therefore be advantageous.

Snakes in this study population emerge in early to late March and consistently move to what is referred to as transition habitat where they may spend days or weeks before moving away from the hibernaculum. For the purpose of my research, I defined transition habitat as the area below hibernacula characterized by south facing slopes, high elevation, and rock outcrops with deep crevices (Browning et al., 2005). In the Ozark Mountains, the previous year's deciduous foliage creates a thick loosely compacted layer on the forest floor. Therefore, transitional habitat is typically characterized by deep deciduous leaf litter.

Snakes use transition habitat in the weeks prior to ingress or following egress. However, during the summer snakes were less frequently seen in LH position but more often observed lying in a depression in the leaf litter or leaf cratered (LC) or, on top (Top) of the leaf litter. Observations of radio tagged snakes indicated a peak in LH behavior in early spring and a second, smaller peak just before ingress. I questioned if the leaf litter layer and associated hiding behavior could serve two purposes. First, the leaf litter may act as an insulating layer against

extreme temperatures. Secondly, Timber Rattlesnakes rely on cryptic coloration and avoid detection even when disturbed. Thus, snakes buried in the leaf litter would be nearly undetectable by potential predators during periods of low temperatures when they are most vulnerable.

We hypothesized the leaf litter would provide a layer of insulation against extreme fluctuations in environmental temperature and that significant differences in snake body temperature exist depending on the position adopted (LC, LH, or Top) in the leaf litter. Because actual snake body temperatures are difficult to collect in a way that directly compares these positions, we elected to study this problem using operative temperature models (OTMs) (Bakken et al., 1985; Stevenson, 1985c).

### ***Purpose of Research***

The goal of my research was to gain an understanding of the mechanisms used by *C. horridus* to balance the competing behaviors of thermoregulation and ambush foraging in a temperate forest. In **Chapter 1**, I investigate the role of thermal conduction in maintaining core body temperature ( $T_b$ ) during thermal extremes caused by intermittent solar exposure (i.e., solar transients). Specifically, whether the rate of conduction was sufficient to offset increases in temperature at the dorsum due to solar transients. In **Chapter 2**, I use fixed videography to investigate the potential trade-off between the behaviors of thermoregulation and an ambush foraging strategy of *C. horridus*. Finally, in **Chapter 3**, I investigated the insulative properties of the deciduous leaf litter layer using OTMs and if the position adopted by *C. horridus* in the leaf litter (Top, LC, or LH) significantly affects model temperature.

## References

- BAKKEN, G. S.** 1976. A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data to field ecology. *Journal of Theoretical Biology*. 60:337-384.
- . 1992. Measurement and application of operative and standard operative temperatures in ecology. *American Zoologist*. 32:194-216.
- BAKKEN, G. S., W. R. SANTEE, AND D. J. ERSKINE.** 1985. Operative and Standard Operative Temperature: Tools for Thermal Energetics Studies. *American Zoologist*. 25:933-943.
- BEAUPRE, D. S. J., AND D. J. DUVAL.** 1998. Integrative Biology of the Rattlesnakes: Contributions to biology and evolution. *Bioscience*. 48:531-538.
- BEAUPRE, S. J.** 1995. Effects of Geographically Variable Thermal Environment on Bioenergetics of Mottled Rock Rattlesnakes. *Ecology*. 76:1655-1665.
- BEAUPRE, S. J., AND L. E. DOUGLAS.** 2009. Snakes as Indicators and Monitors of Ecosystem Properties.
- BLOUIN-DEMERS, G., K. A. PRIOR, AND P. J. WEATHERHEAD.** 2000. Patterns of variation in spring emergence by black rat snakes (*Elaphe obsoleta obsoleta*). *Herpetologica*:175-188.
- BROWNING, D. M., S. J. BEAUPRE, AND L. DUNCAN.** 2005. Using partitioned Mahalanobis D-2(K) to formulate a GIS-based model of timber rattlesnake hibernacula. *Journal of Wildlife Management*. 69:33-44.
- CANNATELLA, D. C., AND R. O. DE SA.** 1993. *Xenopus laevis* as a model organism. *Systematic Biology*. 42:476-507.
- COWLES, R. B., AND C. M. BOGERT.** 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* 83:261-296.
- DUNHAM, A. E., B. W. GRANT, AND K. L. OVERALL.** 1989. Interfaces between Biophysical and Physiological Ecology and the Population Ecology of Terrestrial Vertebrate Ectotherms. *Physiological Zoology*. 62:335-355.
- GARDNER-SANTANA, L. C., AND S. J. BEAUPRE.** 2009. Timber Rattlesnakes (*Crotalus horridus*) Exhibit Elevated and Less Variable Body Temperatures during Pregnancy. *Copeia*. 2009:363-368.
- GRANT, B. W., AND A. E. DUNHAM.** 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology*:167-176.

- GUNDERSON, A. R., AND M. LEAL.** 2016. A conceptual framework for understanding thermal constraints on ectotherm activity with implications for predicting responses to global change. *Ecology Letters*. 19:111-120.
- HARVEY, D. S., AND P. J. WEATHERHEAD.** 2010. Habitat selection as the mechanism for thermoregulation in a northern population of massasauga rattlesnakes (*Sistrurus catenatus*). *Ecoscience*. 17:411-419.
- HARVEY, D. S., AND P. J. WEATHERHEAD.** 2011. Thermal ecology of Massasauga Rattlesnakes (*Sistrurus catenatus*) near their northern range limit. *Canadian Journal of Zoology*. 89:60.
- HERTZ, P. E., R. B. HUEY, AND R. STEVENSON.** 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *American Naturalist*:796-818.
- HOEKSTRA, A. A.** 2015. The influence of habitat on body temperature regulation in the timber rattlesnake (*Crotalus horridus*), p. 47. Vol. 1597442. Middle Tennessee State University, Ann Arbor.
- HUEY, R. B.** 1974. Behavioral thermoregulation in lizards: importance of associated costs. American Association for the Advancement of Science.
- HUEY, R. B.** 1982. Temperature, Physiology, and the Ecology of Reptiles, p. 25-91. *In*: Biology of the Reptilia. Vol. 12. C. Gans and F. H. Pough (eds.). Academic Press, New York.
- . 1991. Physiological Consequences of Habitat Selection. *The American Naturalist*. 137:S91-S115.
- HUEY, R. B., E. R. PIANKA, AND T. W. SCHOENER.** 1983. Lizard ecology: studies of a model organism. Harvard Univ Pr.
- HUEY, R. B., AND M. SLATKIN.** 1976. Cost and benefits of lizard thermoregulation. *Quarterly Review of Biology*:363-384.
- LILLYWHITE, H. B.** 1987. Temperature, energetics, and physiological ecology. *Smith VII*.
- PORTER, W. P., AND D. M. GATES.** 1969. Thermodynamic equilibria of animals with environment. *Ecological monographs*. 39:227-244.
- SEARS, M. W.** 2005. Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia*. 143:25-36.
- SEARS, M. W., M. J. ANGILLETTA, M. S. SCHULER, J. BORCHERT, K. F. DILLIPLANE, M. STEGMAN, T. W. RUSCH, AND W. A. MITCHELL.** 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proceedings of the National Academy of Sciences*.

- SHINE, R., AND T. MADSEN.** 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology*. 69:252-269.
- STEVENSON, R.** 1985a. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*:102-117.
- STEVENSON, R. D.** 1985b. The Relative Importance of Behavioral and Physiological Adjustments Controlling Body-Temperature in Terrestrial Ectotherms. *American Naturalist*. 126:362-386.
- TRACY, C. R.** 1982. Biophysical modeling in reptilian physiology and ecology. *Biology of the Reptilia*. 12:275-321.



# **Chapter 1: Balancing Competing Activities of Foraging and Thermoregulation: Can Thermal Conduction Offset Frequent Exposure to Solar Radiation in a Field Active Ectothermic Ambush Predator?**

## **Abstract**

Terrestrial ectothermic ambush foragers face the unique challenge of balancing thermoregulatory needs with a sedentary foraging strategy in thermally heterogeneous environments. For example, the Timber Rattlesnake (*Crotalus horridus*) must avoid frequent movements to maintain crypsis while tolerating recurring exposure to direct solar radiation (thermal transients) as the sun transits. We examined how an ambush forager like *C. horridus* offsets frequent thermal transients while remaining relatively motionless for hours or days while foraging. We hypothesized that thermal conduction from the body core to the venter-ground interface may play a significant role in countering incoming irradiance heat loads on the dorsal skin surface. We gathered temperature data from adult field active Timber Rattlesnakes along with incoming short-and long-wave radiation and compared conduction rates from the dorsal surface (dorsum) to the body core (core) and from core to the venter-ground interface (ground). Our results suggest Timber Rattlesnakes in closed-canopy deciduous forests like the Ozark Highlands are rarely under thermal stress. Repeated measures correlation analysis did not detect a significant relationship between conduction rates from the dorsum to the core and from the core to the ground within individuals. A cross-correlation analysis was used to test for significant correlations between the two conduction rates at five-minute lag increments from 0-60 mins (approximately three time constants) of  $Q_{cond}$  from dorsum to core. We observed the most significant, yet weak correlations between conduction from the skin to the core and from core to ground after approximately -54, -22, 7, and 9 mins. Timber Rattlesnakes in our population

appear to experience relatively few extreme operative temperatures that could limit the time available for other activities such as foraging. A combination of heat storage and continuous conduction to the ground appear to be important in maintaining a relatively stable core temperature during infrequent periods of intense exposure to direct solar radiation.

## **Introduction**

Allocation of time and energy to competing behavioral and physiological activities is strongly influenced by body temperature ( $T_b$ ) in ectotherms (Huey, 1982; Dunham et al., 1989). Allocating more time to one activity affects time available for other activities (Huey and Slatkin, 1976). In ectotherms, the daily allocation of time to thermoregulation usually takes precedence over all other behaviors (Dunham et al., 1989). However, Hertz et al. (1993) argued that under certain circumstances, many animals simultaneously thermoregulate while performing other activities such as foraging. Many lizards often shuttle between sun and shade while opportunistically feeding on insects (Huey, 1974; Stevenson, 1985b; Grant, 1990). In these situations, the benefits of potentially capturing prey outweigh the consequences of short-term exposure to extreme thermal conditions. However, for an ambush predator a more direct trade-off may occur. Ambush foraging necessitates infrequent movements to maintain crypsis, which exposes the animal to variable environmental conditions. Because body temperature ( $T_b$ ) is a product of complex interactions between the animal and the biophysical environment (Gates, 1980; Grant and Dunham, 1988), ectothermic ambush foragers may frequently face direct tradeoffs between  $T_b$  and foraging.

To understand the relationship between an ectotherm and the biophysical environment we must first quantify the mechanistic interactions at relevant temporal and spatial scales. Heat

transfer mechanisms are dependent on behavioral, morphological, and physiological characteristics of the animal as well as the five fundamental routes of heat transfer ( $Q$ ) between the animal and the environment; convection ( $Q_{conv}$ ), conduction ( $Q_{cond}$ ), evaporation ( $Q_{evap}$ ), metabolism ( $Q_{met}$ ), and radiation ( $Q_{rad}$ ) including solar ( $Q_{sol}$ ) and infra-red ( $Q_{ir}$ ) radiation (Porter and Gates, 1969; Bakken and Gates, 1975). Obtaining empirical data under natural conditions for each route provides a direct comparison of fluxes under different biophysical conditions, and may inform the mechanistic basis of behavioral compensation for thermoregulatory challenges. To date, heat transfer work has focused almost exclusively on small ( $< 100$  g) or large ( $> 5$  kg) reptiles (Spotila et al., 1972; Porter et al., 1973; Stevenson, 1985b; Turner and Tracy, 1985; Huey et al., 1989; Shine et al., 2000; Seebacher and Shine, 2004) although Tracy (1982b) recognized data were lacking that address conduction rates in intermediate sized reptiles.

Thermoregulatory strategies exist along a continuum composed of physiological and behavioral adjustments. However, a general dichotomy is often used to describe the strategy for maintaining suitable  $T_b$ s; active thermoregulation and thermoconforming (Huey and Slatkin, 1976; Hertz et al., 1993; Vickers et al., 2011). According to the cost benefit model introduced by Huey and Slatkin (1976), as energetic cost of thermoregulating increases, individuals should shift to thermoconforming. Support for this dichotomy is well established for small ectotherms (Huey, 1974; Huey and Slatkin, 1976; Congdon et al., 1982; Grant and Dunham, 1988; Hertz et al., 1993; Basson et al., 2016). But the dichotomy may be insufficient because both strategies can be used by the same species or even a single individual, depending on the complexity of the thermal environment and the behavioral context.

Thermoconforming is unconventional in temperate environments because extreme daily and seasonal temperature fluctuations are common (Huey, 1974; Buckley et al., 2015b). Thus, in

more thermally challenging environments ectotherms should spend more time actively thermoregulating by selecting suitable locations at appropriate times (Huey and Pianka, 1977; Vickers et al., 2011). Frequent shuttling behavior and observations of a stable  $T_b$  relative to the frequency distribution of available environmental temperatures, or the operative temperature ( $T_e$ ) distribution (Bakken and Gates, 1975), are often used to characterize active thermoregulation (Grant and Dunham, 1988; Hertz et al., 1993; Wills and Beaupre, 2000). But active thermoregulation may not be energetically efficient for organisms that feed infrequently and rely on prolonged periods of immobility for conserving energy and acquiring prey. Ambush foragers like most rattlesnakes are considered relatively eurythermic in comparison to more heliothermic lizards (Wills and Beaupre, 2000; Alford and Lutterschmidt, 2012). A wide thermal tolerance would allow for infrequent movements that minimize exposure to potential prey (Wills and Beaupre, 2000). However, some studies suggest larger species of rattlesnake maintain relatively stable  $T_b$ s despite highly fluctuating  $T_e$ s (Wills and Beaupre, 2000; Gardner-Santana and Beaupre, 2009). Results like these indicate thermoregulatory mechanisms other than shuttling may be in use.

Despite apparent general adherence to a thermoregulatory strategy, many organisms may frequently shift between strategies to balance trade-offs between thermoregulation and other activities in response to available  $T_e$  (Huey and Slatkin, 1976). Timber rattlesnakes (*Crotalus horridus*) in Arkansas occupy thermally heterogeneous environments where operative temperature distributions do not appear to constrain activity during summer months (Wills and Beaupre, 2000). However, based on  $T_e$  sampling their data suggested that  $T_b$ s of Timber Rattlesnakes were a nonrandom subsample of available thermal microhabitats (Wills and Beaupre, 2000). As the authors suggested, the observed deviation between  $T_e$  and  $T_b$  frequency

distributions may also be due to thermal inertia. The effect of thermal inertia involves three key properties; thermal conductivity, density, and heat capacity ( $C_p$ ) and has a positive relationship with body mass as explained by the heat conservation hypothesis (Meiri, 2011). Heat is dissipated more slowly in larger animals as the surface-area to volume ratio (SA:V) decreases. Thermal inertia provides a stabilizing effect for  $T_b$ s in ectotherms as an increase in body mass produces a decrease in the rate of heat gain and heat loss (Spotila et al., 1973; Bakken, 1976; McNab and Auffenberg, 1976; Bell, 1980; Stevenson, 1985b; Carothers et al., 1997; Seebacher and Shine, 2004; Lutterschmidt and Reinert, 2012). Even moderately sized reptiles like the timber rattlesnake are large enough to be affected by thermal inertia. However, increases in heat flux to the body core must eventually be dissipated by the five routes of heat transfer or  $T_b$  will approach a voluntary thermal maximum ( $VT_{bmax}$ ) resulting from heat storage and insufficient diffusion of heat from the body core, and thus forcing a thermoregulatory adjustment. It appears a combination of thermal inertia, heat storage, and conduction may buffer the animal from transient insolation, and allow continuous foraging in a variable microclimate (Congdon et al., 1982; Dunham et al., 1989).

Ambush foragers like *C. horridus* (Figure 1) rely on crypsis and the ability to remain motionless for extended periods (Huey and Pianka, 1981). In temperate deciduous forests, frequent and repeated exposure to intense heat loads from intermittent solar exposure (i.e., thermal transients) are common. However, the effects of thermal transients are unknown. For example, the frequency, duration, and intensity of thermal transients may have an additive effect producing a significant increase in  $T_b$  over time, forcing a thermoregulatory adjustment. If thermal transients are frequent and intense enough, the snake may be forced to relocate at a frequency that may interfere with maintenance of crypsis, and thus reduce foraging success.

We build upon previous work by addressing theoretical aspects of heat exchange with an empirical approach incorporating field derived data for an intermediate ( $> 300$  g and  $< 1500$  g) sized reptile. Specifically, we investigated how *C. horridus* navigates the two competing behaviors of thermoregulation and ambush foraging by asking, to what extent does conduction from the dorsal skin surface (dorsum) to the venter-ground interface (ground) offset frequent and intense exposure to thermal transients? To answer this question, we first collected in situ temperature and incoming total solar radiation data from field active *C. horridus* in a temperate deciduous environment. These temperatures, and a series of assumptions were used to estimate relevant conduction rates ( $Q_{cond}$ ). We assumed conduction to be linear between the dorsum, core, and ground because the greatest temperature difference would occur between these three points. However, we realize thermal inertia would create a lag in the time it takes for the temperature gradients to become established between the dorsum and the ground. We hypothesized that the magnitude of  $Q_{cond}$  from the core to the ground was sufficient to offset the magnitude of  $Q_{cond}$  from the dorsum to the core during exposure to thermal transients. Secondarily, we tested the assumption that conduction from the dorsum to the ground was disjunct resulting from the effect of heat storage. Increases in thermal inertia due to larger body size should increase the lag in heat transfer from the dorsum to the core and therefore, correspond to a lag in heat transfer from the core to the ground. Any delay in heat transfer from the dorsum to the ground would implicate heat storage.

## Materials and Methods

### *Heat Transfer Model*

In ectotherms, the dorsum is the primary connection between the external environment and the body core via the five routes of heat transfer previously outlined above (Porter and Gates, 1969). Therefore, energy transfer between an animal and its environment under steady state conditions can be expressed as Heat Energy In = Heat Energy Out (Porter and Gates, 1969) in Watts/whole animal or Watts/m<sup>2</sup> as indicated, and illustrated by simplifying the complex processes to a word equation;

$$Q_{met} + Q_{sol} + Q_{ir-in} = Q_{ir-out} + Q_{conv} + Q_{evap} + Q_{cond} + Q_{stored} \quad (\text{Eq. 1}).$$

Variation in any single route may have an immediate effect on  $T_b$  (Porter and Gates, 1969; Bakken, 1976). Identifying the relative contribution of each route allows us to remove the least influential from the equation and focus on those routes with the most significant effect on the thermoregulatory strategy (Dunham et al., 1989).

We provide brief descriptions of each transfer route to outline our approach. For more detailed descriptions Porter and Gates (1969); Bakken and Gates (1975); see; Bakken (1976); and Porter and Tracy (1983). Convective heat transfer is the conduction of heat between the layer of air surrounding an animal, or boundary layer, and the animal's surface (Porter and Gates, 1969). As wind increases the boundary layer surrounding the animal is stripped away, increasing the effect from  $Q_{conv}$  (Porter and Gates, 1969). Additionally, as an animals' profile extends above the ground, convective heat transfer increases as a function of air movement across the exposed surface (Porter and Gates, 1969; Mitchell, 1976). However, snakes often adopt a low profile in the environment (i.e., on the ground) and we commonly observed Timber Rattlesnakes "cratered" in the leaf litter with the dorsal surface of the snake aligned and parallel to the leaf

litter surface, effectively becoming part of the ground and allowing a thicker boundary layer to accumulate (Porter and Gates, 1969; Mitchell, 1976; Withers, 1992; Vogel, 1996). Based on the described geometry, we assumed the effect from  $Q_{conv}$  to be minimal because; 1) wind velocity is often negligible in the understory of temperate hardwood forests especially near the leaf litter surface and, 2) the low profile assumed by many snakes including Timber Rattlesnakes further decreases the effect of  $Q_{conv}$ . To verify these assumptions, we recorded wind speeds at 1 m above the ground surface and at the height of the snakes dorsum and observed negligible (i.e., 0.1 m/s) wind speeds at the ground surface even as windspeeds at 1 m exceeded 8 m/s (Appendix 1). Thus, we assumed convection was minimal and removed convective heat transfer from our equation.

Metabolism and evaporation can significantly contribute to heat balance in some reptiles. However relative to endotherms,  $Q_{met}$  in ectotherms contributes little to heat balance (Seebacher et al., 1999; Seebacher and Franklin, 2005). Likewise, evaporative water loss is often negligible in low energy specialists like *C. horridus* (Wang et al., 1998; Borrell et al., 2005). Tattersall et al. (2006) observed only modest differences between head temperatures and ambient temperatures due to EWL at relative humidity between 40 and 70%, similar to what is often observed in our system. Therefore, we can remove  $Q_{met}$  and  $Q_{evap}$  as significant heat exchange routes for *C. horridus* as both are relatively small fluxes that are believed to effectively balance each other (Bakken, 1992). We are left with a simplified steady state equation modified from equation 1;

$$Q_{sol} + Q_{ir-in} = Q_{ir-out} + Q_{cond} + Q_{stored} \quad \text{Eq. 2.}$$

Substituting quantifiable variables for each factor we arrive at the following equation following Porter and Gates (1969);

$$Q_{sol} + \sigma \bar{a} AT_e^4 = \sigma \epsilon AT_r^4 + \frac{kA(\Delta T)}{x} \quad \text{Eq. 3}$$



with  $\sigma$  denoting the Stefan-Boltzman constant ( $5.67 \times 10^{-8} \text{ Wm}^{-2}\text{K}^{-4}$ ),  $\bar{a}$  is the mean absorptivity of the snakes' surface to incident solar radiation,  $T_{\bar{e}}$  is the mean environmental temperature,  $\varepsilon$  is the emissivity of the organism (0.95) and is dimensionless (Withers, 1992; Tattersall et al., 2004),  $k$  is the thermal conductivity of snake muscle  $0.58 \text{ W/cm/K}^\circ$  (Li et al., 2014),  $A$  is the effective area ( $\text{cm}^2$ ) of a coiled snake (i.e. the dorsal and ventral surfaces),  $\Delta T$  is the change in temperature ( $^\circ\text{C}$ ) between the dorsum temperature ( $T_r$ ) and  $T_b$  or, between  $T_b$  and the venter-ground interface ( $T_g$ ), and  $x$  is the distance (cm) between  $T_r$  and  $T_b$  or, between  $T_b$  and  $T_g$ . The temperatures used for calculating radiation flux are in degrees Kelvin ( $K$ ). Absorptivity to environmental long wave radiation is most often between 0.95 and 1.0 while absorptivity to sunlight and skylight can vary between 0.2 and 1.0 (Porter and Gates, 1969). Incoming  $Q_{sol}$  and  $Q_{ir}$  are directly measured using Apogee Instruments SP-110 pyranometer and SL 510 pyrgeometer connected to a Campbell Scientific® CR300series-WiFi 2D datalogger, respectively. Outgoing  $Q_{ir}$  was calculated for the dorsal and ventral surfaces of the snake, based on surface temperatures measured with an IR thermometer. Once a steady state solution has been calculated, any discrepancy between incoming and outgoing energy is likely a result of heat storage ( $Q_s$ ). Heat storage occurs when the temperature gradient is directed towards the internal body temperature (i.e.  $T_b$  is less than  $T_g$  and  $T_r$ ). Once  $T_b$  has exceeded  $T_r$  or  $T_g$ , conduction reverses the direction of heat flow towards the lowest temperature.

The heat conservation hypothesis describes the positive relationship between body mass ( $M$ ) and heat capacity ( $C$ ) whereby heat transfer is slower in larger animals as the SA:V decreases (Meiri, 2011; Zamora-Camacho et al., 2014). Heat capacity depends on the quantity of material whereas specific heat ( $C_p$ ) indicates the energy needed to change the temperature of a unit mass of a substance by  $1^\circ\text{C}$  ( $\text{J/g K}$ ) (Gates, 1980). As the animal increases in size and thus

mass,  $C$  increases while  $C_p$  remains unchanged because it is specific to the material and not its mass. Therefore, higher  $C_p$  and  $C$  allow an ambush predator to remain motionless for extended periods (Spotila et al., 1973; Tracy, 1982b; Stevenson, 1985b; Cushman et al., 1993; Christian et al., 2006). Specifically,  $Q_s$  is affected by  $M$ , the  $C_p$  of muscle tissue (3.46 J/kg K) (Giering et al., 1996), and the rate of temperature change ( $\frac{dT}{dt}$ ). The following equation;

$$Q_s = MC_p \frac{dT}{dt} \quad (\text{Eq.4}),$$

can therefore be used to quantify  $Q_s$ . Combining equations 3 and 4 provides our final modified equation from Porter and Gates (1969):

$$Q_{sol} + \underbrace{\sigma \epsilon A T_e^4}_{Q_{ir-in}} = \underbrace{\sigma \epsilon A T_r^4}_{Q_{ir-out}} + \underbrace{\frac{kA(\Delta T)}{x}}_{Q_{cond}} + \underbrace{MC_p \frac{dT}{dt}}_{Q_s} \quad (\text{Eq. 5}).$$

The area term associated with  $Q_{cond}$ ,  $Q_{ir-in}$ , and  $Q_{ir-out}$  reveals the direct proportional relationship to surface area through which heat energy is conducted. In addition, solar radiation is absorbed over the effective surface area of the animal and is measured in W/m<sup>2</sup> per unit area. Thus, by directly measuring incoming short and longwave radiation, temperatures at the skin, body core, and venter-ground interface we can estimate the relative magnitude of  $Q_s$  and  $Q_{cond}$  under different scenarios of change in  $Q_{sol}$ .

### Study Site

Data were collected at the Ozark Natural Science Center (ONSC) and Madison County Wildlife Management Area (MCWMA) in Madison County, Arkansas in conjunction with a long-term radio-telemetry project currently entering the 26<sup>th</sup> year of monitoring and research activity (Wills and Beaupre, 2000; Browning et al., 2005; Lind and Beaupre, 2015; Beaupre et al., 2017). The study site is a mix of current and previously managed oak-hickory (*Quercus spp.*-*Carya spp.*) hardwood and pine (*Pinus spp.*) forested areas, cedar (*Juniperus spp.*) glades,

wildlife feed plots, perennial and intermittent streams in the drainages, and limestone and dolomite outcroppings (Browning et al., 2005).

### *General Data Collection*

Rattlesnakes spend a large portion of the active season in a coiled position, likely a behavioral adaptation to facilitate defensive and offensive strikes (Roth, 2003). But coiling may also facilitate other functions relating to heat transfer. For example, a snake can adjust its general shape (elongate to coiled) affecting the surface area (SA) exposed to the incoming heat source or outgoing heat sink thus, influencing the rates of conduction and convection (Ayers and Shine, 1997; Wills and Beaupre, 2000). Coiling behavior was also advantageous for calculating the SA of each snake. Timber Rattlesnakes in our study population are moderate to large sized snakes (adult SVL 81.4 - 117.4 cm, mean = 91.61 cm, mass 310.5 - 1474.5 g, mean = 677.6 g). We treated a coiled snake as a short cylinder and used a tape measure to estimate the height and radius of a sample of snakes in situ to get a range of sizes. We then estimated the SA of the dorsal and ventral portions for each individual.

Snakes were brought into the lab each spring for processing and morphometric data (snout-vent length, tail length, mass, sex, and rattle measurements) were collected and the general health of each snake was assessed. All recaptured and newly captured snakes that met minimum size requirements (transmitter was  $\leq 5\%$  total mass of the snake) were re-implanted/implanted with radio-transmitters equipped with a temperature sensor weighing approximately 13.5 grams and a battery life of 12-30 months (Holohil Systems LTD, model SI-2T) following a modified protocol from Reinert and Cundall (1982). An active pool of approximately 10-15 snakes were maintained for tracking each year and were located an average of every 3-4 days. The majority of snakes used for this study were males to avoid disturbing

gravid females. Due to similar morphologies, foraging behaviors, and basic physiological processes between sexes, we assumed that foraging males and females interacted with the thermal environment identically when females were not gravid. The females used in this study ( $n=3$ ) were not gravid or were post-partum for at least one active season.

### *Temperature Data*

As transient incoming solar radiation heats the dorsal surface of the snake, heat is diffused through the snake's body towards the cooler, more stable ground temperatures. Therefore, we assume heat is most often conducted along a thermal gradient from the dorsum to the venter-ground interface during hot days in the active season. We collected temperatures along a vertical axis perpendicular to the body of a coiled snake (Figure 2). Body temperature data (i.e.  $T_r$ ,  $T_b$ , and  $T_g$ ) were collected opportunistically from May through October, 2017 – 2019 from snakes found coiled in a hunting posture. We simultaneously collected thermal data from the snakes' dorsum to the venter every 1-5 minutes for as long as the snake remained in position.

The radio transmitters' temperature-sensor provided the  $T_b$ . A ThermoWorks<sup>®</sup> Inc. Professional Infrared (IR) thermometer with an accuracy of  $\pm 1^\circ \text{C}$  at 3 m was used to collect the average  $T_r$  from a maximum distance of 0.5 m (Figure 3a). Emissivity was set to 0.95 in accordance to the emissivity of most living tissue (Birkebak et al., 1964; Bakken, 1976; Campbell and Norman, 2012). Finally,  $T_g$  was collected using an Omega<sup>®</sup> handheld digital thermometer with a thermocouple wire fixed to a 0.9 m extendable probe positioned under the snake to the approximate center of the coils (Figure 3c). The thermometer was allowed to equilibrate for a minimum of 5 minutes prior to data collection. Data collection occurred at

different time intervals (1-5 minutes) so we adjusted all calculations to reflect one minute intervals. All temperature units are in °C.

### *Conduction Rates and Heat Storage*

Characterizing a coiled snake as a flattened cylinder allowed us to assume the most relevant heat flux was occurring through a horizontal slab. We calculated whole animal conduction rates (W) using the temperature differences from  $T_r$  to  $T_b$  and from  $T_b$  to  $T_g$  (equation 3) and then adjusted for dorsal and ventral surface area to provide per unit area conduction ( $\text{W}/\text{cm}^2/^\circ\text{C}$ ). Any discrepancy between the two conduction rates would likely result from heat storage and could be quantified using equation 4. To simplify terminology related to conduction, hereafter we refer to dorsal surface as “skin”, body core as “core”, and the venter-ground interface as “ground”.

### *Solar Radiation*

The total effective incoming solar radiation on the dorsum is dependent on the duration and intensity of the thermal transient. Incoming  $Q_{sol}$  and  $Q_{ir}$  data were collected concurrent to temperature data at one and five minute intervals using an Apogee SP-110 Pyranometer and SL-610 Pyrgeometer connected to the CR300 series datalogger from Campbell Scientific® and positioned approximately 0.5 m - 1 m from the snake and approximately 15 cm off the ground (Figure 3b). We assumed  $Q_{sol}$  and  $Q_{ir-in}$  were the greatest sources of incoming heat experienced by a snake at ground level. We calculated outgoing longwave radiation from the snakes’ dorsum using the equation for  $Q_{ir-out}$  and subtracted that from the total incoming longwave radiation. All incoming solar radiation data were converted to whole animal values (W) and are collectively referred to as  $Q_{rad}$ .

### *Mass*

Mass is a significant factor affecting heat transfer in reptiles (Bartholomew and Tucker, 1964; Spotila et al., 1973; McNab and Auffenberg, 1976; Seebacher and Shine, 2004). We attempted to contribute further to this body of literature by considering the effect of mass on thermal conduction in medium-to large-bodied reptiles. We assigned individuals to groups based on their mass rounding to the nearest 100g (e.g. 400g, 500g, etc.) and compared conduction rates across groups.

### *Statistical Analysis*

We used 23 field observations from 15 snakes to derive repeated measures (i.e., multiple observations per individual) temperature data with unbalanced replications (i.e., the time sampled varied per observation). Our primary goal was to establish the relationship between incoming solar radiation and conduction from the dorsum to the core and from the core to the ground. Secondly, we attempted to determine the effect of mass on the rate of which thermal gradients are established under heat loads from incoming solar radiation. Increased body mass should create a lag between the change in temperature at the dorsum to core and a corresponding change in temperature from the core to the ground.

We were also interested in the role thermal conduction has in mitigating the effects of incoming heat loads and the affect body mass has on the time it takes to establish thermal gradients. We used the temperature differences between  $T_r$  to  $T_b$  and  $T_b$  to  $T_g$  to calculate conduction rates. A paired t-test was used to test for differences between mean temperatures and mean conduction rates from dorsum to core and core to ground.

The common correlation methods for paired data (e.g. Pearson, Spearman, Kendall) assume independent observations. By design, our data were collected simultaneously and analyses often included overlapping variables (e.g, conduction from dorsum to core and from

core to ground) and thus, are paired and repeated observations. We used a repeated measures correlation (rmcorr) (Bland and Altman, 1995b; Bland and Altman, 1995a) using the Rmcorr package (Bakdash and Marusich, 2017) in the R statistical package (R Development Core Team, 2018) to examine the within-individual strength and direction of the relationship between  $Q_{cond}$  from the dorsum to the core and from the core to the ground. Rmcorr is calculated using a form of analysis of covariance (ANCOVA) and therefore, follows the same assumptions (Bakdash and Marusich, 2017). Non-independence among observations is resolved using ANCOVA to statistically adjust for inter-individual variability and therefore produces the best linear fit for each individual using equal slopes with varying intercepts (Bakdash and Marusich, 2017). The rmcorr coefficient ( $r_{rm}$ ) is bound by -1 and 1 representing the strength of the linear association between two variables (Bakdash and Marusich, 2017).

Heat storage is dependent on several factors including the mass of the tissue storing the heat, the specific heat of the tissue (J/kg/°C), and the temperature difference. Increased body mass should affect the time to establish the thermal gradients hence, creating a lag between the change in temperature at the dorsum to core and a corresponding change in temperature from the core to the ground. We assigned snakes to one of eight groups based on mass (e.g. 400g, 500g, etc.) and used linear regression to determine if mass had an effect on total rate of conduction. To address the effect of body mass on the time required to establish thermal gradients, we explored the relationship between mass and total conduction rate within individuals using a rmcorr.

Cross-correlation function (CCF) analysis was used to detect conduction covariance over different temporal scales between the two conduction rates by producing a lagged regression where we tested the y-variables' ( $Q_{cond}$  from core to ground) response at present time to stepwise five-minute time lags of the x-variable ( $Q_{cond}$  from dorsum to core). We also used CCF to detect

covariance between each of the conduction rates (y-variables) and total incoming solar radiation (x-variable). The principles of heat transfer are well established (Kreith et al., 2012) as are the relationships between the mechanisms (i.e. conduction, convection, thermal radiation).

Therefore, we expected autocorrelation and non-independence between the two conduction rates and were interested in the strength of those relationships. Ecological field data involving individual sampling often consists of non-stationary time series data (i.e. sample mean and variance are not consistent over time), has a relatively short duration ( $\leq 1$  hour), and small sample sizes that may display trends over time that are often hidden when data are transformed (Bakdash and Marusich, 2017). Stationarity is a stochastic process that occurs when the shape of the distribution remains constants even with a shift in time. One way to achieve stationarity is to calculate the differences between consecutive observations effectively stabilizing the mean of the times series (Arellano and Pantula, 1995). However, stationarity can hide underlying trends. In time-series analyses, filtering or data transformations such as pre-whitening are often used to remove any correlation between variables and set the variance to 1, potentially diminishing significant cross-correlations associated with lag times (Chatfield, 2003; DeWalle et al., 2016). Specifically, we were interested in the lag response of  $Q_{cond}$  from the core to the ground to  $Q_{cond}$  from the dorsum to the core in addition to the relationship between each conduction rate and total incoming solar radiation. Therefore, we chose not to pre-whiten or filter the time series data. We then used linear regression to test whether  $Q_{cond}$  from the core to the ground is a linear function of past lags, or earlier time points of  $Q_{cond}$  from the dorsum to the core. Autocorrelation function analysis and PACF identifies the relationships between each conduction rate against the five-minute time lags. The ACF determines how strongly an observation, and its lags are correlated with each other whereas the PACF removes indirect correlations in the series so only the most



important lags are presented. Autocorrelation function (ACF) analysis was used to summarize the relationships between the first value of  $Q_{cond}$  from the core to the ground with lags of  $Q_{cond}$  from the dorsum to the core. Partial autocorrelation function (PACF) analysis was used to find the correlation of the residuals of  $Q_{cond}$  from the core to the ground with lags of  $Q_{cond}$  from the dorsum to the core. We also used ACF and PACF to assess the relationship between solar radiation and the two conduction rates. Significance of cross-correlation time lag was determined using a 95% confidence threshold.

Data were examined to determine if parametric assumptions were met and residuals were tested for normality. All statistical analyses adopted a type one error rate of 0.05.

## Results

Field measurements yielded 308 sample points for each body temperature ( $T_r$ ,  $T_b$ , and  $T_g$ ) during 83 sampling events ranging from 2 - 110 minutes ( $\bar{x}$  = 12 mins). We only used sampling events  $\geq 10$  mins and on days with minimal cloud cover for this analysis ( $n = 23$ ). Several individuals were sampled multiple times and the sampling frequency ranged from 1-5 minutes and was converted to a one-minute sample frequency for all relevant calculations (Table 1).

### *Temperatures*

Wills and Beaupre (2000) identified 37.4 °C as the voluntary thermal maximum for *C. horridus* in the same population but have no behavioral notes associated with this temperature. We did not observe a  $T_b$  greater than 33.9 °C in this study. Thus, we used 34.0 °C for the  $VT_{bmax}$  when considering the threshold core body temperature that may elicit a thermoregulatory movement. The average  $T_r$  observed for the 23 snakes was  $25.1 \pm 3.4$  °C,  $24.3 \pm 3.3$  °C for  $T_b$ , and  $21.0 \pm 2.8$  °C for  $T_g$  ( $n = 308$ ).

The temperature difference is one factor that directly affects the rate of conduction. We used paired T-tests to compare the means for each body temperature and observed a statistically significant differences between  $T_r$  and  $T_b$  ( $t = 5.61$ ,  $df = 307$ ,  $p < 0.05$ ),  $T_r$  and  $T_g$  ( $t = 13.66$ ,  $df = 599.89$ ,  $p < 0.05$ ), and between  $T_b$  and  $T_g$  ( $t = 26.96$ ,  $df = 307$ ,  $p < 0.05$ ). The immediate effect of transient insolation on the dorsal surface is evident when individual temperature profiles are observed (Figure 4a). Temperatures at the core and ground-venter interface remained relatively stable even as the dorsum experienced increased heat loads from thermal transients.

### *Conduction Rates*

Conduction rates ranged from 0.00 W/min to 16.23 W/min from the dorsum to the core ( $\bar{x} = 3.54$ ,  $s = 3.11$ ) and 0.97 W/min to 23.82 W/min from the core to ground ( $\bar{x} = 5.59$ ,  $s = 3.74$ ). Conduction from the dorsum to the core was often lower than conduction from the core to the ground (Figure 4b). Individual mean conduction rates ranged from 0.42 to 5.85 W ( $\bar{x} = 1.67$ ,  $s = 0.29$ ) from dorsum to core and 0.85 to 11.61 W ( $\bar{x} = 3.03$ ,  $s = 0.22$ ) from core to ground. Average individual heat storage values ranged from 0.08 to 6.18 W ( $\bar{x} = 1.63$ ,  $s = 0.29$ ; Table 2). A Levene's test revealed unequal variances among conduction rates ( $F(1) = 0.546$ ,  $p = 0.460$ ) so we used a Welch's t-test and detected a significant difference between the two conduction rates ( $t = -7.375$ ,  $df = 593.97$ ,  $p < 0.001$ ). However, the within-individual rmcorr analysis did not detect a significant relationship between conduction rates from the dorsum to the core and from the core to the ground ( $r_{rm} = -0.091$ ,  $df = 166$ , 95% CI (-0.627, 0.240),  $p = 0.243$ ; Figure 5).

We used CCF analysis to test for significant correlations between the two conduction rates at five-minute lag increments from 0-60 mins (approximately three time constants) of  $Q_{cond}$  from dorsum to core. The most significant correlations occurred at approximately -54 mins ( $r = -0.180$ ), -22 mins ( $r = -0.190$ ), -7 mins ( $r = -0.215$ ), and 9 mins ( $r = 0.191$ ; Figure 6). A negative

correlation indicates an increase in conduction from dorsum to core leads to an increase in conduction from core to ground approximately 7 mins later.

Five-minute time lags of conduction from the dorsal surface to the body core were used as a predictor of conduction from body core to venter-ground interface in our regression analysis (Table 3). Our model explained 21.7% of the variance and was a significant predictor of conduction from the body core to the venter-ground interface  $F(12, 107) = 2.464, p = 0.007$ . We expected autocorrelation to occur between the two conduction rates and observed a number of significant indirect correlations in the ACF (Figure 7a). The PACF identified significant positive correlations at approximately 7 and 10 minutes (Figure 7b), further supporting that exposure to incoming solar radiation on the dorsum leads to increased conduction from the core to the ground after approximately 7-10 minutes. It should be noted that the correlation we observed between the two conduction rates in this analysis used averages and would vary depending on the mass of the animal (i.e., smaller snakes experiencing a shorter lag time under identical conditions and larger snakes experiencing longer lags).

### *Solar Radiation and Conduction*

Incoming total solar radiation ranged from 452.60 to 1370.40 W/m<sup>2</sup> and averaged 618.57 ± 210.72 W/m<sup>2</sup> ( $0.038 \pm 0.021$  W/m<sup>2</sup>) for our data. Thirteen separate observations exceeded 1000 W/m<sup>2</sup> equating to 10.6% of the 523 mins sampled (Table 4). A rmcrr analysis suggests significant, yet weak negative relationships exist between incoming total solar radiation and conduction from dorsum to the core ( $r_{rm} = -0.232, df = 166, 95\% \text{ CI } (-0.372, -0.083), p = 0.003$ ) and from the core to the ground ( $r_{rm} = -0.293, df = 166, 95\% \text{ CI } (-0.426, -0.147), p = 0.0001$ ) (Figure 8).

A CCF analysis indicated a high correlation exists between incoming solar radiation and an increase in conduction from dorsum to core ( $r = 0.649$ ; Figure 9a). We observed a peak negative correlation at -54 mins ( $r = -0.243$ ) indicating increases in incoming solar radiation to the dorsal surface led to increases in conduction from the body core to the ground after approximately 54 mins (Figure 9b), or approximately two time constants for Timber Rattlesnakes in this population. The discrepancy between the 54 min lag and the 7 min lag response in conduction mentioned earlier likely occurs because more time is required for the effect of radiation on the dorsum to be translated from an increase in temperature to a corresponding increase in conduction.

Multiple regression identified a significant correlation between solar radiation and conduction from dorsum to core after 15 min (Table 5a). However, no correlation was observed between solar radiation and conduction from the core to ground at any time lag (Table 5b). Our regression model for solar radiation and conduction from dorsum to core was not significant and explained 16.0% of the variance  $F(12, 107) = 1.703, p = 0.076$ . Our overall model for solar radiation and conduction from the core to the ground was significant but only explained 21.7% of the variance  $F(12, 107) = 2.472, p = 0.006834$ . Residuals of the ACF and PACF show the relationship between each conduction rate against five-minute time lags of total incoming solar radiation (Figure 10a and 10b). After controlling for indirect correlations using the PACF we observed one significant correlation at 10 minutes between solar radiation and  $Q_{cond}$  from the core to the ground (Figure 10b).

### *Mass and Conduction*

Masses ranged from 417.1 g to 1474.5 g ( $\bar{x} = 823.8 \pm 293.42$  g,  $n=23$ ) and was divided into eight 100g groups beginning with the smallest (e.g., 400g, 500g, etc.; Figure 11). We

observed an effect of mass on the rate of conduction from dorsum to core ( $t = -49.06$ ,  $df = 307.07$ ,  $p < 0.001$ ) and from core to ground ( $t = -48.93$ ,  $df = 307.10$ ,  $p < 0.001$ ). A post-hoc test indicated an effect of mass on the combined conduction rate ( $F(2, 288) = 57.785$ ,  $p < 0.0001$ ).

## Discussion

Snakes in our study experienced extreme thermal transients at low rates of occurrence (i.e., 10% of the time) as indicated by the relative distribution of total incoming solar radiation measured (Table 4). Thus, significant and prolonged thermal transients appear to be uncommon in this system. Temperature profiles from our data indicated  $T_b$  and  $T_g$  remained tightly coupled and relatively stable even when exposed to extreme dorsal thermal transients (Figure 4).

Although the temperature analysis was performed without a time lag, the differences between temperatures indicated a non-linear or time-delayed relationship exists between  $T_r$  and  $T_b$  or  $T_g$ . Interestingly, the means of  $T_r$  ( $\bar{x} = 25.1$ ,  $s = 3.4$  °C) and  $T_b$  ( $\bar{x} = 24.3$ ,  $s = 3.3$  °C) were often more similar compared to the means of  $T_b$  and  $T_g$  ( $\bar{x} = 21.0$ ,  $s = 2.8$  °C) (Figure 4a.), further supporting the assumption that the ground was acting as a heat sink.

Accordingly, we often observed stable and higher  $Q_{cond}$  from the core to the ground compared to  $Q_{cond}$  from dorsum to core, except when subjected to high heat loads on the dorsum (Figure 4b). Negative correlations in the repeated measures analysis is likely a result of the often higher conduction rate from core to ground indicating conduction rates are relatively impervious to solar transients. Multiple regression analysis identified a significant relationship between the two conduction rates and conduction from the dorsum to ground was a significant predictor of conduction from the core to the ground after approximately seven to 10 minutes, although our model explained only a small amount of variance (Table 3).

Regression analysis for five-minute time lags of total incoming solar radiation to each conduction rate indicated no significant linear relationship existed between solar radiation and conduction from the dorsum to the body core. However, a significant relationship was detected between solar radiation and conduction from the core to the ground although the model only explained a small amount of variance (Table 5). We did observe a significant correlation between solar radiation and conduction from dorsum to core after 15 min of radiation exposure indicating 15 mins of exposure to solar radiation will induce a change in conduction from dorsum to core. Conversely, no coupling between incoming radiation and core to ground conduction was observed. Our low sample size or the low occurrence of extreme incoming solar radiation events may be contributing to the lack of strong correlations. Furthermore, a correlation was not detected between lags of solar radiation exposure and the residuals of each conduction rate (i.e. dorsum to core and core to ground). Again, we suspect the low frequency and duration of solar transient events coupled with the effects of heat storage act to reduce the immediate effects of exposure to the conduction rate from the dorsum to the core. The two conduction rates were often decoupled also suggesting heat storage is a significant factor in maintaining relatively stable  $T_{bs}$ .

Negative correlations observed between incoming radiation and conduction rates (Figure 8) are likely a result of the consistently higher conduction rates from core to ground and the relatively short durations of thermal transients. Direct thermal radiation has a positive correlation with conduction rate from dorsum to core among individuals (Figure 9a). However, the negative correlation observed in the rmcrr analysis identifies the within-individual relationship between conduction rate and radiation which suggests conduction rates may covary with another variable

such as mass or heat storage. In fact, mass may be the most significant factor affecting heat exchange in an intermediate sized ectotherm in a closed canopy system.

The relationship between mass and heat storage in large reptiles is well established (McNab and Auffenberg, 1976; Bell, 1980; Lutterschmidt and Reinert, 2012). However, intermediate sized reptiles (e.g., < 2 kg) may face a unique set of conditions with regard to the role of thermal inertia and heat storage. Stored heat is continuously conducted to the ground even during periods of low intensity solar radiation (i.e., shade) as indicated by the consistently greater difference between  $T_b$  and  $T_g$  compared to  $T_r$  and  $T_b$  (Figure 4). The average  $T_b$  of  $24.3 \pm 3.3$  °C in the warmest month of July was approximately 13 °C cooler than the maximum observed in situ  $T_b$  of 37 °C (Wills and Beaupre, 2000) and 10 °C cooler than the maximum observed  $T_b$  in this study. Heat is continuously being fluxed toward the cooler ground, helping maintain a relatively low body core temperature despite variable and sometimes intense solar irradiation.

Theoretically, the effect of mass on heating and cooling rates suggests heat storage acts as a stabilizing factor for  $T_b$  (Bell, 1980; Slip and Shine, 1988; Seebacher and Shine, 2004). We detected an effect of mass on individual and combined conduction rates ( $F(2, 288) = 57.785$ ,  $p < 0.0001$ ). Anecdotally, conduction from core to ground was often higher than from dorsum to core for snakes less than 1000 g. In snakes with higher masses, the conduction rates appear to reverse with conduction from dorsum to core often higher than conduction from core to ground (Figure 11). Furthermore, we detected significant, yet weak negative correlations between mass and each conduction rate. A negative correlation suggests more energy is required to raise the core temperature in larger snakes indicating a greater tolerance to increases in skin temperature.

Clark (2006) suggested Timber Rattlesnakes navigate the environment choosing and remaining at potential ambush sites based on the likelihood of encountering potential prey. Timber Rattlesnakes in our population appear to experience relatively few extreme  $T_e$ s that could limit the time available for other activities such as foraging. Our data appears to support the assertion that Timber Rattlesnakes can navigate their environment with minimal thermal constraints (Clark, 2006).

Multiple mechanisms such as conduction and heat storage often work concurrently to maintain a relatively stable  $T_b$ . In this study, total conduction was not significantly affected by thermal transients. However, when conduction was viewed as two separate rates from dorsum to core and core to ground, we observed significant yet weak correlations between radiation and each conduction rate. The weak, negative relationships are likely an artifact of mass and heat storage delaying the translation of solar transients to thermal conduction from dorsum to core and from core to ground. Additionally, constant and often higher conduction rates observed from core to ground would further delay the effect of the thermal transient until a sufficient thermal gradient has been established. Thus, constant flux to the ground from the body core may be more ecologically relevant in the long term. Perhaps the maximum voluntary body temperature observed by Wills and Beaupre (2000) represented an extreme thermal transient event in which the snake was forced to move. Unfortunately, associated behavioral data are not available.

Several studies address the role of conduction in heat transfer using mathematical models (Bogert, 1949; Spotila et al., 1973; Paladino et al., 1990; Seebacher and Shine, 2004). We provide a different approach for collecting in situ, real-time temperature data from animals under varying natural conditions while experiencing thermal transients that allowed us to calculate ecologically relevant conduction rates. Although many of the mechanisms involved in heat



transfer are relevant and even critical to ectotherms, the degree to which each mechanism operates is specific to the organism and the thermal microhabitat. For an ambush predator, the significance of maintaining a relatively stable body temperature without employing behavioral thermal adjustments like shuttling is a necessity, especially when considering tradeoffs between time and energy allocation. Each behavior has an associated energetic cost and any behavior other than foraging may reduce the total energy budget (Beaupre, 2002). Thus, reducing time and energy associated with non-foraging behaviors like thermoregulation would allow more time for foraging activities, and presumably, increased foraging success.

Tracy (1976) recognized the importance of understanding conductive heat transfer in energy budgets and modeling conduction in reptiles while experiencing thermal transients. The degree of importance of heat transfer in small and large reptiles is also well documented (Spotila et al., 1972; Spotila et al., 1973; Tracy, 1976; Muth, 1977) but data is lacking for intermediate sized reptiles like the Timber Rattlesnake. The analysis presented here represents an attempt at quantifying conduction rates for field active Timber Rattlesnakes in the Ozark Highlands and identifies the relative importance of conductive heat transfer to thermoregulation in an ectothermic ambush predator that exhibits close contact with the substrate. We have presented a novel attempt at assessing the role of conduction in maintaining core body temperature under natural conditions. While heat transfer analyses are relatively common, the methods and conditions under which our data were collected represent a unique quantification of conductive heat transfer in a intermediate sized terrestrial ectotherm. Continued work is needed to augment the current data set including more size classes within the same species and other species from different habitat types (where constraints may be greater). Ambush foraging pitvipers are essentially flattened cylinders of varying surface areas and volumes. Because of their similar

morphologies, valid comparisons of the same mechanisms under different conditions and environments are possible. Comparisons across species, in different habitats, and under different operative environments would provide deeper understanding of the efficacy and plasticity of heat transfer mechanisms. Thus, increased understanding of how heat transfer mechanisms influence tradeoffs between behaviors like foraging and thermoregulation provides better insight into time and energy allocation in ectotherms.

## References

- ABD LATIF, Z., AND G. A. BLACKBURN.** 2010. The effects of gap size on some microclimate variables during late summer and autumn in a temperate broadleaved deciduous forest. *International Journal of Biometeorology*. 54:119-129.
- ADOLPH, S. C., AND W. P. PORTER.** 1993. Temperature, Activity, and Lizard Life Histories. *The American Naturalist*. 142:273-295.
- AGUGLIARO, J.** 2011. Ecophysiology of hibernation in timber rattlesnakes ( *Crotalus horridus*), p. 277. Vol. Ph.D. University of Arkansas, United States -- Arkansas.
- ALFORD, J. G., AND W. I. LUTTERSCHMIDT.** 2012. Modeling energetic and theoretical costs of thermoregulatory strategy. *Journal of Biological Dynamics*. 6:63-79.
- . 2018. From conceptual to computational: Cost and benefits of lizard thermoregulation revisited. *Journal of Thermal Biology*. 78:174-183.
- ARELLANO, C., AND S. G. PANTULA.** 1995. Testing for trend stationarity versus difference stationarity. *Journal of Time Series Analysis*. 16:147-164.
- AYERS, D., AND R. SHINE.** 1997. Thermal influences on foraging ability: body size, posture and cooling rate of an ambush predator, the python *Morelia spilota*. *Functional Ecology*. 11:342-347.
- BAKDASH, J. Z., AND L. R. MARUSICH.** 2017. Repeated Measures Correlation. *Frontiers in Psychology*. 8.
- BAKKEN, G. S.** 1976. A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data to field ecology. *Journal of Theoretical Biology*. 60:337-384.
- . 1992. Measurement and application of operative and standard operative temperatures in ecology. *American Zoologist*. 32:194-216.
- BAKKEN, G. S., AND D. M. GATES.** 1975. Heat-transfer analysis of animals: some implications for field ecology, physiology, and evolution, p. 255-290. *In: Perspectives of biophysical ecology*. Springer.
- BAKKEN, G. S., W. R. SANTEE, AND D. J. ERSKINE.** 1985. Operative and Standard Operative Temperature: Tools for Thermal Energetics Studies. *American Zoologist*. 25:933-943.
- BARTHOLOMEW, G. A., AND V. A. TUCKER.** 1964. Size, body temperature, thermal conductance, oxygen consumption, and heart rate in Australian varanid lizards. *Physiological zoology*. 37:341-354.

- BASSON, C. H., O. LEVY, M. J. ANGILLET, AND S. CLUSELLA-TRULLAS.** 2016. Lizards paid a greater opportunity cost to thermoregulate in a less heterogeneous environment. *Functional Ecology*:n/a-n/a.
- BEATTY, S. W., AND O. D. SHOLES.** 1988. Leaf litter effect on plant species composition of deciduous forest treefall pits. *Canadian Journal of Forest Research*. 18:553-559.
- BEAUPRE, D. S. J., CHAD E. MONTGOMERY.** 2007. The meaning and consequences of foraging mode in snakes. Cambridge University Press.
- BEAUPRE, D. S. J., AND D. J. DUVAL.** 1998. Integrative Biology of the Rattlesnakes: Contributions to biology and evolution. *Bioscience*. 48:531-538.
- BEAUPRE, S. J.** 1995. Effects of Geographically Variable Thermal Environment on Bioenergetics of Mottled Rock Rattlesnakes. *Ecology*. 76:1655-1665.
- BEAUPRE, S. J.** 2002. Modeling time-energy allocation in vipers: individual responses to environmental variation and implications for populations. *Biology of the vipers*:463-481.
- BEAUPRE, S. J.** 2008. Annual variation in time-energy allocation by timber rattlesnakes (*Crotalus horridus*) in relation to food acquisition, p. 111-122. *In: The biology of rattlesnakes*. W. K. Hayes, M. D. Cardwell, K. R. Beaman, and S. P. Bush (eds.). Loma Linda University Press, Loma Linda, California.
- BEAUPRE, S. J., J. AGUGLIARO, J. U. VAN DYKE, AND F. ZAIDAN III.** 2017. Annual Energy Budgets of the Timber Rattlesnake: Advancements, Refinements, and Open Questions, p. 97-108. *In: The Biology of the Rattlesnakes II*. M. J. Dreslik, W. K. Hayes, S. J. Beaupre, and S. P. Mackessy (eds.). ECO Herpetological Publishing and Distribution, Rodeo, New Mexico.
- BEAUPRE, S. J., AND L. E. DOUGLAS.** 2009. Snakes as Indicators and Monitors of Ecosystem Properties.
- BEAUPRE, S. J., AND L. E. DOUGLAS.** 2012. Responses of timber rattlesnakes to fire: lessons from two prescribed burns.
- BEAUPRE, S. J., AND F. ZAIDAN.** 2012. Digestive Performance in the Timber Rattlesnake (*Crotalus horridus*) with Reference to Temperature Dependence and Bioenergetic Cost of Growth. *Journal of Herpetology*. 46:637-642.
- BELL, C. J.** 1980. The Scaling of the Thermal Inertia of Lizards. *Journal of Experimental Biology*. 86:79-85.
- BIRKEBAK, R. C., R. C. BIRKEBAK, AND D. W. WARNER.** 1964. A note on total emittance of animal integuments. *Journal of heat transfer*. 86:287-288.

- BLAND, J. M., AND D. G. ALTMAN.** 1995a. Calculating correlation coefficients with repeated observations: Part 2—Correlation between subjects. *Bmj.* 310:633.
- . 1995b. Statistics notes: Calculating correlation coefficients with repeated observations: Part 1—correlation within subjects. *Bmj.* 310:446.
- BLOUIN-DEMERS, G., K. A. PRIOR, AND P. J. WEATHERHEAD.** 2000a. Patterns of Variation in Spring Emergence by Black Rat Snakes (*Elaphe obsoleta obsoleta*). *Herpetologica.* 56:175-188.
- BLOUIN-DEMERS, G., K. A. PRIOR, AND P. J. WEATHERHEAD.** 2000b. Patterns of variation in spring emergence by black rat snakes (*Elaphe obsoleta obsoleta*). *Herpetologica*:175-188.
- BOGERT, C. M.** 1949. Thermoregulation in Reptiles, A Factor in Evolution. *Evolution.* 3:195-211.
- BORRELL, B. J., T. J. LADUC, AND R. DUDLEY.** 2005. Respiratory cooling in rattlesnakes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology.* 140:471-476.
- BREWSTER, C. L., AND S. J. BEAUPRE.** 2019a. The effect of body posture on available habitat and activity-time in a lizard: Implications for thermal ecology studies. *Journal of Thermal Biology.*
- . 2019b. The effect of body posture on available habitat and activity-time in a lizard: implications for thermal ecology studies. *Journal of thermal biology.* 82:10-17.
- BROWNING, D. M., S. J. BEAUPRE, AND L. DUNCAN.** 2005. Using partitioned Mahalanobis D-2(K) to formulate a GIS-based model of timber rattlesnake hibernacula. *Journal of Wildlife Management.* 69:33-44.
- BRUCKERHOFF, L. A., L. K. KAMEES, A. T. HOLYCROSS, AND C. W. PAINTER.** 2021. Patterns of Survival of a Communally Overwintering Rattlesnake Using an Artificial Hibernaculum. *Ichthyology & Herpetology.* 109:64-74.
- BUCKLEY, L. B., J. C. EHRENBERGER, AND M. J. ANGILLETTA JR.** 2015a. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology.* 29:1038-1047.
- BUCKLEY, L. B., J. C. EHRENBERGER, AND M. J. ANGILLETTA.** 2015b. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology.* 29:1038-1047.
- BUCKLEY, L. B., AND J. G. KINGSOLVER.** 2012. The demographic impacts of shifts in climate means and extremes on alpine butterflies. *Functional Ecology.* 26:969-977.

- BUCKLEY, L. B., AND J. ROUGHGARDEN.** 2005. Effect of species interactions on landscape abundance patterns. *Journal of Animal Ecology*:1182-1194.
- CAMPBELL, G. S., AND J. M. NORMAN.** 2012. An introduction to environmental biophysics. Springer Science & Business Media.
- CANNATELLA, D. C., AND R. O. DE SA.** 1993. *Xenopus laevis* as a model organism. *Systematic Biology*. 42:476-507.
- CAROTHERS, J., H., S. FOX, F., P. MARQUET, A., AND F. JAKSIC, M.** 1997. Thermal characteristics of ten Andean lizards of the genus *Liolaemus* in central Chile. *Revista Chilena de Historia Natural*. 70:297-309.
- CARRUTHERS, N.** 1943. Variations in wind velocity near the ground. *Quarterly Journal of the Royal Meteorological Society*. 69:289-301.
- CHATFIELD, C.** 2003. The analysis of time series: an introduction. Chapman and Hall/CRC.
- CHAZDON, R. L., AND R. W. PEARCY.** 1991. The importance of sunflecks for forest understory plants. *BioScience*. 41:760-766.
- CHRISTIAN, K. A., C. R. TRACY, AND C. R. TRACY.** 2006. Evaluating thermoregulation in reptiles: An appropriate null model. *American Naturalist*. 168:421-430.
- CLARK, A. M., P. E. MOLER, E. E. POSSARDT, A. H. SAVITZKY, W. S. BROWN, AND B. W. BOWEN.** 2003. Phylogeography of the Timber Rattlesnake (*Crotalus horridus*) Based on mtDNA Sequences. *Journal of Herpetology*. 37:145-154.
- CLARK, R. W.** 2006. Fixed Videography to Study Predation Behavior of an Ambush Foraging Snake, *Crotalus Horridus*. *Copeia*. 2006:181-187.
- CLARK, R. W., S. TANGCO, AND M. A. BARBOUR.** 2012. Field video recordings reveal factors influencing predatory strike success of free-ranging rattlesnakes (*Crotalus* spp.). *Animal Behaviour*. 84:183-190.
- CONANT, R., AND J. T. COLLINS.** 1998. A field guide to reptiles & amphibians: eastern and central North America. Houghton Mifflin Harcourt.
- CONGDON, J. D., A. E. DUNHAM, AND D. W. TINKLE.** 1982. Energy Budgets and Life Histories of Reptiles, p. 233-271. *In: Biology of the Reptiles*. Vol. 13. C. Gans and Pough (eds.). Academic Press, New York.
- CORE, T. R.** 2018. R: A language and environment for statistical computing, p. 2013, R Foundation for Statistical Computing, Vienna, Austria.
- COWLES, R. B., AND C. M. BOGERT.** 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* 83:261-296.

- CUSHMAN, J. H., J. H. LAWTON, AND B. F. MANLY.** 1993. Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia*. 95:30-37.
- DEWALLE, D. R., E. W. BOYER, AND A. R. BUDA.** 2016. Exploring lag times between monthly atmospheric deposition and stream chemistry in Appalachian forests using cross-correlation. *Atmospheric environment*. 146:206-214.
- DUNHAM, A. E., B. W. GRANT, AND K. L. OVERALL.** 1989. Interfaces between Biophysical and Physiological Ecology and the Population Ecology of Terrestrial Vertebrate Ectotherms. *Physiological Zoology*. 62:335-355.
- GARDNER-SANTANA, L. C., AND S. J. BEAUPRE.** 2009. Timber Rattlesnakes (*Crotalus horridus*) Exhibit Elevated and Less Variable Body Temperatures during Pregnancy. *Copeia*. 2009:363-368.
- GATES, D. M.** 1980. *Biophysical ecology*. Springer-Verlag, Mineola, NY.
- GIENGER, C., AND D. D. BECK.** 2011. Northern Pacific Rattlesnakes (*Crotalus oreganus*) use thermal and structural cues to choose overwintering hibernacula. *Canadian journal of zoology*. 89:1084-1090.
- GIERING, K., I. LAMPRECHT, AND O. MINET.** 1996. Specific heat capacities of human and animal tissues. SPIE.
- GLOYD, H. K.** 1978. The rattlesnakes, genera *Sistrurus* and *Crotalus*: a study in zoogeography and evolution. Milwaukee: Society for the Study of Amphibians and Reptiles.
- GRANT, B. W.** 1990. Trade-offs in activity time and physiological performance for thermoregulating desert lizards, *Sceloporus merriami*. *Ecology*:2323-2333.
- GRANT, B. W., AND A. E. DUNHAM.** 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology*:167-176.
- GRAVES, B. M., AND D. DUVALL.** 1990. Spring emergence patterns of wandering garter snakes and prairie rattlesnakes in Wyoming. *Journal of Herpetology*:351-356.
- GRIGG, G. C., C. R. DRANE, AND G. P. COURTICE.** 1979. Time constants of heating and cooling in the eastern water dragon, *Physignathus lesueruii* and some generalizations about heating and cooling in reptiles. *Journal of Thermal Biology*. 4:95-103.
- GUNDERSON, A. R., AND M. LEAL.** 2015. Patterns of Thermal Constraint on Ectotherm Activity. *The American Naturalist*. 185:653-664.
- . 2016. A conceptual framework for understanding thermal constraints on ectotherm activity with implications for predicting responses to global change. *Ecology Letters*. 19:111-120.

- HARVEY, D. S., AND P. J. WEATHERHEAD.** 2006. Hibernation site selection by eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) near their northern range limit. *Journal of Herpetology*. 40:66-73.
- . 2010. Habitat selection as the mechanism for thermoregulation in a northern population of massasauga rattlesnakes (*Sistrurus catenatus*). *Ecoscience*. 17:411-419.
- HARVEY, D. S., AND P. J. WEATHERHEAD.** 2011. Thermal ecology of Massasauga Rattlesnakes (*Sistrurus catenatus*) near their northern range limit. *Canadian Journal of Zoology*. 89:60.
- HERTZ, P. E., R. B. HUEY, AND R. STEVENSON.** 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *American Naturalist*:796-818.
- HOEKSTRA, A. A.** 2015. The influence of habitat on body temperature regulation in the timber rattlesnake (*Crotalus horridus*), p. 47. Vol. 1597442. Middle Tennessee State University, Ann Arbor.
- HUEY, R. B.** 1974. Behavioral thermoregulation in lizards: importance of associated costs. *American Association for the Advancement of Science*.
- HUEY, R. B.** 1982. Temperature, Physiology, and the Ecology of Reptiles, p. 25-91. *In: Biology of the Reptilia*. Vol. 12. C. Gans and F. H. Pough (eds.). Academic Press, New York.
- . 1991. Physiological Consequences of Habitat Selection. *The American Naturalist*. 137:S91-S115.
- HUEY, R. B., C. R. PETERSON, S. J. ARNOLD, AND W. P. PORTER.** 1989. Hot Rocks and Not-So-Hot Rocks: Retreat-Site Selection by Garter Snakes and Its Thermal Consequences. *Ecology*. 70:931-944.
- HUEY, R. B., AND E. R. PIANKA.** 1977. Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. *Ecology*:1066-1075.
- HUEY, R. B., AND E. R. PIANKA.** 1981. Ecological consequences of foraging mode. *Ecology*. 62:991-999.
- HUEY, R. B., E. R. PIANKA, AND T. W. SCHOENER.** 1983. Lizard ecology: studies of a model organism. Harvard Univ Pr.
- HUEY, R. B., AND M. SLATKIN.** 1976. Cost and benefits of lizard thermoregulation. *Quarterly Review of Biology*:363-384.
- HUEY, R. B., AND R. STEVENSON.** 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist*. 19:357-366.
- HUTCHISON, B. A., AND D. R. MATT.** 1977. The distribution of solar radiation within a deciduous forest. *Ecological Monographs*. 47:185-207.



- KARSTENSEN, K. A.** 2010. Land-cover change in the Ozark Highlands, 1973-2000. US Geological Survey.
- KOSTEL-HUGHES, F., T. P. YOUNG, AND J. D. WEHR.** 2005. Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation to seed size. *The Journal of the Torrey Botanical Society*. 132:50-61.
- KREITH, F., R. M. MANGLIK, AND M. S. BOHN.** 2012. Principles of heat transfer. Cengage learning.
- LELIEVRE, H., M. LE HÉNANFF, G. BLOUIN-DEMERS, G. NAULLEAU, AND O. LOURDAIS.** 2010. Thermal strategies and energetics in two sympatric colubrid snakes with contrasted exposure. *Journal of Comparative Physiology B*. 180:415-425.
- LEVINE, B. A.** 2013. Genetic structure of the copperhead (*Viperidae: Agkistrodon contortrix mokasen*) at its most northern distribution, p. 78. Vol. 1535348. University of Arkansas, Ann Arbor.
- LI, L., M. LIANG, B. YU, AND S. YANG.** 2014. Analysis of thermal conductivity in living biological tissue with vascular network and convection. *International Journal of Thermal Sciences*. 86:219-226.
- LILLYWHITE, H. B.** 1980. Behavioral thermoregulation in Australian elapid snakes. *Copeia*:452-458.
- . 1987. Temperature, energetics, and physiological ecology. *Smith VII*.
- LIND, C. M., AND S. J. BEAUPRE.** 2015. Male Snakes Allocate Time and Energy according to Individual Energetic Status: Body Condition, Steroid Hormones, and Reproductive Behavior in Timber Rattlesnakes, *Crotalus horridus*. *Physiological and Biochemical Zoology*. 88:624-633.
- LUTTERSCHMIDT, W. I., AND H. K. REINERT.** 2012. Modeling body temperature and thermal inertia of large-bodied reptiles: Support for water-filled biophysical models in radiotelemetric studies. *Journal of Thermal Biology*. 37:282-285.
- MACARTNEY, J. M., AND P. T. GREGORY.** 1988. Reproductive Biology of Female Rattlesnakes (*Crotalus viridis*) in British Columbia. *Copeia*. 1988:47-57.
- MACARTNEY, J. M., K. W. LARSEN, AND P. T. GREGORY.** 1989. Body temperatures and movements of hibernating snakes (*Crotalus* and *Thamnophis*) and thermal gradients of natural hibernacula. *Canadian Journal of Zoology*. 67:108-114.
- MARTIN, W., AND T. TYNING.** 1992. The timber rattlesnake: its distribution and natural history. *Conservation of the Timber Rattlesnake in the Northeast*:13-22.

- MCNAB, B. K., AND W. AUFFENBERG.** 1976. The effect of large body size on the temperature regulation of the komodo dragon, *Varanus komodoensis*. *Comparative Biochemistry and Physiology Part A: Physiology*. 55:345-350.
- MEIRI, S.** 2011. Bergmann's Rule—what's in a name? *Global Ecology and Biogeography*. 20:203-207.
- MICHAEL, P.** 2019. A Conversion Guide: Solar Irradiance and Lux Illuminance., IEEE Dataport.
- MITCHELL, J. W.** 1976. Heat transfer from spheres and other animal forms. *Biophysical Journal*. 16:561-569.
- MUTH, A.** 1977. Thermoregulatory postures and orientation to the sun: a mechanistic evaluation for the zebra-tailed lizard, *Callisaurus draconoides*. *Copeia*:710-720.
- NAKAGAWA, S., AND H. SCHIELZETH.** 2013. A general and simple method for obtaining R<sup>2</sup> from generalized linear mixed-effects models. *Methods in ecology and evolution*. 4:133-142.
- PALADINO, F. V., M. P. O'CONNOR, AND J. R. SPOTILA.** 1990. Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature*. 344:858.
- PARKER, W. S., AND W. S. BROWN.** 1973. Species composition and population changes in two complexes of snake hibernacula in northern Utah. *Herpetologica*:319-326.
- PETERSON, C. R., A. R. GIBSON, AND M. E. DORCAS.** 1993. Snake thermal ecology: the causes and consequences of body-temperature variation, p. 241-314. *In: Snakes, ecology and behavior*. R. A. Seigel and J. T. Collins (eds.). McGraw-Hill, New York.
- PORTER, W. P., AND D. M. GATES.** 1969. Thermodynamic equilibria of animals with environment. *Ecological monographs*. 39:227-244.
- PORTER, W. P., J. W. MITCHELL, W. A. BECKMAN, AND C. B. DEWITT.** 1973. Behavioral implications of mechanistic ecology. *Oecologia*. 13:1-54.
- PORTER, W. P., AND C. TRACY.** 1983. Biophysical analyses of energetics, time-space utilization, and distributional limits. *Lizard ecology: Studies of a model organism*:55-83.
- POUGH, F. H., AND C. GANS.** 1982. Physiology C: Physiologocial Ecology, p. 17-23. *In: Biology of the Reptilia*. Vol. 12. C. Gans (ed.). Academic Press, New York, New York.
- PUTMAN, B. J., AND R. W. CLARK.** 2017. Behavioral thermal tolerances of free-ranging rattlesnakes (*Crotalus oreganus*) during the summer foraging season. *Journal of thermal biology*. 65:8-15.
- R DEVELOPMENT CORE TEAM.** 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

- REINERT, H. K., AND D. CUNDALL.** 1982. An improved surgical implantation method for radio-tracking snakes. *Copeia*. 1982:702-705.
- ROTH, E. D.** 2003. 'Handedness' in snakes? Lateralization of coiling behaviour in a cottonmouth, *Agkistrodon piscivorus leucostoma*, population. *Animal Behaviour*. 66:337-341.
- SCHANTZ, K. A.** 2009. Characterization of landscape-scale habitat use by timber rattlesnakes (*Crotalus horridus*) within the ridge and valley and highlands regions of New Jersey, p. 195. Vol. 1470007. Rutgers The State University of New Jersey - New Brunswick, Ann Arbor.
- SEARS, M. W.** 2005. Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia*. 143:25-36.
- SEARS, M. W., M. J. ANGILLET, M. S. SCHULER, J. BORCHERT, K. F. DILLIPLANE, M. STEGMAN, T. W. RUSCH, AND W. A. MITCHELL.** 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proceedings of the National Academy of Sciences*.
- SEEBACHER, F., R. M. ELSEY, AND P. L. TROSCLAIR III.** 2003. Body temperature null distributions in reptiles with nonzero heat capacity: seasonal thermoregulation in the American alligator (*Alligator mississippiensis*). *Physiological and Biochemical Zoology*. 76:348-359.
- SEEBACHER, F., AND C. E. FRANKLIN.** 2005. Physiological mechanisms of thermoregulation in reptiles: a review. *Journal of Comparative Physiology B*. 175:533-541.
- SEEBACHER, F., G. C. GRIGG, AND L. BEARD.** 1999. Crocodiles as dinosaurs: behavioural thermoregulation in very large ectotherms leads to high and stable body temperatures. *Journal of Experimental Biology*. 202:77-86.
- SEEBACHER, F., AND R. SHINE.** 2004. Evaluating Thermoregulation in Reptiles: The Fallacy of the Inappropriately Applied Method. *Physiological and Biochemical Zoology*. 77:688-695.
- SHINE, R., P. S. HARLOW, M. J. ELPHICK, M. M. OLSSON, AND R. T. MASON.** 2000. Conflicts between courtship and thermoregulation: The thermal ecology of amorous male garter snakes (*Thamnophis sirtalis parietalis*, Colubridae). *Physiological and Biochemical Zoology*. 73:508-516.
- SHINE, R., AND T. MADSEN.** 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology*. 69:252-269.

- SLIP, D. J., AND R. SHINE.** 1988. Thermophilic response to feeding of the diamond python, *Morelia s. spilota* (Serpentes: Boidae). *Comparative Biochemistry and Physiology Part A: Physiology*. 89:645-650.
- SPOTILA, J. R., P. W. LOMMEN, G. S. BAKKEN, AND D. M. GATES.** 1973. A mathematical model for body temperatures of large reptiles: Implications for dinosaur ecology. *American Naturalist*:391-404.
- SPOTILA, J. R., O. H. SOULE, AND D. M. GATES.** 1972. The biophysical ecology of the alligator: heat energy budgets and climate spaces. *Ecology*:1094-1102.
- STEARNS, S. C., AND S. C. STEARNS.** 1992. *The Evolution of Life Histories*. OUP Oxford.
- STEVENSON, R.** 1985a. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*:102-117.
- STEVENSON, R. D.** 1985b. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*:102-117.
- . 1985c. The Relative Importance of Behavioral and Physiological Adjustments Controlling Body-Temperature in Terrestrial Ectotherms. *American Naturalist*. 126:362-386.
- STICKLEY, S. F., AND J. M. FRATERIGO.** 2021. Understory vegetation contributes to microclimatic buffering of near-surface temperatures in temperate deciduous forests. *Landscape Ecology*. 36:1197-1213.
- TATTERSALL, G. J., V. CADENA, AND M. C. SKINNER.** 2006. Respiratory cooling and thermoregulatory coupling in reptiles. *Respiratory physiology & neurobiology*. 154:302-318.
- TATTERSALL, G. J., W. K. MILSOM, A. S. ABE, S. P. BRITO, AND D. V. ANDRADE.** 2004. The thermogenesis of digestion in rattlesnakes. *Journal of Experimental Biology*. 207:579-585.
- TRACY, C. R.** 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. *Ecological Monographs*. 46:293-326.
- . 1982a. Biophysical modeling in reptilian physiology and ecology. *Biology of the Reptilia*. 12:275-321.
- TRACY, C. R.** 1982b. Biophysical modelling in reptilian physiology and ecology, p. 275-321. *In: Biology of the Reptilia*. Vol. 12. C. Gans, and Pough, F. H. (ed.). Academic Press, New York.
- TURNER, J., AND C. TRACY.** 1985. Body size and the control of heat exchange in alligators. *Journal of thermal biology*. 10:9-11.

- UMMENHOFER, C. C., AND G. A. MEEHL.** 2017. Extreme weather and climate events with ecological relevance: a review. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372:20160135.
- VICKERS, M., C. MANICOM, AND L. SCHWARZKOPF.** 2011. Extending the cost-benefit model of thermoregulation: high-temperature environments. *The American Naturalist*. 177:452-461.
- VOGEL, S.** 1996. *Life in moving fluids: the physical biology of flow*. Princeton University Press.
- WANG, T., A. S. ABE, AND M. L. GLASS.** 1998. Effects of temperature on lung and blood gases in the South American rattlesnake *Crotalus durissus terrificus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 121:7-11.
- WILLS, C. A., AND S. J. BEAUPRE.** 2000. An application of randomization for detecting evidence of thermoregulation in timber rattlesnakes (*Crotalus horridus*) from northwest Arkansas. *Physiological and Biochemical Zoology*. 73:325-334.
- WITHERS, P. C.** 1992. *Comparative animal physiology*. Saunders College Pub.
- ZAIDAN III, F., AND D. S. J. BEAUPRE.** 2003. Effects of Body Mass, Meal Size, Fast Length, and Temperature on Specific Dynamic Action in the Timber Rattlesnake (*Crotalus horridus*). *Physiological and Biochemical Zoology*. 76:447-458.
- ZAMORA-CAMACHO, F., S. REGUERA, AND G. MORENO-RUEDA.** 2014. Bergmann's Rule rules body size in an ectotherm: heat conservation in a lizard along a 2200-metre elevational gradient. *Journal of evolutionary biology*. 27:2820-2828.

## Appendix

### Tables and Figures

**Table 1.** Individual snake morphometrics and duration of each sampling event. Estimated height and radius (rounded to the nearest cm) were determined by measuring individuals with a meter stick when coiled. Individuals with repeat observations are identified by matching superscripts in the ID.

ID	Mass (g)	SVL (cm)	TL (cm)	Minutes Sampled	Height (cm)	Radius (cm)	Surface Area (cm <sup>2</sup> )	Volume (cm <sup>3</sup> )	SA/V
56 <sup>1</sup>	948.9	87.7	93.6	10	7	12	1431.8	3165.1	0.5
61 <sup>2</sup>	646.7	87.7	93.6	10	5	10	942	1570	0.6
62 <sup>1</sup>	948.9	87.7	93.6	35	7	12	1431.8	3165.1	0.5
63 <sup>2</sup>	646.7	87.7	93.6	16	5	10	942	1570	0.6
66 <sup>2</sup>	646.7	87.7	93.6	15	5	10	942	1570	0.6
67 <sup>3</sup>	504.4	87.1	93.6	50	5	10	942	1570	0.6
69 <sup>4</sup>	540.8	90	97	16	5	10	942	1570	0.6
71 <sup>5</sup>	547.9	87.9	95.4	14	5	10	942	1570	0.6
72 <sup>4</sup>	540.8	90	97	12	5	10	942	1570	0.6
73 <sup>6</sup>	1145.6	103.5	111.7	62	8	14	1934.2	4923.5	0.4
74 <sup>4</sup>	540.8	90	97	47	5	10	942	1570	0.6
75 <sup>7</sup>	612.7	90	96.6	60	5	10	942	1570	0.6
76 <sup>8</sup>	417.1	83.3	88	25	5	10	942	1570	0.6
77 <sup>9</sup>	480	94.2	99.9	25	5	10	942	1570	0.6
78 <sup>10</sup>	568.4	90	96.6	41	5	10	942	1570	0.6
79 <sup>11</sup>	832.9	98.6	106.3	52	5	10	942	1570	0.6
80 <sup>12</sup>	1269.4	103.5	111.7	66	8	14	1934.2	4923.5	0.4
81 <sup>13</sup>	1173	104.7	112.2	110	8	14	1934.2	4923.5	0.4
82 <sup>14</sup>	936.4	92.8	100.8	40	7	12	1431.8	3165.1	0.5
83 <sup>15</sup>	653.1	87.90	111.7	25	7	10	1067.6	2198.0	0.5

**Table 2.** Means and standard deviations for conduction rates ( $Q_{cond}$ ) and heat storage ( $Q_s$ ) using raw data in units of W adjusted for the surface area of a coiled *C. horridus*.

ID	$Q_{cond}$ $T_r - T_b$	$Q_{cond}$ $T_r - T_b$	$Q_{cond}$ $T_b - T_g$	$Q_{cond}$ $T_b - T_g$	$Q_s$	$Q_s$
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
56	5.85	1.56	1.87	0.18	1.77	1.08
61	0.42	0.14	4.56	0.14	1.34	1.50
62	0.92	0.31	3.52	0.18	0.65	0.72
63	1.58	1.06	1.37	0.21	1.19	1.07
64	2.23	0.17	1.15	0.07	4.54	1.84
66	2.68	0.98	3.23	0.53	2.15	2.25
67	0.51	0.38	0.87	0.25	4.86	4.06
69	1.34	0.62	0.85	0.07	2.03	1.71
70	3.03	0.96	2.37	0.07	1.77	1.08
71	0.88	0.35	3.48	0.06	6.18	4.78
72	0.45	0.23	1.59	0.06	0.91	0.81
73	3.87	1.12	1.94	0.50	0.61	0.55
74	1.11	0.67	2.18	0.08	2.28	1.47
75	0.55	0.35	3.59	0.33	0.54	0.61
76	0.62	0.60	2.34	0.03	0.19	0.24
77	1.18	0.31	3.07	0.28	0.08	0.11
78	1.31	0.52	2.89	0.10	0.43	0.42
79	0.50	0.44	2.70	0.17	0.90	1.75
80	2.57	1.71	1.71	0.37	1.20	1.40
81	1.47	0.61	6.33	0.68	1.40	2.12
82	0.77	0.57	3.44	0.27	0.44	0.20
83	3.00	0.49	11.61	0.25	0.38	0.29

**Table 3.** Regression analysis for five-minute time lags of  $Q_{cond}$  from dorsum to core ( $Q_{cond} T_r T_b$ ) to time zero of  $Q_{cond}$  from core to ground. Significance was determined using a 95% confidence threshold.

$Q_{cond} T_r T_b$ Lag (mins)	Coefficient	SE	<i>t</i>	<i>p</i>
(Intercept)	12.023	1.321	9.098	< 0.001
5	-0.309	0.35	-0.883	0.38
10	0.244	0.339	0.719	0.47
15	0.514	0.311	1.652	0.1
20	-0.695	0.31	-2.24	0.027*
25	-0.375	0.295	-1.271	0.21
30	-0.22	0.232	-0.946	0.35
35	0.013	0.219	0.061	0.95
40	0.243	0.205	1.186	0.24
45	0.009	0.2	0.045	0.96
50	-0.304	0.198	-1.533	0.13
55	-0.458	0.19	-2.411	0.018*
60	-0.053	0.187	-0.282	0.78

Residual standard error: 4.227 on 107 degrees of freedom

Multiple R-squared: 0.2165, Adjusted R-square: 0.1287

F-statistic: 2.464 on 12 and 107 df, *p*-value: 0.007013

**Table 4.** Total minutes of incoming solar radiation ( $W/m^2$ ) observed during data collection for 12 snakes. Sampling occurred from May-September 2016-2018. A total of 523 minutes was sampled.

	Range of Total Radiation ( $W/m^2$ ) Observed						
	400- 499	500- 599	600- 699	700- 799	800- 899	900- 999	>1000
Total Time (min.)	19	82	13	5	7	0	15
% Occurrence	13.5	58.2	9.2	3.5	5.0	0.0	10.6

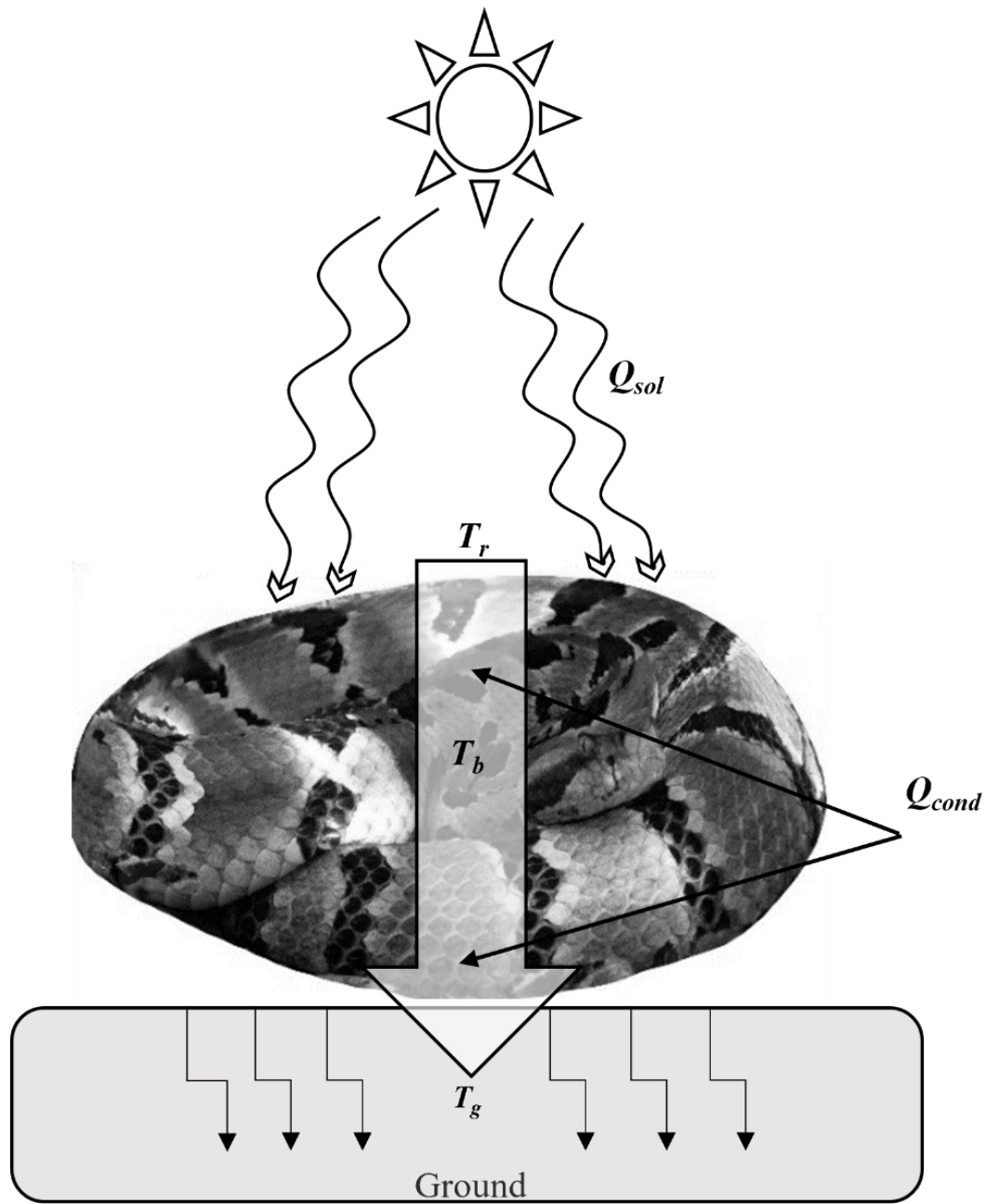


**Table 5.** Regression analysis for five-minute time lags of total incoming solar radiation to time zero conduction from the dorsum to the core (a.) and from the core to the ground (b.). Significance was determined using a 95% confidence threshold.

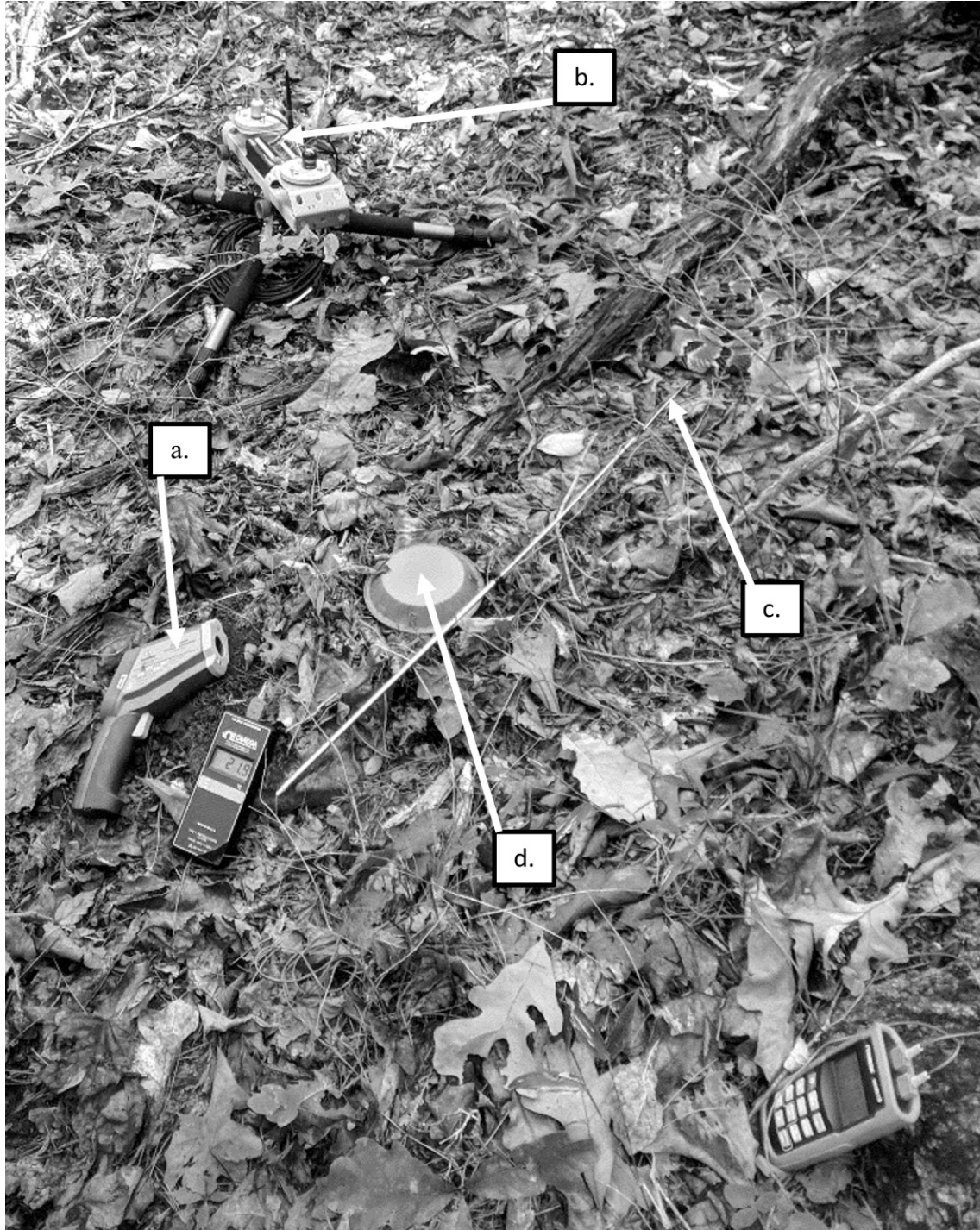
a.					b.				
Radiation Lag (mins)	Coefficient	SE	<i>t</i>	<i>p</i>	Radiation Lag (mins)	Coefficient	SE	<i>t</i>	<i>p</i>
(Intercept)	1.268	1.011	1.255	0.212	(Intercept)	8.114	2.861	2.836	0.005*
5	0.047	0.024	1.946	0.054	5	0.098	0.068	1.44	0.153
10	-0.033	0.024	-1.392	0.167	10	-0.008	0.068	-0.118	0.906
15	0.051	0.023	2.155	0.033*	15	0.117	0.066	1.759	0.081
20	0.017	0.022	0.748	0.456	20	0.048	0.063	0.767	0.445
25	-0.017	0.017	0.748	0.456	25	-0.072	0.037	-1.526	0.13
30	-0.004	0.016	-0.249	0.804	30	-0.008	0.046	-0.176	0.851
35	-0.023	0.015	-1.484	0.141	35	-0.49	0.043	-1.136	0.29
40	0.004	0.0114	0.275	0.784	40	0.016	0.04	0.405	0.687
45	-0.005	0.014	-0.348	0.728	45	-0.027	0.039	-0.699	0.486
50	-0.009	0.013	-0.71	0.479	50	-0.043	0.037	-1.157	0.25
55	-0.006	0.013	-0.493	0.623	55	-0.016	0.036	-0.443	0.659
60	0.015	0.011	1.389	0.168	60	-0.024	0.031	-0.763	0.447
Residual standard error: 1.493 on 107 degrees of freedom Multiple R-squared: 0.1604, Adjusted R-squared: 0.0662 F-statistic: 1.703 on 12 and 107 DF, p-value: 0.076					Residual standard error: 4.225 on 107 degrees of freedom Multiple R-squared: 0.2171, Adjusted R-squared: 0.1293 F-statistic: 2.472 on 12 and 107 DF, p-value: 0.006834				



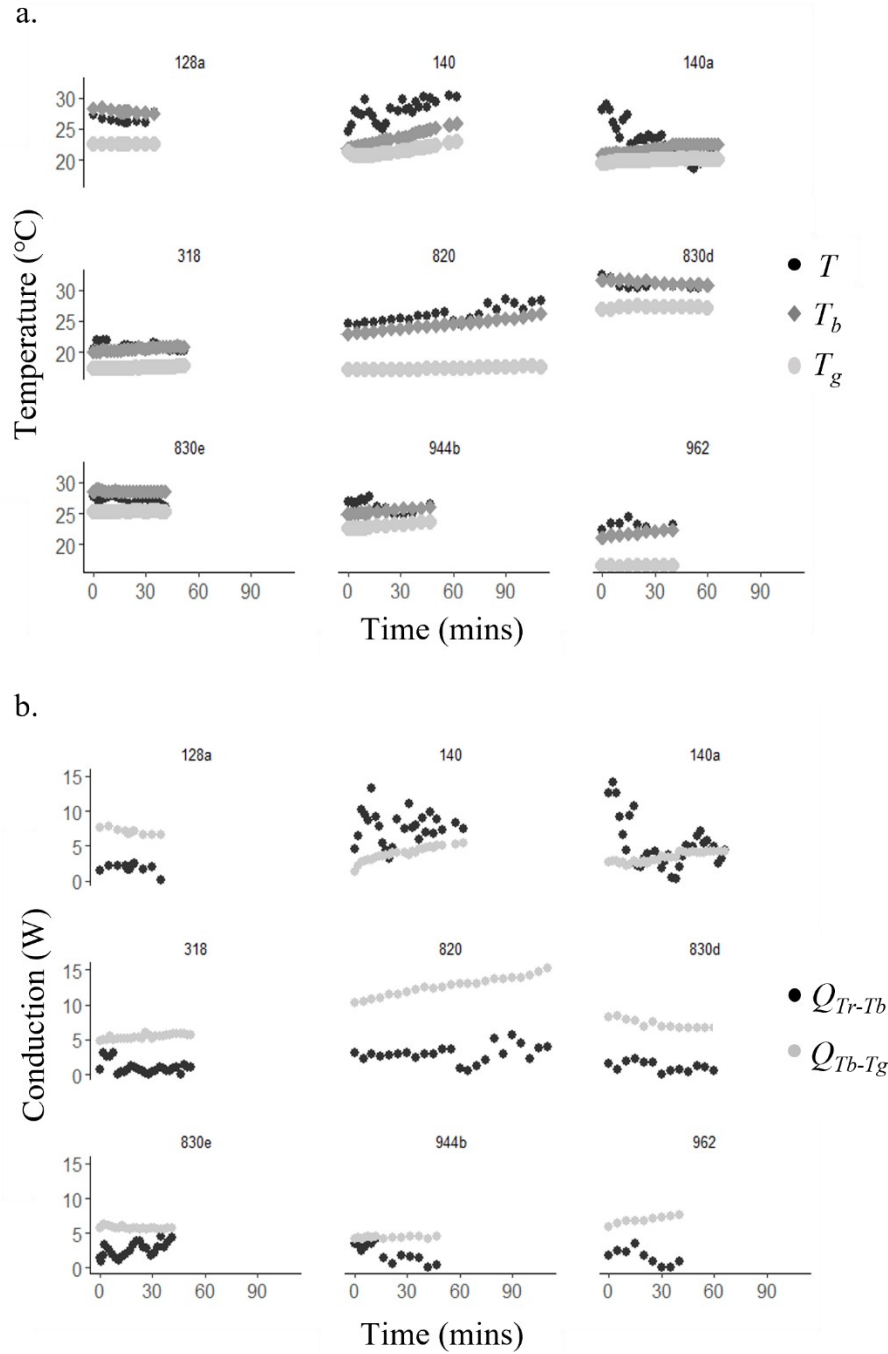
**Figure 1.** Typical ambush foraging posture of a Timber Rattlesnake (*Crotalus horridus*) from the Ozark Highlands in Northwest Arkansas. Thermal transients on the snake's dorsum may produce temporary, extreme heat loads.



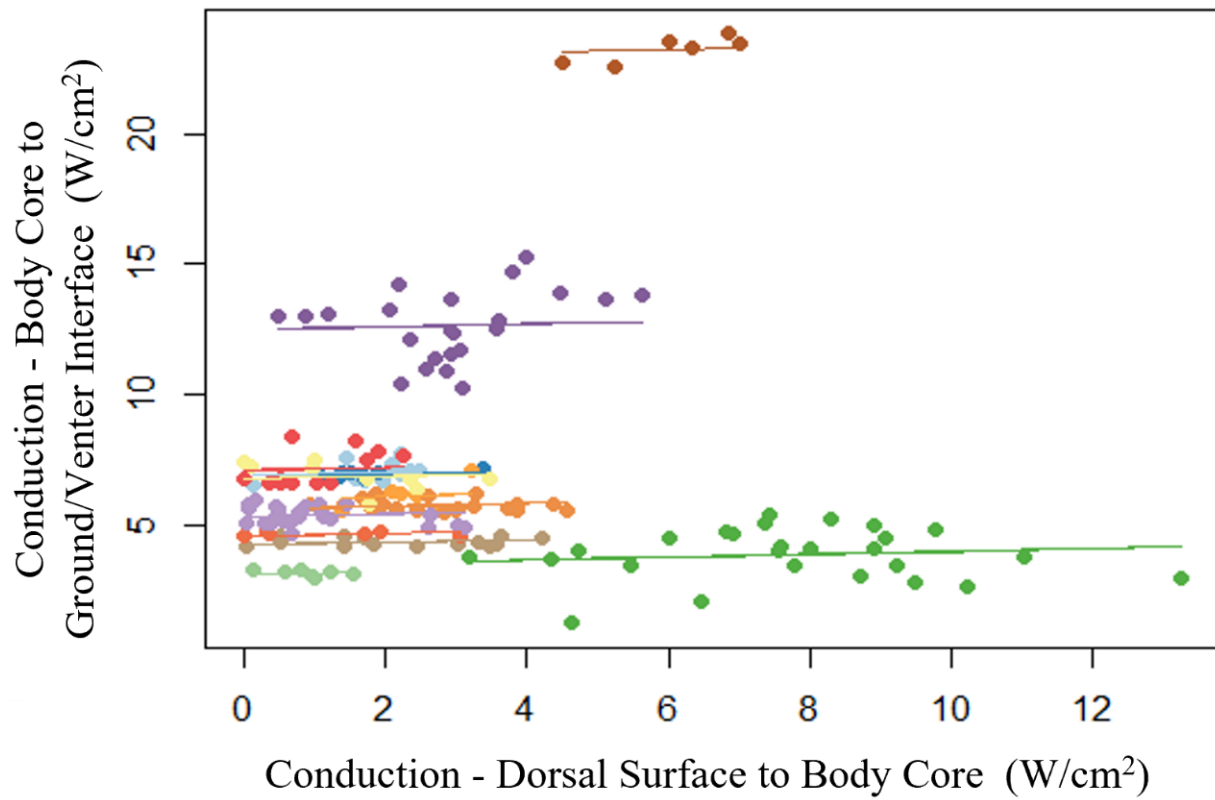
**Figure 2.** The three temperatures ( $T_r$ ,  $T_b$ , and  $T_g$ ) required to calculate thermal conduction from incoming solar radiation hitting the dorsum. In a snake experiencing increased heat loads at  $T_s$  and the increased conduction between  $T_r$  and  $T_b$  may be offset by conduction from  $T_b$  to  $T_g$ .



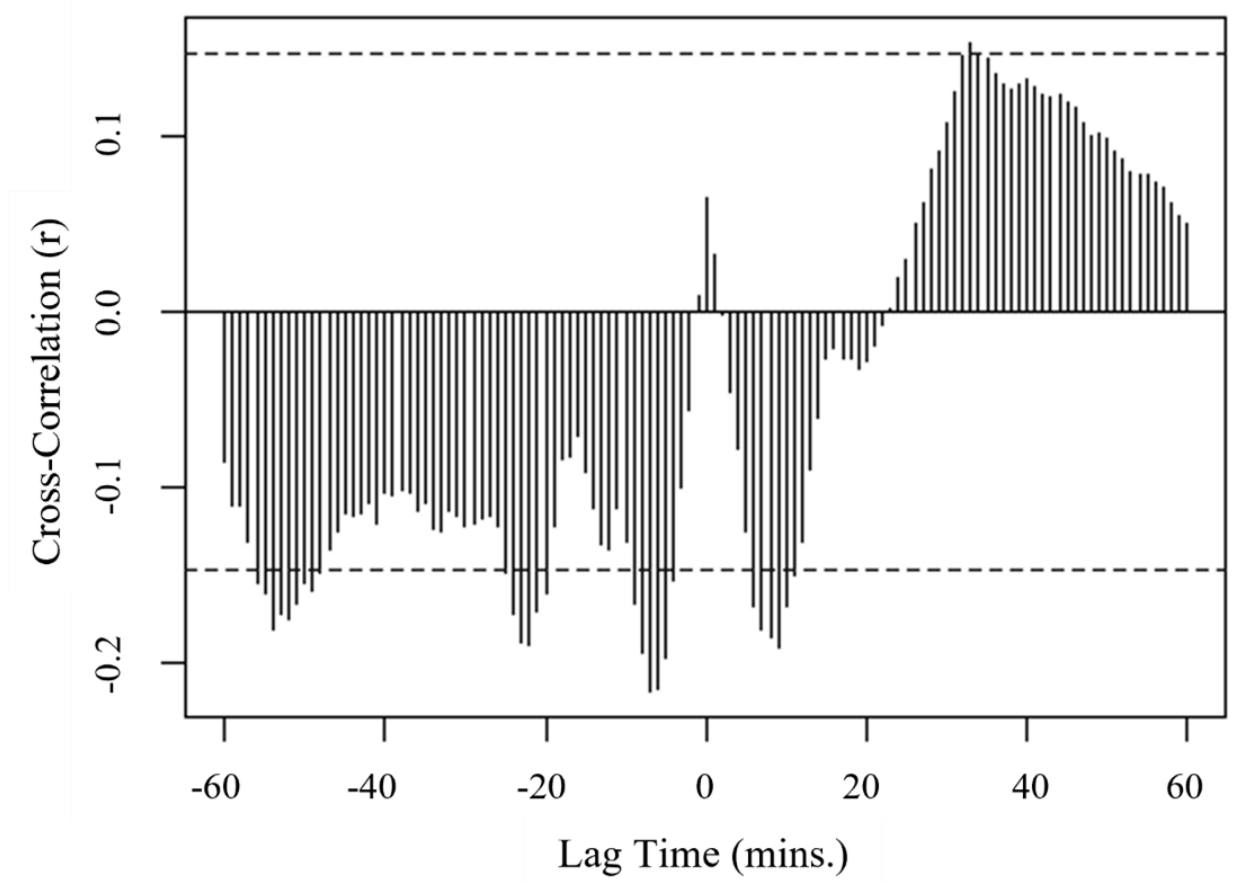
**Figure 3.** In situ data collection using an IR thermometer (a) for  $T_r$ , pyrgometer and pyranometer (b) for incoming longwave and shortwave radiation respectively, and a thermocouple wire fixed to a probe and positioned under the snake coils(c) for  $T_g$ . Body temperatures were collected using radio transmitters (Wildlife Materials TRX 1000s receiver with a Yagi three-element directional antenna). Additional data were collected to validate the operative temperature models (d) for another aspect of this study.



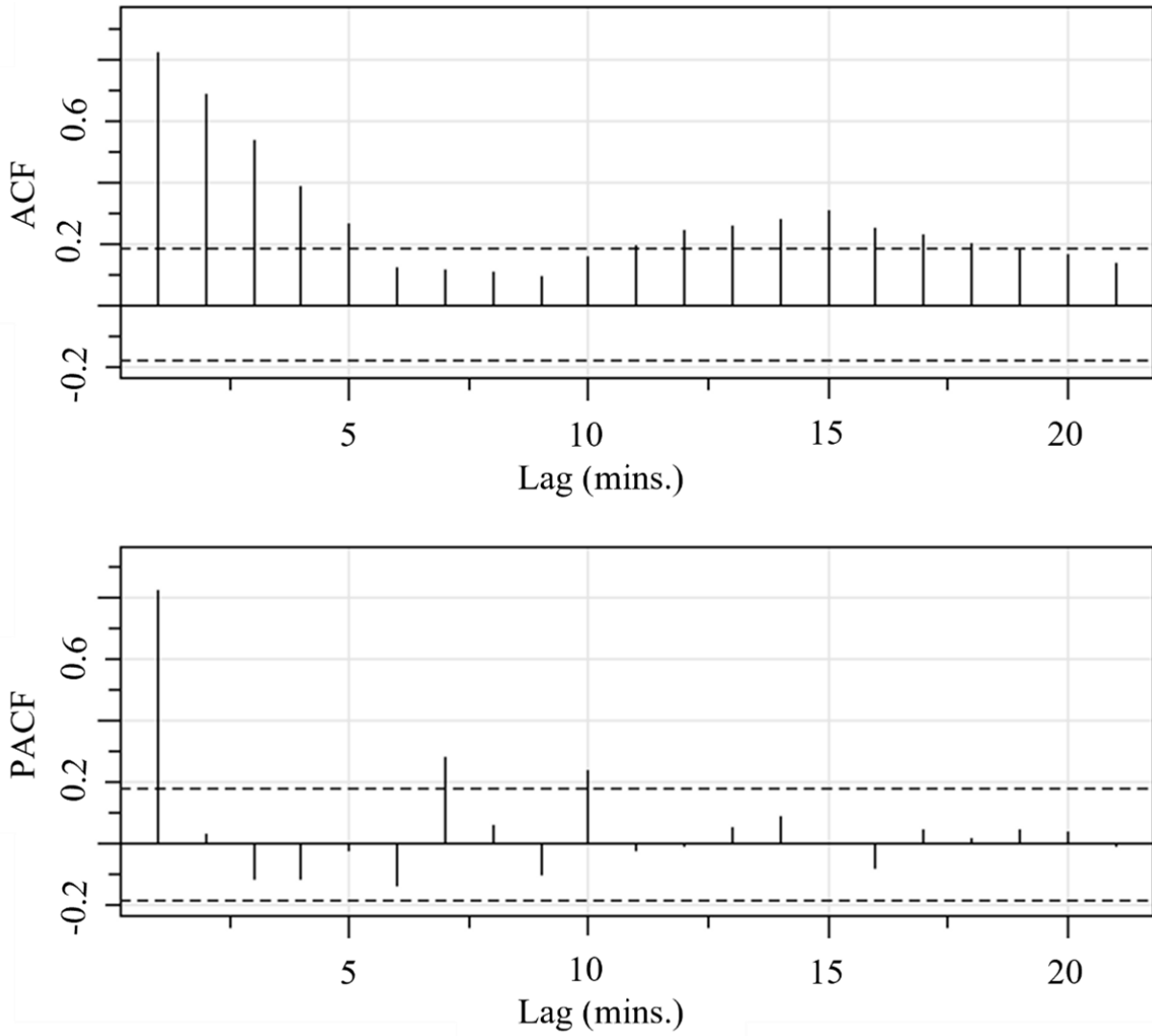
**Figure 4.** Raw temperature data (a.) and corresponding conduction data (b.) for a subsample of *C. horridus* during exposure to thermal transients. Numbers above each plot identify individual snakes with the same individuals shown in each graph. For this example, individuals were sampled for  $\geq 30$  mins. The top plot (a.) illustrates the relationship between  $T_r$ ,  $T_b$ , and  $T_g$ . Note the thermal gradient between  $T_b$  and  $T_g$  remains relatively stable even during transient heat events at the dorsum. Conduction data (b.) from core to the ground also exhibited a stable trend during events of increased conduction from the dorsum to core.



**Figure 5.** Repeated measures correlation plot of intra-individual conduction rates represented by different colors with corresponding regression lines indicating rmcrr fit for each individual. Each point represents separate observations of  $Q_{cond}$  from dorsum to core and core to ground. The lack of a significant correlation indicates conduction from the core to ground does not play a significant role in compensating for increases in conduction from the dorsum to the core.

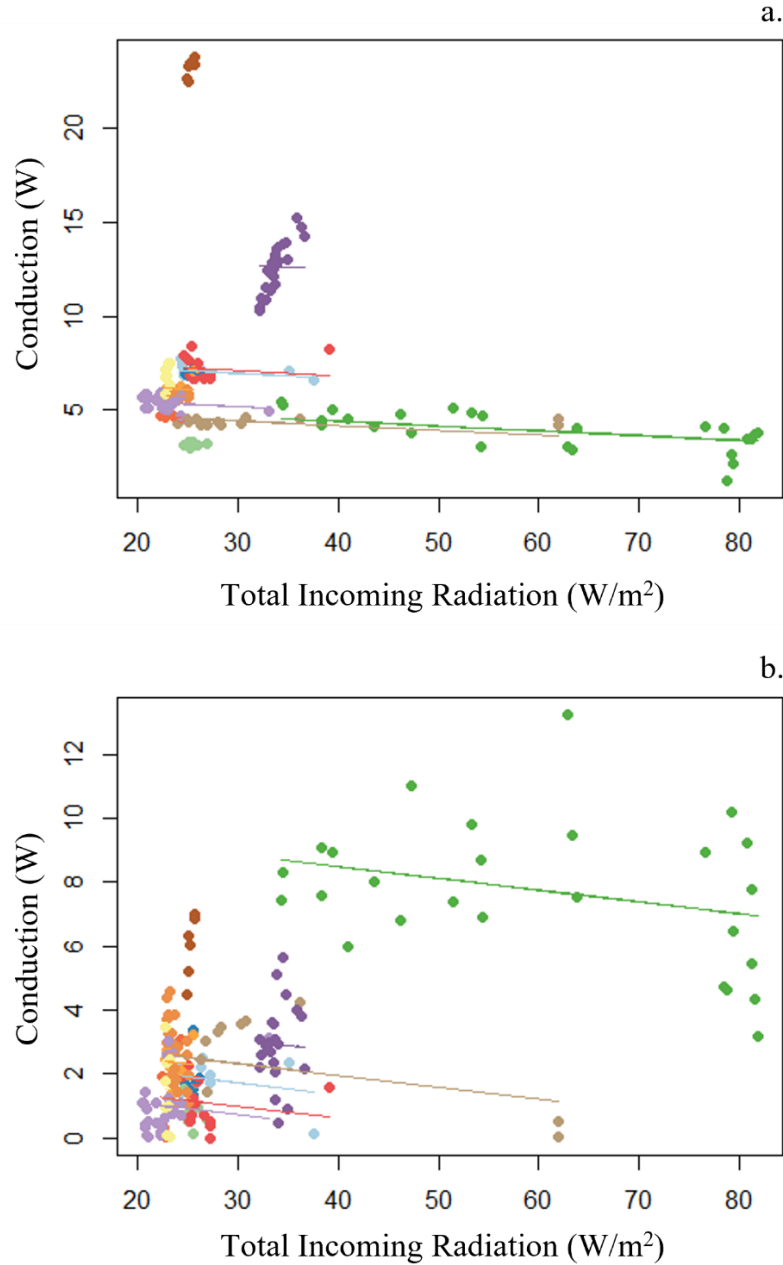


**Figure 6.** Cross-correlation analysis using five-minute lags identifying significant correlations at lags, 9, -7, -22 and -54 minutes where increases in  $Q_{cond}$  from  $T_s$  to  $T_b$  are followed by increases in  $Q_{cond}$  from  $T_b$  to  $T_g$ . Horizontal dotted lines identify the 95% confidence intervals.

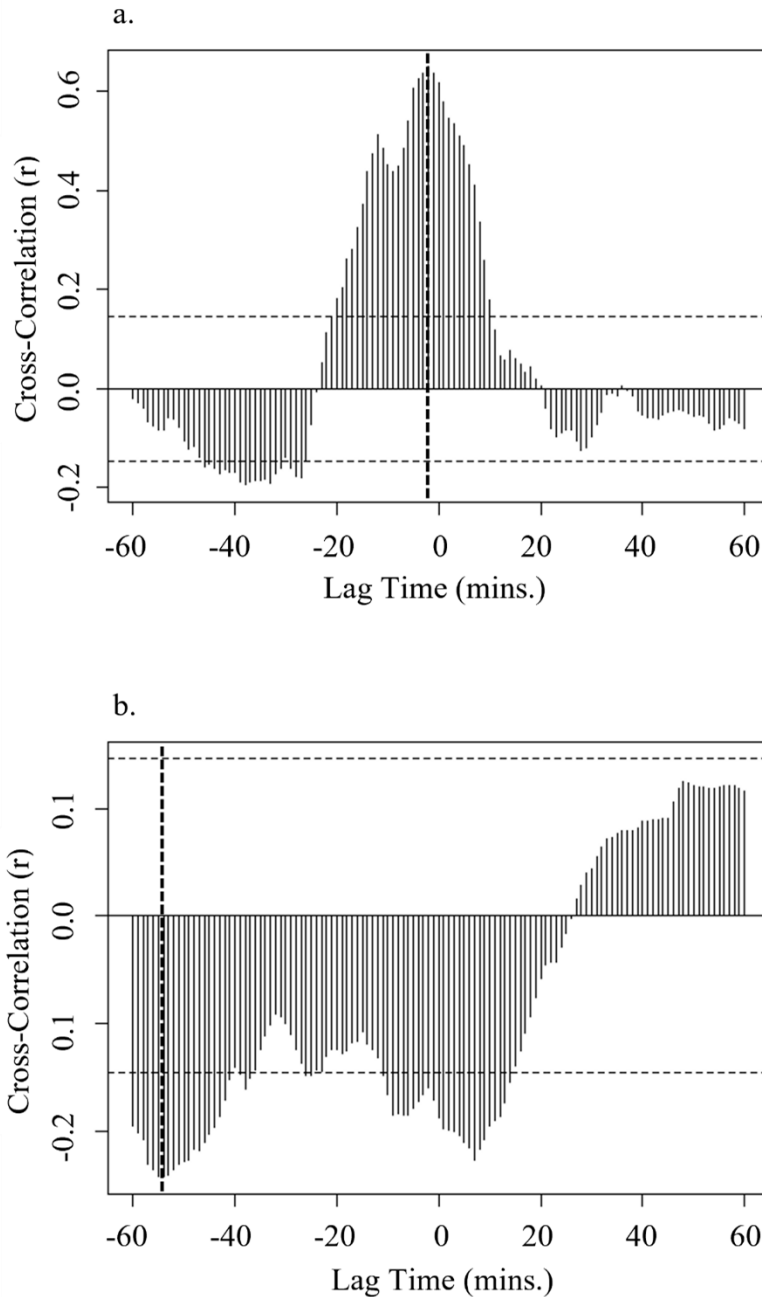


**Figure 7.** Residuals of the autocorrelation (ACF) and partial autocorrelation (PACF) functions for  $Q_{cond}$  from dorsum to core and from core to ground against five-minute lags. Dotted lines indicated 95% confidence intervals.

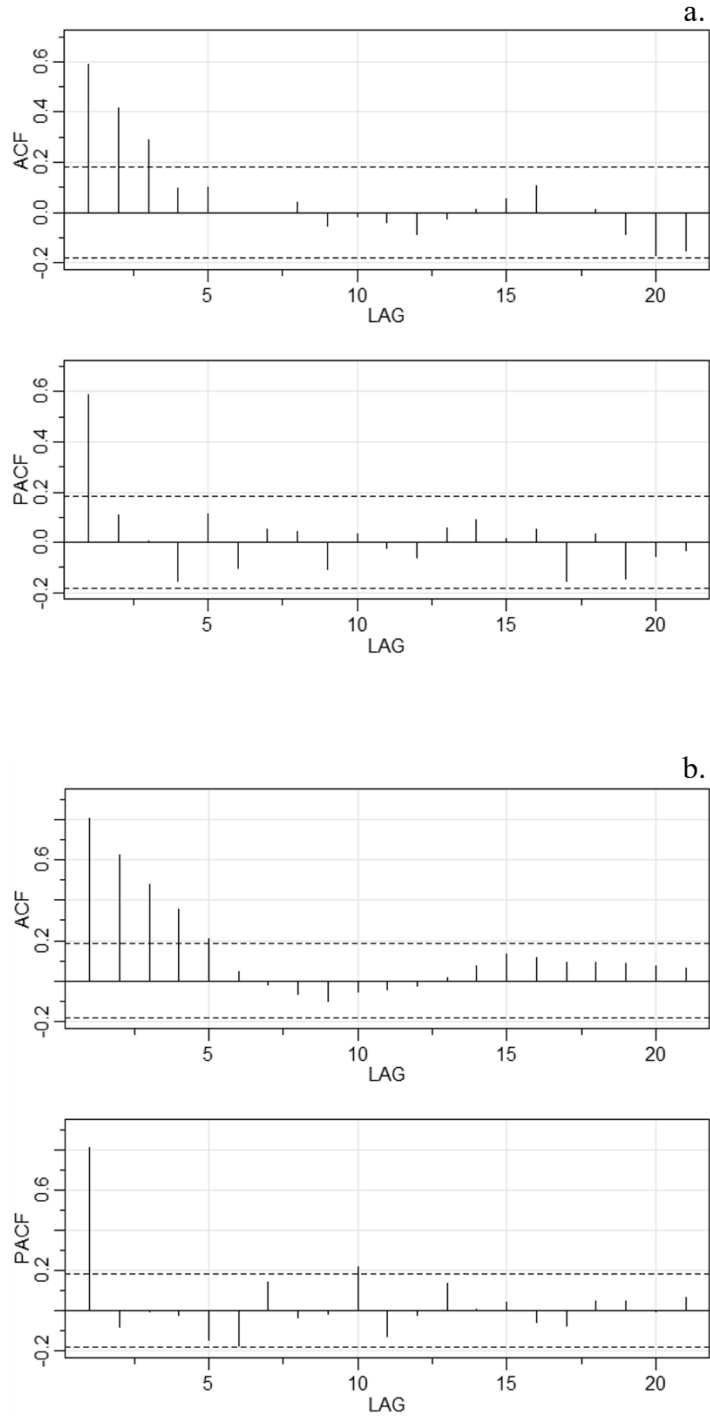




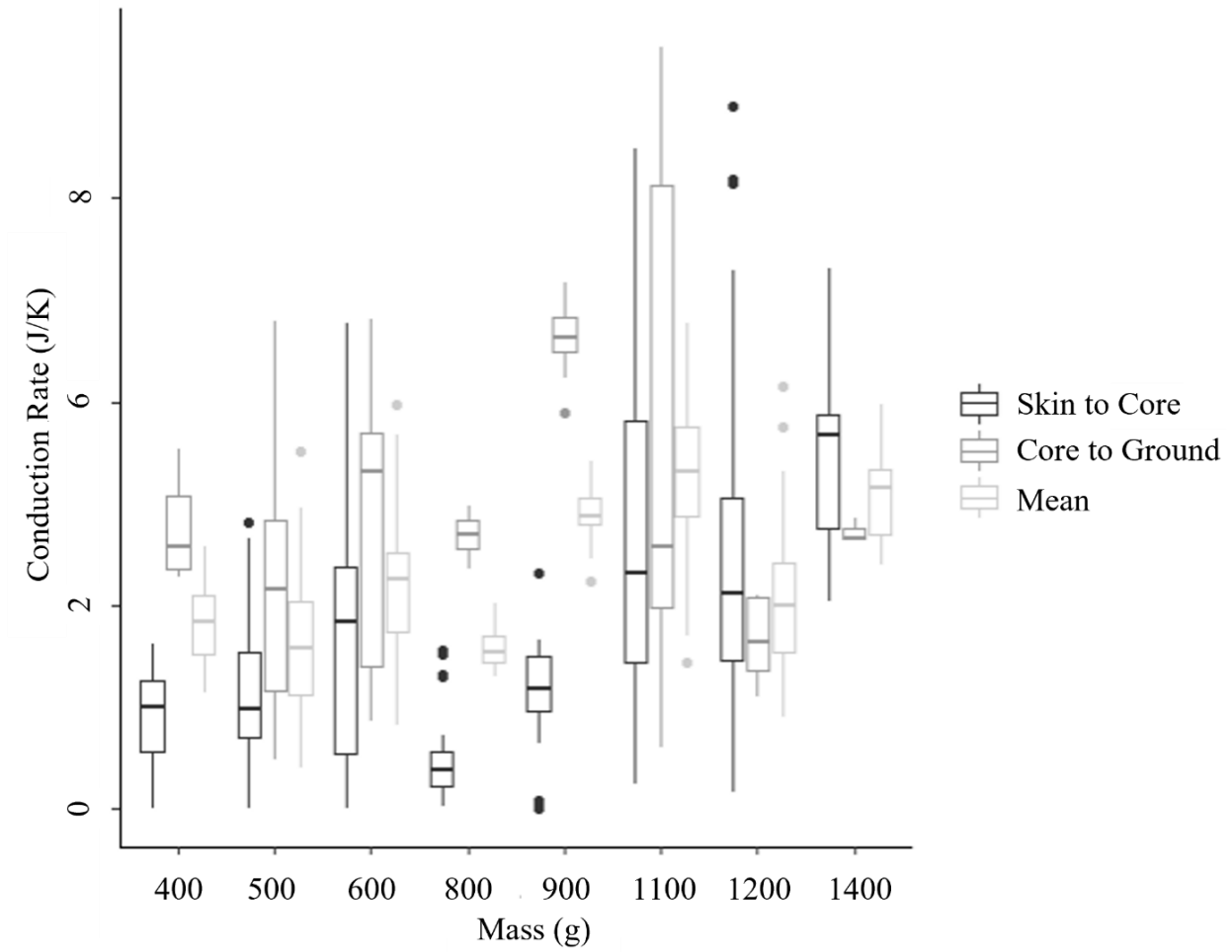
**Figure 8.** The within-individual relationship between total incoming radiation and conduction from the dorsum to the core (a) and from the core to ground (b). Each point represents the corresponding conduction rate to a simultaneous measurement of incoming total radiation, color identifies individual observations, and colored lines show rmcorr fit for each observation. Negative correlations are likely a result of often higher rates of conduction from the body core to the ground throughout many of the observation periods. Incoming radiation events were intense, but lasted only for a short time (i.e., minutes).



**Figure 9.** Cross-correlation plots of incoming total radiation (x-axes) and conduction (y-axes) from the dorsum to the core (a.) identified a significant positive correlation at -2 mins (black dashline) indicating increased exposure to direct solar radiation leads to a significant increase in conduction from the dorsum to the core after two minutes. Likewise, a significant although weaker correlation was detected between incoming solar radiation and conduction from the core to the ground at -54 min (black dash line) (b.) indicating significant increases in conduction from the core to the ground occurs after prolonged exposure to direct solar radiation. Horizontal dotted lines identify the 95% confidence intervals.



**Figure 10.** Residuals of the autocorrelation and partial autocorrelation functions for five-minute lags of total incoming radiation and  $Q_{cond}$  from the dorsum to core (a.) and from the core to ground (b.). Dotted lines indicated 95% confident intervals. One significant correlation was observed in the PACF after a 10 min lag between incoming radiation at the dorsum and  $Q_{cond}$  from core to ground (b.).



**Figure 11.** Snakes were assigned to 100g groups based on mass. Linear regression indicated a statistically significant difference between mass and the combined dependent variables of conduction from dorsum to core and core to ground ( $F(2, 288) = 57.785, p < 0.0001$ ).

## Chapter 2: The Effect of Transient Insolation on the Competing Behaviors of Thermoregulation and Ambush Foraging in Timber Rattlesnakes

### Abstract

Unprecedented changes to habitat structure and terrestrial communities are occurring around the globe due to continued unsustainable land-use methods, landscape scale management practices and more recently, extreme stochastic events often involving large scale changes to vegetation communities. Vegetation structure and composition significantly affect the frequency, duration, and intensity of solar insolation at ground level. For many diurnal ectotherms, the amount of solar insolation exposure can significantly affect surface activity. Reptile thermoregulation can impose significant constraints on daily activities and under extreme conditions, may require tradeoffs that could have implications to life-history traits. We considered the effects of patches of sunlight or transient insolation, on the core body temperature of the ambush foraging Timber Rattlesnake (*Crotalus horridus*) in the Ozark Mountains of northwest Arkansas. We hypothesized that exposure to frequent and intense solar insolation could cause a thermoregulatory response (thermal movement) and if frequent enough, these movements may negatively affect the time available for foraging. We used continuous videography to simultaneously collect body core temperature ( $T_b$ ), incoming solar radiation, and behavior data. Both the rate of change in  $T_b$  and the frequency of transient insolation were significant indicators of whether a thermal move occurred. We concluded that frequency of transient insolation was the most significant factor determining whether a thermal move occurred in a dense canopy forest because duration and intensity are less important if solar transients are infrequent. We used these data to predict the length of time a snake of a given mass under varying incoming solar conditions could remain in position before being forced to make a

thermal move. However, daytime activity in our population does not appear to be thermally constrained. The observed forced thermal movements we did observe only occurred during the months of June, July, and August and were centered approximately around solar noon. However, snakes would also forage at night during these months, effectively compensating for any lost foraging time. Therefore, our data suggest the tradeoff between ambush foraging and thermoregulation is negligible in our study population. However, management activities that open the canopy such as thinning and prescribed fire could have unanticipated impacts on the thermal environment that may influence the daily activity budget of an apex predator like the Timber Rattlesnake.

## **Introduction**

Environmental change can influence time-energy allocation trade-offs among fitness components such as growth, reproduction, and survivorship that ultimately affect life history strategies (Stearns and Stearns, 1992). Organisms use an array of behaviors to allocate time to competing daily functions such as foraging, thermoregulation, predator avoidance, or reproductive activities (Dunham et al., 1989). Energy acquired must also be balanced between the four competing functions of growth, reproduction, maintenance, and storage (Dunham et al., 1989). A major impact on mass/energy allocation comes from effects of the biophysical environment on core body temperature ( $T_b$ ) and behavior (Dunham et al., 1989; Buckley and Kingsolver, 2012). However, understanding how ectotherms balance daily heat and mass budgets requires detailed information about foraging strategies and thermoregulatory requirements (Dunham et al., 1989).

In most ectotherms, meeting daily thermal requirements takes precedence over all other activities (Grant and Dunham, 1988; Dunham et al., 1989). However, short-term unpredictability in ambient heat load can influence ectotherm body temperature regulation and activity patterns (Dunham et al., 1989). Frequent thermoregulatory movements would reduce the time available for biologically necessary activities such as foraging or reproduction (Huey and Slatkin, 1976; Grant and Dunham, 1988; Dunham et al., 1989) and thus, potentially affect fitness. For example, if regional environmental thermal distributions shift in response to climate change or large scale stochastic events such as wildfire, ectotherms must meet the new thermal challenges by shifting activity patterns (Beaupre, 2002). With the intensity, frequency, and duration of temperature and precipitation extremes expected to increase as a result of a rapidly changing climate (Ummenhofer and Meehl, 2017), understanding how these changes translate to life history trait expression is critical for management activities (Brewster and Beaupre, 2019b).

During the active season, reptile daily activities are most often constrained by voluntary maximum body temperatures ( $VT_{max}$ ) (Pough and Gans, 1982). Identifying  $VT_{max}$  for field active animals provides information necessary for interpreting daily activity allocation patterns (Congdon et al., 1982; Grant and Dunham, 1988; Grant, 1990; Adolph and Porter, 1993; Beaupre, 1995). However, thermal activity thresholds are lacking for most organisms and are difficult to estimate for species in temperate habitats (Gunderson and Leal, 2015) for two reasons. First, individuals rarely allow themselves to be thermally challenged in situ. Second, thermal thresholds determined in a laboratory setting may not reflect behavioral responses to body temperature under natural conditions. Constraints on activity induced by thermal thresholds often involve physiological limits (Buckley and Roughgarden, 2005; Buckley et al., 2015a), but ectotherms are rarely active at or near thermal thresholds (Huey and Stevenson, 1979). Porter et

al. (1973) asserted that different activities often have different temperature thresholds. But if thermoregulation and foraging occur as part of a single behavioral strategy then identifying a field-based temperature threshold should provide an accurate estimate of potential constraints on both activities.

It is expected that foraging mode will affect thermoregulatory opportunities and constraints (Dunham et al., 1989; Beaupre, 1995; Ayers and Shine, 1997; Brewster and Beaupre, 2019b). Although meeting thermal requirements generally takes precedence over other daily activities in reptiles, heat and mass balance can often be achieved concurrently by active foraging (i.e. simultaneously shuttling between sun and shade while opportunistically feeding) (Dunham et al., 1989). However, an ambush foraging reptile such as the Timber Rattlesnake (*Crotalus horridus*) is necessarily constrained by both upper thermal thresholds and the associated requirements of an ambush foraging strategy. A successful ambush forager remains relatively motionless for an extended period (hours or days) which is counter to perceptions of active reptile thermoregulation (e.g., shuttling or active searching) (Huey and Pianka, 1981; Beaupre, 2007). Remaining motionless for extended periods would require a thermoregulatory strategy that favors a wide thermal tolerance or utilizes habitat structure and composition that minimizes opportunities for thermal stress. However, Wills and Beaupre (2000) observed Timber Rattlesnakes in the same population as our study population exhibited significant departures from available operative temperatures during most times of day in August and eight of 24 hours in September. The average  $T_b$  was less than the average environmental operative temperature ( $T_e$ ) from 0900 to 1600 hours in August and from 1000 to 1700 hours in September. Their results suggest behavioral thermoregulation that would be counter-productive to an ambush foraging strategy (Wills and Beaupre, 2000). Furthermore, frequent thermoregulatory movements could



reveal a snake in ambush to potential prey or increase the risk of predation. Therefore, a strategy is required that maintains body temperature within normal limits while the animal remains relatively motionless in ambush for extended time periods (Huey and Slatkin, 1976; Grant and Dunham, 1988; Dunham et al., 1989).

We investigated the potential trade-off between thermoregulation and an ambush foraging strategy in Timber Rattlesnakes in a deciduous hardwood forest using fixed videography by testing the following hypotheses: 1) Transient solar insolation causes significant increases in the  $T_b$ ; 2) The rate of change in  $T_b$  prompts a thermoregulatory response rather than achieving a thermal maximum; and 3) transient insolation can constrain ambush foraging activities in a moderate sized ambush forager by increasing the frequency of thermoregulatory movements, thus prompting a trade-off between thermoregulation and foraging. To characterize natural patterns of thermal load on ambush foraging in Timber Rattlesnakes, we quantified the average duration, intensity, and frequency of transient insolation for each video observation in five-minute intervals and adjusted data to reflect one-minute intervals. We quantified  $VT_{max}$  by examining thermal profiles of foraging Timber Rattlesnakes and assessed the impact of insolation on changes in  $T_b$  through concurrent measurements of in situ  $T_b$  and incident solar irradiance. For each snake, we calculated the rate of change in  $T_b$  and tested for a significant effect on the likelihood of observing a thermoregulatory movement. To assess the potential for transient insolation to constrain foraging activities, we used the insolation data and the rate of change in  $T_b$  to identify snakes that made thermoregulatory movements. We then considered whether the direct trade-off between thermoregulation and foraging as a function of time allocated to each activity has occurred.

## Methods

### *Study Organism*

Timber rattlesnakes are wide-ranging pit vipers historically distributed across the eastern half of the United States from Ontario, Canada south into Florida (Gloyd, 1978; Martin and Tynning, 1992; Clark et al., 2003) (Conant and Collins, 1998). Currently, *C. horridus* occupies a fraction of the historic range with populations becoming increasingly fragmented (Clark et al., 2003).

Our research population is part of a long-term monitoring and research project currently entering its 27<sup>th</sup> year and includes a broad scope of research topics including time and energy allocation (Beaupre, 2008; Lind and Beaupre, 2015), digestive performance (Beaupre and Zaidan, 2012), response to fire (Beaupre and Douglas, 2012), metabolism (Zaidan III and Beaupre, 2003), and thermoregulation (Wills and Beaupre, 2000). The field site is located in northwest Arkansas at the Ozark Natural Science Center (ONSC)/Madison County Wildlife Management Area (MCWMA), Madison County, Arkansas. Typical features of the area include sedimentary rock formations such as limestone and dolomite dominated by oak-hickory-pine forests and cedar glades characterized by diverse landscapes and endemic plant species. For a comprehensive description of the region the reader is referred to (Karstensen, 2010). The study population occupies a mix of managed and unmanaged forested areas with fire, brush removal, and selective thinning used as the primary management tools for controlling the density of understory vegetation. When fires are implemented, the thermal landscape is abruptly altered from a densely forested area to a shaded, closed-canopy system with an open understory.

For the current study, data were collected over five years (2015-2019). Adult *C. horridus* weighing 300 g or more were implanted with a temperature sensing SI-2T Holohil Systems Ltd.

radio transmitter following a modified protocol described by Reinert and Cundall (1982). Standard morphometrics including snout-vent length (SVL), head length (HL), head width (HW), tail length (TL), and body weight (mass) were collected each year upon emergence (April-May). Length data were measured in cm and mass in g. Data consisted of repeated observations (n=20) of 16 individuals (9 males and 7 females) for this analysis.

#### *Data Collection and Processing*

We used videography to collect in situ foraging behavior and associated  $T_b$  from *C. horridus*. Throughout the active season, Spy Tec STI-AEON OT mini bullet outdoor waterproof video cameras with audio were mounted on tripods and powered by a 12V 18-amp battery housed in a plastic ammo box were positioned to record snakes found in a typical foraging posture. A typical foraging *C. horridus* is often tightly coiled with the head resting on the body just beyond the coil perimeter resting on a log (Figure 1). The cameras were equipped with internal 128 GB SD storage cards and capable of recording at 30 fps for approximately 34 hours. A telemetry receiver (Communication Specialists Inc. Model R1000) housed in a Seal Line® 5L vinyl-coated waterproof “dry” bag was set to the snake’s telemetry frequency and positioned next to the camera to facilitate audio recordings of the temperature related pulses (Figure 1). We could then determine the snake’s  $T_b$  throughout the video recording.

Each camera was equipped with a microphone that recorded the transmitters pulse rate and thus, the corresponding internal  $T_b$ . The video system could record continuously up to 36 hours, although snakes often moved more frequently. Cameras were monitored a minimum of once daily and could be reset as the snake moved to other foraging locations. A HOBO pendent® temperature/light intensity data logger from Onset® Computer Corporation (HOBO) was positioned within 0.5 m of the snake to collect incoming solar intensity (0-323,000 lumens/m<sup>2</sup>

[0-30,000 foot candles (lumens/ft<sup>2</sup>)] and ambient temperature ( $T_a$ ) at substrate level every five minutes. Solar intensity was converted to watts/m<sup>2</sup> using the approximate conversion 0.00833 W/m<sup>2</sup> per lux (Michael, 2019).

The body posture and position a reptile adopts in its environment can significantly influence the effects of solar insolation on body temperature (Brewster and Beaupre, 2019a). Each snakes' position was characterized as: 'leaf hiding' (LH) - snake's body completely buried just below the leaf litter; 'leaf cratered' (LC) - snake had created a depression in the leaf litter with the body flush with the top of the leaf litter, or on top with the snake resting on the surface of the substrate with the body extending vertically above the leaf litter. We only used snakes that were leaf cratered or on top of the leaf litter for this analysis because they received direct solar insolation. We verified the assumption that convective heat loss would be negligible because wind velocity at ground level is minimal due to the increased friction drag associated with the ground surface layer (Carruthers, 1943). Wind velocity data (m/s) were collected at 1m above the ground and at ground level using a hot wire anemometer (Omega<sup>®</sup> HHF42). We measured wind velocity at 1 m ( $\bar{x} = 0.336$  m/sec, SD = 0.451) and at ground level ( $\bar{x} = 0.133$  m/sec, SD = 0.842) and found velocity at ground level to be significantly less  $t(119) = -2.5788, p = 0.0057$ .

#### *Categorization of Movements and Body Temperature Analysis*

We reviewed each video at five-minute increments from the time the camera was set and collected core body temperatures from the implanted radio transmitter. We only used video footage of snakes that remained in position for a minimum of 45 min (approximately two time-constants [26 min] for a 350 g *C. horridus*) (Wills and Beaupre, 2000) and on days with minimal cloud cover. The radio transmitters are fitted with a sensor calibrated by the manufacturer that produces a temperature-dependent pulse rate. The initial or starting  $T_b$ ,  $T_b$  at the time a response

occurred, and the maximum  $T_b$  observed were recorded for each video. We also calculated the rate of change in  $T_b$  leading to a response using the slope formula at one-minute increments and then converted to five-minute increments for the analyses. Snake movements were grouped into two categories; interpreted thermoregulatory response (hereafter referred to as a thermal movement for simplicity) and movements that occurred but were not associated with obvious thermal stress (i.e.,  $T_b$  never approached  $VT_{max}$ ) or instances where the snake remained immobile for the duration of the observation (referred to as non-thermal movement). Any departure from the original position was considered a response. Once movements were categorized into one of two groups, we compared: 1) the time of day a response occurred, 2) whether  $T_b$  at the time of response was approaching  $VT_{max}$ , 3) the rate of change in  $T_b$  and, 4) the intensity, frequency, and duration of transient insolation leading to a movement. We assumed most thermal movements would occur at the peak of solar intensity (11:00 – 16:00 hr). However, we extended the timeframe for the analysis to include hours between 07:00 – 17:00 hr because repeated exposure to solar transients even at low angles and intensities could initiate a thermal movement. In some instances, only one criterion was achieved such as approaching or reaching  $VT_{max}$  but did so at such a slow rate of change that it either did not move or did so without haste. Typically, snakes that made interpreted thermal movements achieved all criteria because insolation and body temperature data are often closely associated.

We report the mean  $T_b$  among individuals by pooling the observed  $T_b$ s for each individual from the start of the observation until either a thermal movement was initiated or until 19:00 hr. We tested for differences in  $T_b$  between thermal and non-thermal movements at the time a response occurred and average rate of change in  $T_b$  leading to a movement. Temperatures are reported in degrees Celsius (°C), and rates are expressed in degrees per minute (deg/min).

### *Transient Insolation Analysis*

Radiation data indicated solar exposure was negligible after approximately 19:00 hours (7:00pm). We used video data collected between 07:00 and 19:00 hours and on days with minimal cloud cover. Assuming short duration exposures would be insufficient to cause significant changes in body temperature, we only considered direct transient sun exposures greater than 30s in duration. We assumed shorter duration exposures would be insufficient to cause significant changes in body temperature. The duration of each solar transient (rounded to the nearest 30 sec) was determined by visually inspecting each video and identifying transient insolation that struck the snake's dorsum. Then, the duration of each transient on the dorsal surface of the snake was observed frame by frame until the transient no longer struck the dorsal surface. Frequency of transient insolation for each video was calculated using the total minutes of solar exposure divided by the total observation time (min) and expressed as a frequency between 0 and 1. Solar intensity was collected using the HOBO data logger.

Some snakes are lacking accompanying insolation data due to malfunctions with the HOBO data loggers. Summary statistics are reported only for those snakes with associated insolation data including the average intensity ( $\text{W/m}^2$ ), the total minutes solar insolation striking the snake's dorsum during the observation period. Frequent transient insolation lasting longer than 30 seconds should have a compounding effect on  $T_b$ .

### *Estimating Time to $VT_{max}$*

We used the mean maximum  $T_b$  observed in snakes that made thermal movements to identify  $VT_{max}$  because we assumed snakes that made thermal movements would have higher  $T_b$ s. Mean  $VT_{max}$  is less than  $CT_{max}$ , because snakes rarely, if ever allow  $T_b$  to approach  $CT_{max}$ . However, we assume some plasticity exists for  $VT_{max}$  and that a small range of body

temperatures will initiate a thermoregulatory movement. We calculated the time ( $t$ ) to reach the  $VT_{max}$  for each snake using equation 2 and then compared different thermal related variables (e.g., initial  $T_b$ , rate of change in  $T_b$ , and mean  $T_b$  observed) between snakes that made thermal movements with those that did not. The time to reach the  $VT_{max}$  is influenced by a complex combination of factors. By focusing on the major contributor (direct solar radiation) driving the variation in warming time and using  $T_e$  we may be able to estimate the time a snake with a given mass and under varying conditions of insolation can remain in position before  $T_b$  reaches the  $VT_{max}$ .

### *Statistical Analyses*

Body temperature distributions are often non-normally distributed, although estimated sample means should be approximately normal according to the Central Limit Theorem (Beaupre, 1995), and therefore, parametric tests were used when appropriate. Assumptions of normality, homoscedasticity, and independence were tested using the residuals from each analysis. When data failed to meet assumptions, non-parametric tests were used. Continuous variables with normal distributions are presented as mean (standard deviation [SD]) and non-normal variables are reported as median (1<sup>st</sup> and 3<sup>rd</sup> interquartile range [IQR]). We compared the rate of change and maximum observed  $T_b$ s between snakes that made thermal movements and snakes that did not using a Welch's T-Test. We also tested for differences between the average intensity, duration, and frequency of transient insolation between thermal movements and non-thermal movements. Statistical analyses were conducted using R Studio v3.5.0 (R Core Core, 2018) and we adopted a type-I error rate of  $\alpha = 0.05$ .

## Results

Forty-four in situ video recordings were collected between May and August over a 5-year period (2015-2019). After filtering the videos, a total of 7,865 minutes of video footage from 20 video recordings involving nine males and five females were used for this analysis. However, because environmental conditions varied widely among repeated samples of the same individual, we assumed they were independent observations.

### *Body Temperatures and Rate of Change*

A total of 1,245  $T_b$  observations were collected from 20 individuals at 5 min sampling intervals distributed across the active season. The seasonal distribution of  $T_b$  observations included 24 in May, 475 in June, 359 in July, 302 in August, and 85 in September. We detected a significant difference between mean  $T_b$ s at the time a thermal movement occurred ( $\bar{x} = 33.3$  °C, SD = 1.5 °C,  $n = 7$ ) versus non-thermal movements ( $\bar{x} = 27.2$  °C, SD = 3.7 °C,  $n = 13$ ) ( $t = -4.1354$ ,  $df = 18$ ,  $p = 0.0006213$ ; Table 1). The mean maximum  $T_b$  for snakes that made thermal movements was used to calculate  $VT_{max}$  (34.6°C, SD = 1.3 °C,  $n = 7$ ). Individuals that made thermal movements exhibited initial  $T_b$ s ranging from 20.0 °C to 31.3 °C ( $\bar{x} = 25.4$  °C, SD = 3.5 °C,  $n = 7$ ), while snakes that did not make thermal movements had initial  $T_b$ s ranging from 15.3 °C to 33.6 °C ( $\bar{x} = 24.8$  °C, SD = 4.8 °C,  $n = 13$ ). The initial  $T_b$  between the two groups did not differ ( $t(18) = -0.2937$ ,  $p = 0.7724$ ). A maximum  $T_b$  of 36.5 °C was recorded in July at 15:11 h. The same snake also failed at a foraging attempt earlier in the day (11:11 h) with a  $T_b$  of 34.5°C but remained in the same location for 1.35 hr until forced to make a thermal movement with a  $T_b$  of 35.5°C. Thermoregulatory movements occurred most frequently between 12:50 and 14:00 h while non-thermal movements primarily happened after 1600 h (Figure 2). The single instance of a snake making a thermal movement with an associated negative rate of change in  $T_b$  at 13:13 h



(-0.007 °C/min) resulted from a high initial  $T_b$  (31.3 °C) and thus, a small temperature difference between the body core and dorsal surface temperatures. Another thermoregulatory response could be prompted by the new thermal conditions.

We compared both the rate of change in  $T_b$  and maximum  $T_b$  observed for snakes that made thermal moves and those that did not. Rate of change in  $T_b$  was significantly higher in snakes that made thermal movements ( $\bar{x} = 0.03$ , SD = 0.02,  $n = 7$ ) compared to snakes that did not ( $\bar{x} = 0.01$ , SD = 0.01,  $n = 13$ ;  $t = -3.4046$ ,  $df = 7.97$ ,  $p = 0.0093$ ,  $d = 1.9$ , 95% CI[0.81, 2.99]; Figure 3a). Similarly, the mean maximum  $T_b$  observed was significantly higher in snakes that made thermal movements ( $\bar{x} = 34.56$ , SD = 1.31,  $n = 7$ ) compared to snakes that made non-thermal movements ( $\bar{x} = 30.87$ , SD = 2.23,  $n = 13$ ;  $t = -4.6558$ ,  $df = 17.72$ ,  $p = 0.0002$ ,  $d = 1.87$ , 95% CI[0.79, 2.96]; Figure 3b).

Overall, snakes rarely achieved  $VT_{max}$  and in fact, only attained or exceeded  $VT_{max}$  265 min of the total 7,865 min (3 %) observed. Snakes that made thermal movements achieved or exceeded  $VT_{max}$  only 11 % of the total observable minutes (265 of 2,340 min) versus 0 of 5,525 min for non-thermal movements. A chi-square test of independence detected a significant relationship between thermal movements and the frequency of achieving  $VT_{max}$ ,  $X^2(1, N = 7,865) = 644.04$ ,  $p < 0.001$ .

### *Transient Insolation*

Frequency, duration, and intensity of transient insolation varied considerably across samples (Table 2). The frequency of exposure to transient insolation was significantly higher in snakes that made thermal movements ( $\bar{x} = 0.57$  min, SD = 0.23 min,  $n = 7$ ) compared to non-thermal movements ( $\bar{x} = 0.26$  min, SD = 0.16 min,  $n = 13$ ;  $t(8.95) = -3.1872$ ,  $p = 0.01113$ ) (Figure 4a). The duration of transient insolation of thermal ( $\bar{x} = 8.3$  min, SD = 2.9 min,  $n = 7$ )

and non-thermal movements ( $\bar{x} = 5.65$  min,  $SD = 3.19$  min,  $n = 13$ ) did not differ between the two groups ( $t(13.4) = -1.8715$ ,  $p = 0.08327$ ; Figure 4b). Difference in transient insolation intensity for thermal movements ( $\bar{x} = 96.3$  watts/m<sup>2</sup>,  $SD = 100.1$  watts/m<sup>2</sup>,  $n = 5$ ) and non-thermal movements ( $\bar{x} = 32.73$  watts/m<sup>2</sup>,  $SD = 40.01$  watts/m<sup>2</sup>,  $n = 11$ ) were also not significant ( $t(4.6) = -1.3709$ ,  $p = 0.2336$ ; Figure 4c).

#### *Estimated Time to $VT_{max}$*

We estimated the time to  $VT_{max}$  for each snake using in situ body temperatures and incoming solar radiation data. Figure 5 illustrates the relationship between  $T_b$  and transient insolation for a 732 g snake on a typical day in July. The average rate of change leading to a thermal movement in this individual was 0.04 °C/min over a 170 min period. Although thermal movements were often preceded by observed increases in frequency, duration, and intensity of transient insolation (Figure 5c and 5d), only insolation frequency was identified as a significant factor in leading to a thermal movement (Figure 5). Snakes were able to remain in position for most of the day except for thermal movements that occurred between approximately 12:30 h and 14:00 h when transient insolation intensity, duration, and frequency were highest.

## **Discussion**

Thermal constraints on reptile activity have been studied extensively (Cowles and Bogert, 1944; Spotila et al., 1972; Huey and Pianka, 1977; Bakken et al., 1985; Stevenson, 1985b; Grant, 1990; Beaupre, 1995; Vickers et al., 2011; Sears et al., 2016) and support the assertion that thermoregulatory needs represent a significant constraint on the allocation of time to competing activities (Dunham et al., 1989; Wills and Beaupre, 2000). Differences between the distribution of available  $T_e$ s and observed  $T_b$ s may be a necessary, but not sufficient condition to demonstrate

active thermoregulation (Porter et al., 1973; Bakken and Gates, 1975; Grant and Dunham, 1988; Hertz et al., 1993; Wills and Beaupre, 2000). But departures between  $T_b$  and  $T_e$  may also occur for reasons other than thermoregulation such as sampling error, inaccurate characterization of available thermal habitat, time lags associated with thermal inertia, or a lack of appropriate spatial and temporal sampling (Wills and Beaupre, 2000). We attempted to capture in situ thermoregulatory behavior in Timber Rattlesnakes using videography and assess the potential effects on time spent foraging. Advancements in technology such as videography provide access to greater detail of daily activities in animals that have been difficult to capture using intermittent observations (Clark et al., 2012).

Twenty video observations capturing  $T_b$  and solar insolation data for 13 snakes over a five-year period were used to assess the effect of transient insolation on  $T_b$ . We identified a voluntary thermal maximum of 34.6 °C and found that even in the snakes identified as ones that made thermal movements,  $VT_{max}$  was exceeded 26% of the total minutes of observed (3,005 min). Activity appears to only be thermally constrained between approximately 12:00 and 14:00 h (Fig. 2), which aligns with results from Wills and Beaupre (2000), who found surface activity expressed as a percent of  $T_e$  observations between 5.5° and 37.4°C, was constrained only during midday during August (i.e., 78% of  $T_e$  s available). The single instance of a snake making a thermal movement with an associated negative rate of change in  $T_b$  at 13:13 h (-0.007 °C/min) resulted from a high initial  $T_b$  (31.3 °C) and thus, a small temperature difference between the body core and dorsal surface temperatures (Figure 2). It is possible we interrupted a thermal movement when setting up the camera. Our presence may have caused the snake to delay its response or, we approached just after a response occurred and we observed a second thermal movement. However, we included this observation for several reasons. First, the snake remained

in position for more than an hour after setting the camera before relocating. We assumed a snake under thermal stress would be forced to move. All other criteria listed above were met. Finally, thermal movements can occur successively and conditions that cause a thermal movement are dependent on the thermal environment at the new location and the thermal history of the individual. For example, if the snake had made a thermal movement prior to setting a video camera, the same thermal constraints apply but in a new thermal microclimate. Our data suggest that  $T_b$  at the time a response occurred, the rate of change in  $T_b$ , and the mean maximum  $T_b$  are significant indicators of whether a snake made a thermal move (Fig. 3).

Body core temperature is undoubtedly influenced by the intensity, duration, and frequency of solar insolation however, only the frequency of transient insolation was a significant factor in whether snakes made a thermal movement (Fig. 5b and 5c). The lack of a relationship between solar insolation duration or intensity and thermal movements may be a result of our small sample size ( $n = 20$  of 49 total video recordings). Transient insolation is affected by the density of primary and secondary canopy cover. Thus, more frequent transient insolation would be required to significantly affect  $T_b$  (Fig. 5a).

We sought video footage that tracked a foraging snake's  $T_b$  throughout a cloudless day as it was exposed to transient insolation. We were especially interested in situations where solar exposure caused rapid enough heating to force the snake to make a thermoregulatory movement. As expected, obtaining a sample size sufficient for analyses proved to be difficult because the conditions described above and the conditions that would initiate a thermoregulatory response appear to be rare events at our forested study site.

Of the 6,215 min of total observations, 1,941.5 min or 31% of the time resulted in exposure to transient insolation. Subsequently, of the 1,941.5 min of solar transient exposure,

756.5 min or 39 % resulted in a thermal movement (Table 2). A total of 302 solar transients were recorded for all video observations with 218 associated with non-thermal movements and 84 associated with thermal movements.

The amount of transient insolation reaching the ground in deciduous forests is affected by the canopy density (Kostel-Hughes et al., 2005; Stickley and Fraterrigo, 2021). Likewise, dense ground vegetation can also reduce the effect of insolation on the thermal environment by further decreasing the amount of insolation striking the ground (Hutchison and Matt, 1977; Stickley and Fraterrigo, 2021). The ground is also covered in a thick insulating layer of leaf litter that traps moisture and insulates the ground from direct solar radiation (Beatty and Sholes, 1988; Kostel-Hughes et al., 2005). Timber Rattlesnakes spend a considerable amount of time during the active season partially buried or cratered in the leaf litter. The ground temperature is typically lower than the snakes dorsal skin temperature during the day and appears to act as a heat sink drawing heat from the snake's body core (in prep). Partially burying the body in leaf litter or leaf cratering, may reduce the effects of direct solar radiation by minimizing the amount of exposed skin surface area and increasing thermal conduction to the cooler ground and surrounding leaf litter.

Total exposure to transient insolation was 1,941 min or 31% of the 6,245 total observation min. Total minutes of solar exposure in snakes that made thermal movements accounted for 756.5 min (8%) of the 6,215 min total foraging time observed (Table 2). In a 12 h day (07:00 – 19:00 hr) there are 720 min of potential solar exposure. If we assumed 8% of a typical day involves solar exposure that leads to a thermal movement, approximately 58 min of the available 720 min of potential daytime foraging is lost to thermal movements. Our results suggest snakes are thermally limited during mid-day for less than two hours.

The contribution of transient insolation to total radiative flux varies greatly by forest type with the size, shape, duration, intensity, and frequency of the transient insolation influenced by the canopy density and composition (Chazdon and Pearcy, 1991). The temporal and spatial distribution of transient insolation is highly variable and dependent on leaf size and canopy structure of overstory and understory plants (Chazdon and Pearcy, 1991). Within our study site, only frequency of transient insolation was a significant factor associated with the likelihood of a snake making a thermal movement (Figure 5).

We cannot disregard the effect of mass on body core temperature heating and cooling rates (Grigg et al., 1979; Bell, 1980; Ayers and Shine, 1997; Seebacher et al., 2003; Lutterschmidt and Reinert, 2012). As mass increases in ectotherms, the likelihood of the animal reaching thermal equilibrium with the environment decreases because of frequent temporal fluctuations in the daytime thermal environment (Seebacher et al., 2003). Adult timber rattlesnakes in our sample weighed between 350-1474.5g. The average mass of snakes that made thermal movements was 530.4 g (SD = 144.5g,  $n = 6$ ) versus 713.2 (SD = 384.4g,  $n = 9$ ) for those making non-thermal moves. As solar transients move across the dorsal surface, the  $T_b$  heating and cooling rates are reduced due in part to increased mass. Furthermore, the cooling rate is typically less than the heating rate in large bodied reptiles (Grigg et al., 1979). On a typical cloudless summer day, if the total time of solar exposure equaled the total time shaded the core body temperature should display a step increase. Thus, a snake exposed to frequent solar transients should eventually approach the  $VT_{max}$ . However, in our system the low frequency of transient insolation striking the snake throughout a typical day is not sufficient to cause a thermal movement. The delayed increase in  $T_b$  due to the effects of mass on heating and cooling rate

among other factors (i.e., conduction and leaf cratering), appears to allow ambush foraging Timber Rattlesnakes to remain in foraging position most of the day.

Like many ecological field studies our sample is relatively small and requires cautious interpretation because the events we were trying to capture are rare. Our results concur with Wills and Beaupre (2000) that snakes in this population do not appear to be severely limited in activity during the day due to thermal stress although thermal movements did increase during mid-day in July and August. Timber Rattlesnakes in deciduous forests appear to be buffered from the effects of transient insolation by body mass and the dense canopy. However, our results suggest transient insolation should be considered in thermoregulatory studies and that management activities (e.g., prescribed fires or brush removal) or extreme abiotic events (e.g., wildfires or hurricanes) that open the canopy in deciduous forests could have adverse effects. For example, opening the canopy to promote understory growth may expose snakes more frequently to solar insolation. A rattlesnake forced to relocate must appropriate time and energy that could be invested into other activities, such as foraging. In closed-canopy deciduous forests if the frequency of transient insolation increases, an increase in the number of forced thermal relocations could result in negative short-term ecological consequences for Timber Rattlesnakes. It follows that increased thermal movements could affect foraging opportunities and lead to a decrease in the total energy budget (Beaupre, 2002). However, opening the canopy in deciduous hardwood forests influences regeneration, plant diversity (Abd Latif and Blackburn, 2010), and seed and herbaceous production. The corresponding temporary increase in prey species may increase foraging opportunities for Timber Rattlesnakes and outweigh the short-term consequences of frequent thermal movements (Beaupre and Douglas, 2012). However, if changes in habitat structure result in relatively permanent loss of habitat (i.e., road cuts,

development, or silviculture practices) then Timber Rattlesnakes must adjust their activity budgets to compensate.

It is well-understood that thermal constraints can significantly influence patterns of reptile surface activity (Beaupre, 1995; Shine and Madsen, 1996; Sears, 2005; Brewster and Beaupre, 2019a). More open habitats like those found in the desert southwest or open plains would provide an interesting contrast. Thermal constraints can be exceptionally severe in desert systems (Grant and Dunham, 1988; Beaupre, 1995). Rattlesnakes in these habitats are often thermally restricted to foraging at night during the hottest part of active season (Beaupre, 1995; Wills and Beaupre, 2000; Putman and Clark, 2017). The significance of environmental heat load and shade related to vegetation structure is likely important in determining the relative importance of thermally-induced movements. For example, in hotter drier habitats of the American southwest, it is likely that thermally-induced movements are much more common than in our focal Ozark Mountain habitat. Additional work in similar systems that vary in heat load and vegetation structure would assist in discerning the general importance of tradeoffs between ambush foraging and thermoregulation.



## References

- ABD LATIF, Z., AND G. A. BLACKBURN.** 2010. The effects of gap size on some microclimate variables during late summer and autumn in a temperate broadleaved deciduous forest. *International Journal of Biometeorology*. 54:119-129.
- ADOLPH, S. C., AND W. P. PORTER.** 1993. Temperature, Activity, and Lizard Life Histories. *The American Naturalist*. 142:273-295.
- AYERS, D., AND R. SHINE.** 1997. Thermal influences on foraging ability: body size, posture and cooling rate of an ambush predator, the python *Morelia spilota*. *Functional Ecology*. 11:342-347.
- BAKKEN, G. S., AND D. M. GATES.** 1975. Heat-transfer analysis of animals: some implications for field ecology, physiology, and evolution, p. 255-290. *In: Perspectives of biophysical ecology*. Springer.
- BAKKEN, G. S., W. R. SANTEE, AND D. J. ERSKINE.** 1985. Operative and Standard Operative Temperature: Tools for Thermal Energetics Studies. *American Zoologist*. 25:933-943.
- BEATTY, S. W., AND O. D. SHOLES.** 1988. Leaf litter effect on plant species composition of deciduous forest treefall pits. *Canadian Journal of Forest Research*. 18:553-559.
- BEAUPRE, D. S. J., CHAD E. MONTGOMERY.** 2007. The meaning and consequences of foraging mode in snakes. Cambridge University Press.
- BEAUPRE, S. J.** 1995. Effects of Geographically Variable Thermal Environment on Bioenergetics of Mottled Rock Rattlesnakes. *Ecology*. 76:1655-1665.
- BEAUPRE, S. J.** 2002. Modeling time-energy allocation in vipers: individual responses to environmental variation and implications for populations. *Biology of the vipers*:463-481.
- BEAUPRE, S. J.** 2008. Annual variation in time-energy allocation by timber rattlesnakes (*Crotalus horridus*) in relation to food acquisition, p. 111-122. *In: The biology of rattlesnakes*. W. K. Hayes, M. D. Cardwell, K. R. Beaman, and S. P. Bush (eds.). Loma Linda University Press, Loma Linda, California.
- BEAUPRE, S. J., AND L. E. DOUGLAS.** 2012. Responses of timber rattlesnakes to fire: lessons from two prescribed burns.
- BEAUPRE, S. J., AND F. ZAIDAN.** 2012. Digestive Performance in the Timber Rattlesnake (*Crotalus horridus*) with Reference to Temperature Dependence and Bioenergetic Cost of Growth. *Journal of Herpetology*. 46:637-642.
- BELL, C. J.** 1980. The Scaling of the Thermal Inertia of Lizards. *Journal of Experimental Biology*. 86:79-85.

- BREWSTER, C. L., AND S. J. BEAUPRE.** 2019a. The effect of body posture on available habitat and activity-time in a lizard: implications for thermal ecology studies. *Journal of thermal biology*. 82:10-17.
- . 2019b. The effect of body posture on available habitat and activity-time in a lizard: Implications for thermal ecology studies. *Journal of Thermal Biology*.
- BUCKLEY, L. B., J. C. EHRENBERGER, AND M. J. ANGILLET JR.** 2015. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology*. 29:1038-1047.
- BUCKLEY, L. B., AND J. G. KINGSOLVER.** 2012. The demographic impacts of shifts in climate means and extremes on alpine butterflies. *Functional Ecology*. 26:969-977.
- BUCKLEY, L. B., AND J. ROUGHGARDEN.** 2005. Effect of species interactions on landscape abundance patterns. *Journal of Animal Ecology*:1182-1194.
- CARRUTHERS, N.** 1943. Variations in wind velocity near the ground. *Quarterly Journal of the Royal Meteorological Society*. 69:289-301.
- CHAZDON, R. L., AND R. W. PEARCY.** 1991. The importance of sunflecks for forest understory plants. *BioScience*. 41:760-766.
- CLARK, A. M., P. E. MOLER, E. E. POSSARDT, A. H. SAVITZKY, W. S. BROWN, AND B. W. BOWEN.** 2003. Phylogeography of the Timber Rattlesnake (*Crotalus horridus*) Based on mtDNA Sequences. *Journal of Herpetology*. 37:145-154.
- CLARK, R. W., S. TANGCO, AND M. A. BARBOUR.** 2012. Field video recordings reveal factors influencing predatory strike success of free-ranging rattlesnakes (*Crotalus* spp.). *Animal Behaviour*. 84:183-190.
- CONANT, R., AND J. T. COLLINS.** 1998. A field guide to reptiles & amphibians: eastern and central North America. Houghton Mifflin Harcourt.
- CONGDON, J. D., A. E. DUNHAM, AND D. W. TINKLE.** 1982. Energy Budgets and Life Histories of Reptiles, p. 233-271. *In: Biology of the Reptiles*. Vol. 13. C. Gans and Pough (eds.). Academic Press, New York.
- CORE, T. R.** 2018. R: A language and environment for statistical computing, p. 2013, R Foundation for Statistical Computing, Vienna, Austria.
- COWLES, R. B., AND C. M. BOGERT.** 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* 83:261-296.
- DUNHAM, A. E., B. W. GRANT, AND K. L. OVERALL.** 1989. Interfaces between Biophysical and Physiological Ecology and the Population Ecology of Terrestrial Vertebrate Ectotherms. *Physiological Zoology*. 62:335-355.

- GLOYD, H. K.** 1978. The rattlesnakes, genera *Sistrurus* and *Crotalus*: a study in zoogeography and evolution. Milwaukee: Society for the Study of Amphibians and Reptiles.
- GRANT, B. W.** 1990. Trade-offs in activity time and physiological performance for thermoregulating desert lizards, *Sceloporus merriami*. *Ecology*:2323-2333.
- GRANT, B. W., AND A. E. DUNHAM.** 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology*:167-176.
- GRIGG, G. C., C. R. DRANE, AND G. P. COURTICE.** 1979. Time constants of heating and cooling in the eastern water dragon, *Physignathus lesueruii* and some generalizations about heating and cooling in reptiles. *Journal of Thermal Biology*. 4:95-103.
- GUNDERSON, A. R., AND M. LEAL.** 2015. Patterns of Thermal Constraint on Ectotherm Activity. *The American Naturalist*. 185:653-664.
- HERTZ, P. E., R. B. HUEY, AND R. STEVENSON.** 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *American Naturalist*:796-818.
- HUEY, R. B., AND E. R. PIANKA.** 1977. Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. *Ecology*:1066-1075.
- HUEY, R. B., AND E. R. PIANKA.** 1981. Ecological consequences of foraging mode. *Ecology*. 62:991-999.
- HUEY, R. B., AND M. SLATKIN.** 1976. Cost and benefits of lizard thermoregulation. *Quarterly Review of Biology*:363-384.
- HUEY, R. B., AND R. STEVENSON.** 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist*. 19:357-366.
- HUTCHISON, B. A., AND D. R. MATT.** 1977. The distribution of solar radiation within a deciduous forest. *Ecological Monographs*. 47:185-207.
- KARSTENSEN, K. A.** 2010. Land-cover change in the Ozark Highlands, 1973-2000. US Geological Survey.
- KOSTEL-HUGHES, F., T. P. YOUNG, AND J. D. WEHR.** 2005. Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation to seed size. *The Journal of the Torrey Botanical Society*. 132:50-61.
- LIND, C. M., AND S. J. BEAUPRE.** 2015. Male Snakes Allocate Time and Energy according to Individual Energetic Status: Body Condition, Steroid Hormones, and Reproductive Behavior in Timber Rattlesnakes, *Crotalus horridus*. *Physiological and Biochemical Zoology*. 88:624-633.

- LUTTERSCHMIDT, W. I., AND H. K. REINERT.** 2012. Modeling body temperature and thermal inertia of large-bodied reptiles: Support for water-filled biophysical models in radiotelemetric studies. *Journal of Thermal Biology*. 37:282-285.
- MARTIN, W., AND T. TYNING.** 1992. The timber rattlesnake: its distribution and natural history. *Conservation of the Timber Rattlesnake in the Northeast*:13-22.
- MICHAEL, P.** 2019. A Conversion Guide: Solar Irradiance and Lux Illuminance., IEEE Dataport.
- PORTER, W. P., J. W. MITCHELL, W. A. BECKMAN, AND C. B. DEWITT.** 1973. Behavioral implications of mechanistic ecology. *Oecologia*. 13:1-54.
- POUGH, F. H., AND C. GANS.** 1982. Physiology C: Physiologocial Ecology, p. 17-23. *In: Biology of the Reptilia*. Vol. 12. C. Gans (ed.). Academic Press, New York, New York.
- PUTMAN, B. J., AND R. W. CLARK.** 2017. Behavioral thermal tolerances of free-ranging rattlesnakes (*Crotalus oreganus*) during the summer foraging season. *Journal of thermal biology*. 65:8-15.
- REINERT, H. K., AND D. CUNDALL.** 1982. An improved surgical implantation method for radio-tracking snakes. *Copeia*. 1982:702-705.
- SEARS, M. W.** 2005. Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia*. 143:25-36.
- SEARS, M. W., M. J. ANGILLET, M. S. SCHULER, J. BORCHERT, K. F. DILLIPLANE, M. STEGMAN, T. W. RUSCH, AND W. A. MITCHELL.** 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proceedings of the National Academy of Sciences*.
- SEEBACHER, F., R. M. ELSEY, AND P. L. TROSCLAIR III.** 2003. Body temperature null distributions in reptiles with nonzero heat capacity: seasonal thermoregulation in the American alligator (*Alligator mississippiensis*). *Physiological and Biochemical Zoology*. 76:348-359.
- SHINE, R., AND T. MADSEN.** 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology*. 69:252-269.
- SPOTILA, J. R., O. H. SOULE, AND D. M. GATES.** 1972. The biophysical ecology of the alligator: heat energy budgets and climate spaces. *Ecology*:1094-1102.
- STEARNS, S. C., AND S. C. STEARNS.** 1992. *The Evolution of Life Histories*. OUP Oxford.
- STEVENSON, R. D.** 1985. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*:102-117.

- STICKLEY, S. F., AND J. M. FRATERRIGO.** 2021. Understory vegetation contributes to microclimatic buffering of near-surface temperatures in temperate deciduous forests. *Landscape Ecology*. 36:1197-1213.
- UMMENHOFER, C. C., AND G. A. MEEHL.** 2017. Extreme weather and climate events with ecological relevance: a review. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372:20160135.
- VICKERS, M., C. MANICOM, AND L. SCHWARZKOPF.** 2011. Extending the cost-benefit model of thermoregulation: high-temperature environments. *The American Naturalist*. 177:452-461.
- WILLS, C. A., AND S. J. BEAUPRE.** 2000. An application of randomization for detecting evidence of thermoregulation in timber rattlesnakes (*Crotalus horridus*) from northwest Arkansas. *Physiological and Biochemical Zoology*. 73:325-334.
- ZAIDAN III, F., AND D. S. J. BEAUPRE.** 2003. Effects of Body Mass, Meal Size, Fast Length, and Temperature on Specific Dynamic Action in the Timber Rattlesnake (*Crotalus horridus*). *Physiological and Biochemical Zoology*. 76:447-458.

## Appendix

### Tables and Figures

**Table 1.** Summary of body temperature-related data for individual observations highlighting some of the parameters used to identify thermal movements. The parameters highlighted here include the time of day a response occurred (Relocate time),  $T_b$  at the beginning of video recording or initial  $T_b$  (Init.  $T_b$ ),  $T_b$  at the time a response occurred (Relocate  $T_b$ ), maximum  $T_b$  observed ( $TV_{max}$ ) for that individual, and the individual rate of change in  $T_b$  leading to a response ( $T_b$  rate). An asterisk indicates a two-sample t-test identified a significant relationship between the parameter and the probability of a thermal movement occurring (*see* Figure 4).

ID	Sex	Mass (g)	Relocate time	Init. $T_b$ (°C)	Relocate $T_b$ (°C)*	Max. $T_b$ (°C)	$T_b$ rate (°C/min)*	Obs. move (y/n)	Thermal move (y/n)
181	m	1175.1	10:45	25.4	27.4	31.5	0.0197	y	n
606	m	1474.5	15:56	23.7	30.1	30.4	0.0002	y	n
140	m	1175.1	16:10	28.6	29.6	31.3	0.012	y	n
222	m	788.1	17:13	21.7	25.4	26.2	0.01	n	n
81b	m	547.9	17:26	24.5	27.2	32.3	0.011	n	n
943	m	540.8	17:50	26.1	29.1	32	0.007	n	n
81a	m	547.9	18:07	30.8	28.4	30.8	-0.006	y	n
801	m	732.4	18:33	24.1	27.7	33.1	0.016	n	n
398	f	450	18:44	25.7	26.2	27.7	0.004	y	n
245	f	360.1	19:00	18.1	25	32.5	0.013	n	n
942	m	540	19:00	24.5	29.1	30.6	0.0001	n	n
606b	m	1474.5	19:00	33.6	31.8	34.2	-0.006	y	n
081c	m	547.9	12:51	23.6	35.5	36.5	0.043	y	y
961	m	650	13:05	23.7	33.9	34.8	0.046	y	y
245b	f	360.1	13:07	20	31.4	32.9	0.042	y	y
830	m	500	13:13	31.3	31.5	33	-0.007	y	y
698	f	392.2	13:15	27.4	34.2	34.9	0.0372	y	y
801b	m	732.4	13:31	26.1	34.1	35.6	0.037	y	y
801c	m	732.4	14:00	25.6	32.5	34.2	0.038	y	y

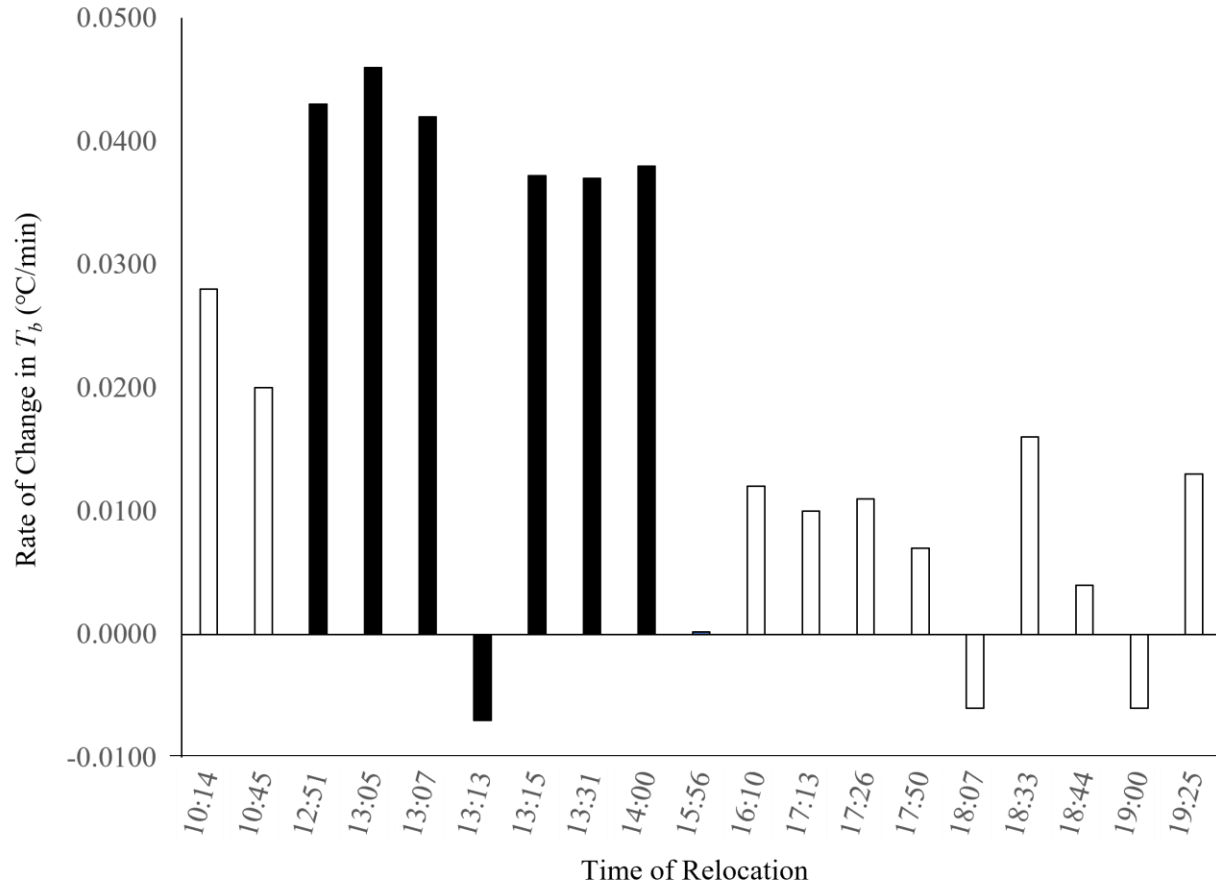
**Table 2.** Summary of transient insolation-related data for individual video observations including total minutes observed until a response (Total obs. time pre-move), total minutes the snake was exposed to transient insolation (Total solar exposure), the average number of minutes a snake was exposed to transient insolation (Ave. duration), frequency of solar transients expressed as a percentage of the total time observed (Solar transient frequency), and the average solar intensity of the observation period (Ave. solar intensity). The actual observed behavior categorized as response (y) or stationary (n) (Obs. move) and movements interpreted as thermal or non-thermal (Thermal Move) are also included for comparison. Dashes indicate data were not available. A significant effect (indicated by an asterisk) was detected between sunspot frequency and the likelihood of making a thermal movement (Figure 5).

ID	Month	Relocate time	Total obs. time pre-move (min)	Total solar exposure (min)	Ave. duration (min)	Solar transient frequency (%)*	Ave. solar intensity (W/m <sup>2</sup> )	Obs. move (y/n)	Thermal move (y/n)
160	Sept.	10:14	50	0	0	0	--	y	n
181	Aug.	10:45	110	67	11.2	0.61	72.7	y	n
606	Aug.	15:56	400	93.5	5.5	0.23	11.6	y	n
140	June	16:10	65	31	3.4	0.48	15.8	y	n
222	Aug.	17:13	470	88.5	6.3	0.19	6.25	n	n
81b	Aug.	17:26	510	129	7.6	0.25	7.21	n	n
943	June	17:50	430	87.5	4.2	0.2	9.7	n	n
81a	June	18:07	350	34	4.9	0.1	68.16	y	n
801	July	18:33	545	165	8.3	0.3	23.78	n	n
398	Sept.	18:44	365	93	4.7	0.26	--	y	n
245	June	19:00	640	227	10.8	0.35	128.7	n	n
942	June	19:00	595	110.5	2.3	0.19	11.9	n	n
606b	July	19:00	265	59	4.2	0.23	4.21	y	n
081c	July	12:51	340	172	8.2	0.51	209.08	y	y
961	July	13:05	220	51	4.6	0.24	--	y	y
245b	June	13:07	220	156	9	0.71	202.3	y	y
830	May	13:13	65	63	12.6	0.97	--	y	y
698	June	13:15	210	135	11.3	0.66	23.99	y	y
801b	July	13:31	225	96.5	6.9	0.43	13.64	y	y
801c	July	14:00	170	83	5.5	0.5	32.47	y	y

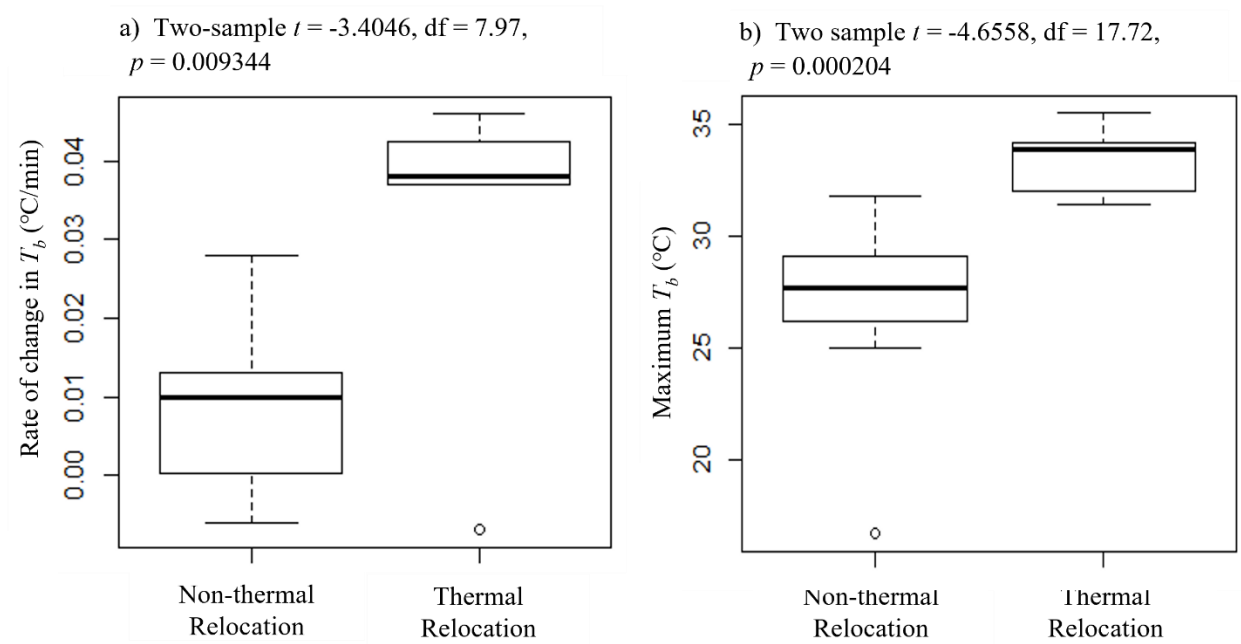


**Figure 1.** A typical videography setup illustrating the audio equipped surveillance video camera (a.) positioned on a snake (b.) and HOBO pendent<sup>®</sup> data logger (c.). The battery (d.) was housed in the orange dry box and the telemetry receiver was housed in a waterproof bag (e.). The system was highly mobile, waterproof and could run continuously for approximately 36 hours depending on camera settings. However, most snakes relocated within 24 hours after camera setup, or the battery was replaced approximately every 24 hours.

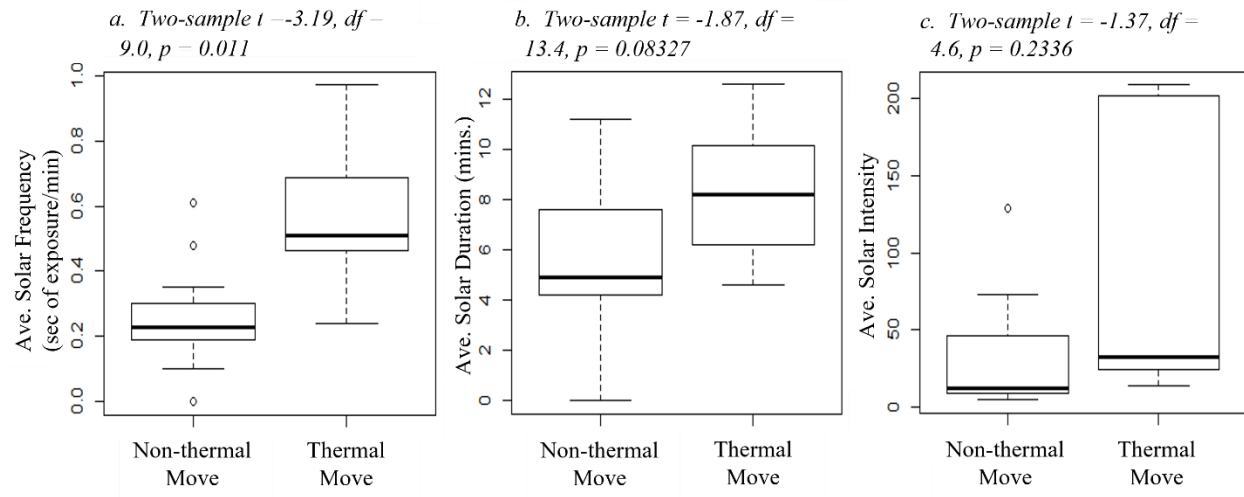




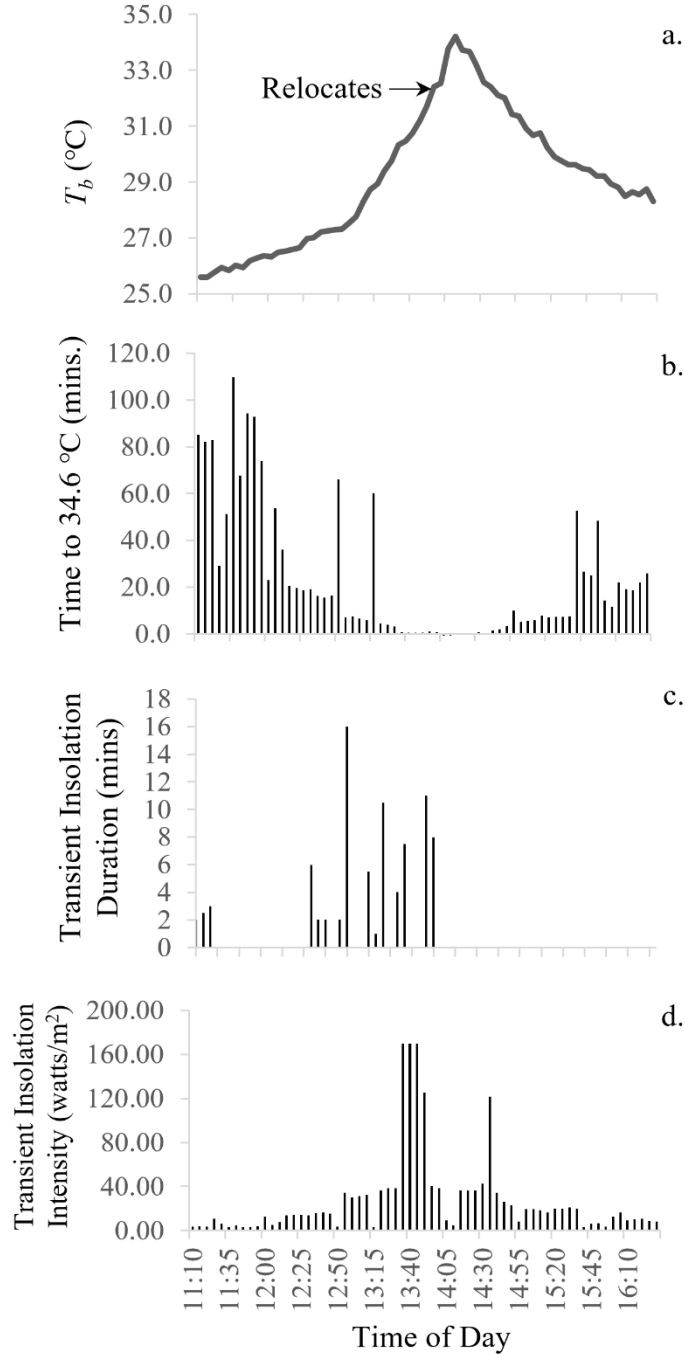
**Figure 2.** Temporal distribution of thermal (black bars) and non-thermal movements (white bars) as a function of the average rate of change in  $T_b$  at the time a response occurred. Negative values indicate an average decrease in body temperature when the response occurred but does not reflect the overall trend in  $T_b$ . Two individuals relocated at 19:00 hr, with one only being slightly positive ( $0.0001^{\circ}\text{C/min}$ ).



**Figure 3.** A Welch's t-test indicated snakes that made thermal movements had significantly higher rate of change in  $T_b$  (a) and mean maximum  $T_b$  observed (Maximum  $T_b$ ) (b) compared to snakes that made non-thermal movements. We found no difference in the results with the outliers removed.



**Figure 4.** Welch's t-test for unequal variances comparing solar intensity, duration, and frequency between thermal and non-thermal movements. We found a significant difference in the frequency of solar exposure (seconds of direct radiation per minute) between thermal and non-thermal movements (a.).



**Figure 5.** Sample data for a single event involving a 732 g snake. The four plots illustrate the relationship between body temperature and transient insolation. Specifically, the effect of transient insolation on the increase in  $T_b$  (a.) and the time for  $T_b$  to reach  $VT_{max}$  (b.) with respect to the duration (c.) and intensity (d.). Estimated time to a forced relocation ranged from 12.2 min at 11:10 h to 0.4 min. The individual in this example relocated at 14:00 h. Oscillations in the time to reach  $VT_{max}$  (b.) illustrate the influence of transient insolation duration (Figure 5 a and c). Time of Day on the x-axes is the same for all graphs and is in five-minute increments.

### **Chapter 3: The effect of leaf litter on Timber Rattlesnake (*Crotalus horridus*) operative temperatures during Spring emergence**

#### **Abstract**

Understanding how an organism interacts with its environment to meet basic physiological needs is fundamental to ecology. One of the most extreme and widespread thermoregulatory behaviors in ectotherms inhabiting temperate climates is hibernation. However, ectotherms emerging from hibernacula are often faced with highly variable environmental temperatures. Mechanisms for dealing with a variable thermal environment often involve behavioral thermoregulatory strategies such as shuttling or postural adjustments. However, ectotherms with life history traits facilitated by crypsis and reduced mobility may rely on other behavioral strategies. Timber Rattlesnakes (*Crotalus horridus*) in dense deciduous forests were often observed buried in the leaf litter during the weeks immediately following Spring emergence. We hypothesized the leaf litter would provide significant protective insulation against extreme environmental temperatures. To test our hypothesis, we used temperature data from operative temperature models and set in one of three positions; leaf hiding (LH), leaf cratered (LC), and on the surface of the leaf litter (Top). Leaf hiding model temperatures often departed from LC and Top model temperatures with lower temperatures during the day and higher temperatures at night. Leaf hiding model temperatures were significantly less variable when compared to models on top of the leaf litter. Leaf cratered models also showed an intermediate response with more stable temperatures and less variability when compared to models on top of the leaf litter. We conclude that model position, and therefore the position adopted by *C. horridus* in the leaf litter, can significantly impact model and body temperatures. Timber Rattlesnakes in temperate deciduous forests are subjected to extreme and highly variable

surface thermal regimes in early Spring following emergence. Leaf hiding behavior offers protection from the thermal extremes during acclimatization to warmer conditions.

## **Introduction**

Ectotherm activities such as foraging, mate searching, and emergence are often thermally constrained in temperate climates (Shine and Madsen, 1996; Sears, 2005; Gunderson and Leal, 2016). For some species, hibernation or brumation represents the most extreme form of thermal constraint accounting for more than half of the life of an individual (Blouin-Demers et al., 2000a). Research involving hibernating snakes has focused on hibernacula site selection (Browning et al., 2005; Harvey and Weatherhead, 2006), physiological mechanisms and thermal profiles of hibernating snakes (Macartney et al., 1989; Agugliaro, 2011), emergence patterns (Graves and Duvall, 1990), or demographics (Parker and Brown, 1973; Levine, 2013; Bruckerhoff et al., 2021). However, few studies have addressed environmental factors influencing the timing of spring emergence (Blouin-Demers et al., 2000a). For some species, the potential advantages of a longer active season (e.g., higher growth rates or earlier sexual maturity), assuming food is not limited, outweigh the risk associated with early emergence (e.g., exposure to extreme environmental temperatures) (Blouin-Demers et al., 2000a). But unpredictable and potentially lethal environmental temperatures in spring present a challenge for snakes emerging from hibernacula. Exposure to extreme low temperatures can be lethal or debilitate snakes and increase the risk of predation if caught outside the hibernaculum. A thermoregulatory strategy that would reduce the effects of extreme temperatures, provide concealment from predators, and extend surface activity time would therefore be advantageous.

Mechanisms for dealing with extreme environmental temperatures vary but often have a common theme. Among reptiles, behavioral mechanisms are the most common and include shuttling between sun and shade, reducing activity time during thermal extremes, and adjusting body posture (Huey and Slatkin, 1976; Alford and Lutterschmidt, 2018; Brewster and Beaupre, 2019b). Shuttling behavior is associated with more active species such as lizards (Brewster and Beaupre, 2019b) and Colubrids (Lelievre et al., 2010), and Elapids (Lillywhite, 1980). Ambush predators like the Timber Rattlesnake (*Crotalus horridus*) rely on cryptic, stationary behavior for long periods (e.g., hours or days; Wills and Beaupre, 2000). Therefore, shuttling behavior is not conducive to a stationary lifestyle. For a more sedentary species, selecting suitable thermal sites would be advantageous.

Choosing a suitable site for hibernation may involve choosing sites with appropriate above ground temperatures as well as below ground temperatures relative to other sites (Gienger and Beck, 2011). Gienger and Beck (2011) found Northern Pacific Rattlesnakes (*Crotalus oreganus*) selected hibernacula with significantly higher mean above ground environmental temperatures compared to random sites, although differences in below ground temperatures were not significant when compared to random sites. Hibernacula are often on south/southwest facing slopes where they receive a higher proportion of direct solar radiation during winter, fall, and spring (Browning et al., 2005; Harvey and Weatherhead, 2006; Schantz, 2009). South facing sites warm quickly and possess high thermal inertia due to the large component of rock often associated with hibernacula (Browning et al., 2005; Gienger and Beck, 2011). Snakes emerging from hibernacula should therefore have more thermally advantageous microclimates. Snakes in our study population emerge in early to late March and consistently move to what we refer to as transition habitat where they may spend days or weeks before moving away from the

hibernaculum. For the purpose of our research, we define transition habitat as the area typically just below hibernacula characterized by south facing slopes, high elevation, and rock outcrops with deep crevices (Browning et al., 2005). In the Ozark Mountains, the previous year's deciduous foliage creates a thick loosely compacted layer on the forest floor. Therefore, transitional habitat is typically characterized by deep deciduous leaf litter.

Snakes use transition habitat in the weeks prior to ingress or following egress. We often observed *C. horridus* coiled beneath the leaf litter or leaf hiding (LH) in early spring and late fall. However, during the summer snakes were less frequently seen in LH position but more often observed lying in a depression in the leaf litter or leaf cratered (LC) or, on top (Top) of the leaf litter (Figure 1). Observations of radio tagged snakes indicated a peak in LH behavior in early spring and a second, smaller peak just before ingress (Figure 2). The leaf litter and associated hiding behavior could serve two purposes. First, the leaf litter may act as an insulating layer providing a thermal buffer to extreme temperatures. Secondly, Timber Rattlesnakes rely strongly on cryptic coloration and avoid detection even when disturbed. Thus, snakes buried in the leaf litter would be nearly undetectable by potential predators during periods of low temperatures when they are most vulnerable.

We hypothesized that leaf litter provides a layer of insulation against extreme fluctuations in environmental temperature and that significant differences in snake body temperature exist depending on the position adopted (LC, LH, or Top) in the leaf litter. Because actual snake body temperatures are difficult to collect in a way that directly compares these positions, we elected to study this problem using operative temperature models (OTMs) (Bakken et al., 1985; Stevenson, 1985c).



## Methods

### *Study Site*

Our study site includes the Ozark Natural Science Center (ONSC), Bear Hollow Natural Area (Arkansas Heritage Commission), and McIlroy Madison County Wildlife Management Area (MMCWMA) in Madison County, Arkansas and is part of a long-term radio-telemetry project currently entering the 27<sup>th</sup> year of monitoring and research activity (Wills and Beaupre, 2000; Zaidan III and Beaupre, 2003; Browning et al., 2005; Lind and Beaupre, 2015; Beaupre et al., 2017). The dominant habitats include a mix of current and previously managed oak-hickory (*Quercus spp.*- *Carya spp.*) hardwood and pine (*Pinus spp.*) forested areas, cedar (*Juniperus spp.*) glades, wildlife feed plots, perennial and intermittent streams in the drainages, and limestone and dolomite outcroppings (Browning et al., 2005).

### *General Data Collection*

Timber Rattlesnakes in our study population are moderate to large-sized snakes with the average adult (males and females combined,  $n = 809$ ) in our population with SVL approximately 52.7 – 126.5 cm, mean = 84.4 cm (-0.43 SE) and mass 93.6 – 2017.65 g, mean = 563.7 g (-11.86 SE). Snakes were brought into the lab each spring to collect morphometric measurements (e.g., snout-vent length, tail length, mass, sex, and rattle measurements), to implant/re-implant telemetry transmitters (Reinert and Cundall, 1982), and assess the general health of each snake. Approximately 10-15 snakes were actively tracked each year and were located an average of every 3-4 days. We used these radio-telemetered snakes to locate hibernaculum and associated transition habitat.

### *Model Construction*

The adult *C. horridus* in our population are moderate in size with a maximum length of approximately 116-cm snout-vent length (SVL) and less than 2000 g in mass. We estimated the diameter and height of an average-sized coiled snake using a ruler. Operative temperature models were constructed of two six-inch (~15 cm) aluminum pie tins painted with primer to approximate the skin reflectivity of *C. horridus* (Peterson et al., 1993). One of the pie tins was fitted with a small strip of insulation around the edge to prevent condensation build up or water from entering. A length of wooden craft stick (approximately 3 cm wide) cut to approximately 7.5 cm lengths, or half the diameter of the pie tin, was used to secure a Maxim ibutton® at the approximate center of the pie tin using a small amount of molding clay. The pie tins were held together by 4 micro binder clips (2.8 x 2.8 x 1.9 in/7.0 x 7.0 x 4.8 cm; Figure 3). We attempted to design the models to reflect the thermal properties of a coiled snake and recognize that the OTM temperatures may not match actual  $T_b$  due to thermal inertia and microclimate variability. However, we do expect broad patterns of model position on  $T_e$  to be reflective of actual snake  $T_b$ . We calculated the time constant for our model and for three *C. horridus* carcasses weighing approximately 232g, 469g, and 573g. We cooled the carcass and the model to a common starting temperature, then placed both in a convection free, room temperature chamber and allowed them both to warm to equilibrium. Snake and model temperatures were measured every minute. We measured the internal temperature of the snake using a thermocouple wire inserted through the cloaca to the approximate center of the body. From these data, thermal time constants were calculated following Grigg et al. (1979).

### *OTM Data Collection*

Models were randomly assigned as LH, LC, or Top and placed in groups of three in random locations approximately 10 m apart within transition habitat just below a known hibernaculum. Seven sets of the three model positions were deployed in 2020-2022 for a week between mid-March and mid-May of each year. Because we were comparing  $T_e$ s of the different model positions under extreme thermal conditions, we chose days with the most extreme temperatures from our sampling periods (i.e., 2022).

### *Statistical Analysis*

We used R (R Core Team, 2019) and the lme4 package (Bates, Maechler & Bolker, 2012) to perform a linear mixed effects analysis of the relationship between temperature and OTM position in the leaf litter. Model position (LH, LC, and Top) was designated as a fixed effect. It is reasonable to assume that time of day affects the temperature of each model, so we converted time to a categorical (i.e., 24 one-hour blocks) random variable. We then fit a model that includes a by-hour random intercept position. Mixed-effects models are usually described using information criterion (e.g., Akaike Information Criterion [AIC]). We present marginal and conditional  $R^2$  values to describe the amount of variance explained by our random and fixed factors (Nakagawa and Schielzeth, 2013). The general linear mixed model (GLMM) marginal  $R^2$  ( $R^2_{GLMM(m)}$ ) describes the variance explained by the fixed factors while GLMM conditional  $R^2$  ( $R^2_{GLMM(c)}$ ) provides estimates of the variance explained by both fixed and random factors (Nakagawa and Schielzeth, 2013).

Visual inspection of residual plots did not reveal extreme deviations from homoscedasticity or normality. Significance values were obtained by likelihood ratio tests of the

full model with OTM position against a null model without OTM position included. All statistical analyses adopted a type one error rate of 0.05.

## Results

### *Operative Temperatures*

The thermal time constant for the operative temperature model was approximately 3 min. Time constants and mass for the three snake carcasses in a coiled position were 29 min (230g), 84 min (469g), and 94 min (573g). Body temperature would therefore lag a minimum of 29 mins behind  $T_e$  under transient conditions. However, our primary interest was to compare how model position affects the  $T_e$  of available microclimates and not predict snake  $T_b$ .

To directly address our question, we chose the two coldest and warmest days from our sample and separated the data into night (i.e., 00:00 hr- 06:00 hr) and day (11:00 hr – 17:00 hr) and compared  $T_e$ s for each position.

### *Nighttime Operative Temperatures Comparison*

Leaf hiding OTM  $T_e$ s averaged 2.5°C (SD = 0.9°C), -0.2°C (SD = 1.2°C) for LC, and -1.3°C (SD = 1.2°C) for Top models (Table 1). Mean  $T_e$  for LH models had a less variable temperature distribution and remained above freezing compared to LC and Top position temperatures (Figure 4).

Model position had a significant effect on temperature ( $F = 5537.3$ ,  $df = 2$ ,  $p < 0.001$ ). Thus, we fit a linear mixed model (estimated using the maximum likelihood (ML) and nonlinear minimization subject to Box Constraints optimizers) because we were interested in the temperature variance associated with OTM position (formula: [temperature ~ position]). Our random intercept model included hour-of-day (hour) and group as random effects (formula: +

[~1|hour] + [~1|group]) to account for baseline-differences in temperature by hour of day and in model group location. The full model's explanatory power (conditional  $R^2$ ) was 0.91 while the variance explained by model position (marginal  $R^2$ ) was 0.673 indicating that most of the variability was associated with hour of day and group. The model's intercept, corresponding to the Top position, was -1.30 (95% CI [-2.04, -0.57],  $t(11.45) = -3.48$ ,  $p = 0.005$ ). Within our model the effect of LH position was significant and positive (coefficients = 1.07, 95% CI [1.00, 1.15],  $t(1506) = 28.83$ ,  $p = 0.001$ ; standardized coefficients = 0.56, 95% CI [0.52, 0.59]). We compared our full model with OTM position (fixed effect) against a reduced model without OTM position. The high marginal  $R^2$  (0.673) emphasizes the effect of position on  $T_e$  in the absence of solar radiation. Leaf hiding affected model temperature ( $\chi^2(2) = 11075$ ,  $p < 0.001$ ), increasing it by approximately  $3.8^\circ\text{C} \pm 0.22$  (SE) (Table 2).

#### *Daytime Operative Temperature Comparison*

During the day (i.e., 11:00 – 17:00 hr), LH  $T_e$ s were also less variable (Figure 5) and with a lower mean temperature of  $25.9^\circ\text{C}$  (SD =  $5.4^\circ\text{C}$ ) than LC ( $\bar{x} = 33.4^\circ\text{C}$  [SD =  $8.2^\circ\text{C}$ ]) and Top models ( $\bar{x} = 36.3^\circ\text{C}$  [SD =  $8.2^\circ\text{C}$ ]) for Top models (Table 3). Model position had a significant effect on temperature ( $F = 760.3$ ,  $df = 2$ ,  $p < 0.001$ ) during the day. Our mixed model for the daytime comparison used the same variables and formula as the night model with OTM position as our fixed effect and temperature as the dependent variable (formula: temperature ~ position). The random intercepts included hour-of-day (hour) and group as random effects (formula: ~1|hour + ~1|group). The full model's explanatory power (conditional  $R^2$ ) was 0.75 while the variance explained by model position (marginal  $R^2$ ) was 0.253. When compared to the higher marginal  $R^2$  of the night model (0.673) in the absence of the effects of solar radiation, we can see the strong effect of direct solar radiation on  $T_e$ . Within our model the effect of LH position was

significant and negative (coefficients = -10.32, 95% CI [-10.85, -9.78],  $t(1506) = -37.72$ ,  $p = 0.001$ ; standardized coefficients = 0.-1.21, 95% CI [-1.27, -1.14]. We again compared our full model against a reduced model without the fixed effect and found leaf hiding to significantly affect model temperature ( $\chi^2(2) = 1520.6$ ,  $p < 0.001$ ), decreasing it by approximately -10.32 °C  $\pm 0.27$  (SE) for every 1 °C increase in the Top model temperature (Table 4).

## Discussion

The goal of this study was to assess the effect of position in the leaf litter (i.e., LH, LC, or Top) on *C. horridus* body temperature using OTMs. Timber Rattlesnakes were often observed in the LH position during spring and fall (Figure 1) when changes in  $T_a$  over 24-hours can be unpredictable and extreme. In early Spring canopy cover is minimal because mid-and upper-story vegetation have not fully leafed-out (Hutchison and Matt, 1977). The increase in direct solar radiation on OTMs commonly produces temperatures exceeding 58 °C, well above the maximum voluntary  $T_b$  for *C. horridus* ( $\bar{x} = 34.6$  °C, SD = 1.3 °C,  $n = 7$ ).

Our data indicate position in the leaf litter can have a significant effect on the temperature of *C. horridus*. Models in the LH position generally remained cooler during the day and warmer at night when comparing hourly averages. The maximum temperature observed for LH (42.5 °C) was 12.5 °C less than the Top model (55.0 °C) (Table 1). Over the two days (12 total hours) of our sample data, we recorded 519 observations exceeding the maximum voluntary  $T_b$  (34.6 °C) compared to 993 observations less than 34.6 °C. The high number of observations exceeding 34.6 °C demonstrates the protective nature of LH. In general, LH OTM mean hourly temperatures were approximately 10.3 °C (0.3 SE) lower than models in the Top position during the day. Leaf cratering also appears to reduce the effect of extreme temperatures with LC OTM temperatures during the day approximately 0.6 °C (0.2 SE) less than the Top OTM temperatures.

During nights when Top  $T_{es}$  were below freezing the leaf litter also buffered heat loss for LH models by approximately 3.8 °C (0.2 SE) when compared to Top OTM mean hourly night temperatures. During the two nights (12 total hours) of our sample data, 738 observations of temperatures  $\leq 0.0$  °C were recorded compared to 774 observations of temperatures above freezing. Again, emphasizing the protective nature of the leaf litter from extreme low temperatures.

Reptiles in temperate climates are behaviorally and physiologically constrained by the thermal environment (Porter et al., 1973; Grant and Dunham, 1988; Beaupre, 2002). Thermal constraints imposed upon the seasonal activity of *C. horridus* could have important biological implications. The annual energy acquired determines life history attributes such as age at sexual maturity, clutch size, and reproductive frequency (Macartney and Gregory, 1988). Extending the active season by even a few weeks could increase foraging opportunities and thus, energy allocated toward production once maintenance costs are met (Congdon et al., 1982). In fact, one snake was observed successfully foraging while leaf hiding in the first few weeks following emergence. Snakes were often observed over consecutive days in the LH position during this project and were video recorded for another aspect of this study. Some individuals were observed without a food bolus one day and subsequently observed on video camera with a bolus on the next day before returning to a LH position.

The importance of postural adjustments for the biophysical properties of animals (Muth, 1977; Stevenson, 1985c), thermal ecology (Ayers and Shine, 1997), and for available time and space (Brewster and Beaupre, 2019b) has been demonstrated in other species. Brewster and Beaupre (2019b) successfully demonstrated that the available time and space for surface activity of *Crotaphytus collaris* from the Ozark Mountains was significantly increased by optimal

adjustments in posture. We have demonstrated similar effects of position in the leaf litter on *C. horridus*  $T_b$  using OTMs. However, additional data are needed to determine if the benefits of emerging earlier outweigh the potential risks (Blouin-Demers et al., 2000b). Timber Rattlesnakes in our study population do not appear to be thermally constrained by environmental thermal distributions during the primary active season (i.e., May – September) (Wills and Beaupre, 2000). However, the weeks following emergence and preceding ingress typically have high environmental temperature variability. Our data show that *C. horridus* can reduce the effects of extreme environmental temperatures during Spring emergence using adjustments in position within the leaf litter. Our findings suggest that thermoregulatory strategies may be subtle, requiring detailed knowledge of the mechanisms used to cope with extreme environmental temperature fluctuations. Furthermore, these same mechanisms may provide insight into an organism's ability to cope with the localized effects of climate change and associated impacts to life history.



## References

- ABD LATIF, Z., AND G. A. BLACKBURN.** 2010. The effects of gap size on some microclimate variables during late summer and autumn in a temperate broadleaved deciduous forest. *International Journal of Biometeorology*. 54:119-129.
- ADOLPH, S. C., AND W. P. PORTER.** 1993. Temperature, Activity, and Lizard Life Histories. *The American Naturalist*. 142:273-295.
- AGUGLIARO, J.** 2011. Ecophysiology of hibernation in timber rattlesnakes ( *Crotalus horridus*), p. 277. Vol. Ph.D. University of Arkansas, United States -- Arkansas.
- ALFORD, J. G., AND W. I. LUTTERSCHMIDT.** 2012. Modeling energetic and theoretical costs of thermoregulatory strategy. *Journal of Biological Dynamics*. 6:63-79.
- . 2018. From conceptual to computational: Cost and benefits of lizard thermoregulation revisited. *Journal of Thermal Biology*. 78:174-183.
- ARELLANO, C., AND S. G. PANTULA.** 1995. Testing for trend stationarity versus difference stationarity. *Journal of Time Series Analysis*. 16:147-164.
- AYERS, D., AND R. SHINE.** 1997. Thermal influences on foraging ability: body size, posture and cooling rate of an ambush predator, the python *Morelia spilota*. *Functional Ecology*. 11:342-347.
- BAKDASH, J. Z., AND L. R. MARUSICH.** 2017. Repeated Measures Correlation. *Frontiers in Psychology*. 8.
- BAKKEN, G. S.** 1976. A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data to field ecology. *Journal of Theoretical Biology*. 60:337-384.
- . 1992. Measurement and application of operative and standard operative temperatures in ecology. *American Zoologist*. 32:194-216.
- BAKKEN, G. S., AND D. M. GATES.** 1975. Heat-transfer analysis of animals: some implications for field ecology, physiology, and evolution, p. 255-290. *In: Perspectives of biophysical ecology*. Springer.
- BAKKEN, G. S., W. R. SANTEE, AND D. J. ERSKINE.** 1985. Operative and Standard Operative Temperature: Tools for Thermal Energetics Studies. *American Zoologist*. 25:933-943.
- BARTHOLOMEW, G. A., AND V. A. TUCKER.** 1964. Size, body temperature, thermal conductance, oxygen consumption, and heart rate in Australian varanid lizards. *Physiological zoology*. 37:341-354.

- BASSON, C. H., O. LEVY, M. J. ANGILLET, AND S. CLUSELLA-TRULLAS.** 2016. Lizards paid a greater opportunity cost to thermoregulate in a less heterogeneous environment. *Functional Ecology*:n/a-n/a.
- BEATTY, S. W., AND O. D. SHOLES.** 1988. Leaf litter effect on plant species composition of deciduous forest treefall pits. *Canadian Journal of Forest Research*. 18:553-559.
- BEAUPRE, D. S. J., CHAD E. MONTGOMERY.** 2007. The meaning and consequences of foraging mode in snakes. Cambridge University Press.
- BEAUPRE, D. S. J., AND D. J. DUVAL.** 1998. Integrative Biology of the Rattlesnakes: Contributions to biology and evolution. *Bioscience*. 48:531-538.
- BEAUPRE, S. J.** 1995. Effects of Geographically Variable Thermal Environment on Bioenergetics of Mottled Rock Rattlesnakes. *Ecology*. 76:1655-1665.
- BEAUPRE, S. J.** 2002. Modeling time-energy allocation in vipers: individual responses to environmental variation and implications for populations. *Biology of the vipers*:463-481.
- BEAUPRE, S. J.** 2008. Annual variation in time-energy allocation by timber rattlesnakes (*Crotalus horridus*) in relation to food acquisition, p. 111-122. *In: The biology of rattlesnakes*. W. K. Hayes, M. D. Cardwell, K. R. Beaman, and S. P. Bush (eds.). Loma Linda University Press, Loma Linda, California.
- BEAUPRE, S. J., J. AGUGLIARO, J. U. VAN DYKE, AND F. ZAIDAN III.** 2017. Annual Energy Budgets of the Timber Rattlesnake: Advancements, Refinements, and Open Questions, p. 97-108. *In: The Biology of the Rattlesnakes II*. M. J. Dreslik, W. K. Hayes, S. J. Beaupre, and S. P. Mackessy (eds.). ECO Herpetological Publishing and Distribution, Rodeo, New Mexico.
- BEAUPRE, S. J., AND L. E. DOUGLAS.** 2009. Snakes as Indicators and Monitors of Ecosystem Properties.
- BEAUPRE, S. J., AND L. E. DOUGLAS.** 2012. Responses of timber rattlesnakes to fire: lessons from two prescribed burns.
- BEAUPRE, S. J., AND F. ZAIDAN.** 2012. Digestive Performance in the Timber Rattlesnake (*Crotalus horridus*) with Reference to Temperature Dependence and Bioenergetic Cost of Growth. *Journal of Herpetology*. 46:637-642.
- BELL, C. J.** 1980. The Scaling of the Thermal Inertia of Lizards. *Journal of Experimental Biology*. 86:79-85.
- BIRKEBAK, R. C., R. C. BIRKEBAK, AND D. W. WARNER.** 1964. A note on total emittance of animal integuments. *Journal of heat transfer*. 86:287-288.

- BLAND, J. M., AND D. G. ALTMAN.** 1995a. Calculating correlation coefficients with repeated observations: Part 2—Correlation between subjects. *Bmj.* 310:633.
- . 1995b. Statistics notes: Calculating correlation coefficients with repeated observations: Part 1—correlation within subjects. *Bmj.* 310:446.
- BLOUIN-DEMERS, G., K. A. PRIOR, AND P. J. WEATHERHEAD.** 2000a. Patterns of Variation in Spring Emergence by Black Rat Snakes (*Elaphe obsoleta obsoleta*). *Herpetologica.* 56:175-188.
- BLOUIN-DEMERS, G., K. A. PRIOR, AND P. J. WEATHERHEAD.** 2000b. Patterns of variation in spring emergence by black rat snakes (*Elaphe obsoleta obsoleta*). *Herpetologica*:175-188.
- BOGERT, C. M.** 1949. Thermoregulation in Reptiles, A Factor in Evolution. *Evolution.* 3:195-211.
- BORRELL, B. J., T. J. LADUC, AND R. DUDLEY.** 2005. Respiratory cooling in rattlesnakes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology.* 140:471-476.
- BREWSTER, C. L., AND S. J. BEAUPRE.** 2019a. The effect of body posture on available habitat and activity-time in a lizard: implications for thermal ecology studies. *Journal of thermal biology.* 82:10-17.
- . 2019b. The effect of body posture on available habitat and activity-time in a lizard: Implications for thermal ecology studies. *Journal of Thermal Biology.*
- BROWNING, D. M., S. J. BEAUPRE, AND L. DUNCAN.** 2005. Using partitioned Mahalanobis D-2(K) to formulate a GIS-based model of timber rattlesnake hibernacula. *Journal of Wildlife Management.* 69:33-44.
- BRUCKERHOFF, L. A., L. K. KAMEES, A. T. HOLYCROSS, AND C. W. PAINTER.** 2021. Patterns of Survival of a Communally Overwintering Rattlesnake Using an Artificial Hibernaculum. *Ichthyology & Herpetology.* 109:64-74.
- BUCKLEY, L. B., J. C. EHRENBERGER, AND M. J. ANGILLETTA JR.** 2015a. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology.* 29:1038-1047.
- BUCKLEY, L. B., J. C. EHRENBERGER, AND M. J. ANGILLETTA.** 2015b. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology.* 29:1038-1047.
- BUCKLEY, L. B., AND J. G. KINGSOLVER.** 2012. The demographic impacts of shifts in climate means and extremes on alpine butterflies. *Functional Ecology.* 26:969-977.

- BUCKLEY, L. B., AND J. ROUGHGARDEN.** 2005. Effect of species interactions on landscape abundance patterns. *Journal of Animal Ecology*:1182-1194.
- CAMPBELL, G. S., AND J. M. NORMAN.** 2012. An introduction to environmental biophysics. Springer Science & Business Media.
- CANNATELLA, D. C., AND R. O. DE SA.** 1993. *Xenopus laevis* as a model organism. *Systematic Biology*. 42:476-507.
- CAROTHERS, J., H., S. FOX, F., P. MARQUET, A., AND F. JAKSIC, M.** 1997. Thermal characteristics of ten Andean lizards of the genus *Liolaemus* in central Chile. *Revista Chilena de Historia Natural*. 70:297-309.
- CARRUTHERS, N.** 1943. Variations in wind velocity near the ground. *Quarterly Journal of the Royal Meteorological Society*. 69:289-301.
- CHATFIELD, C.** 2003. The analysis of time series: an introduction. Chapman and Hall/CRC.
- CHAZDON, R. L., AND R. W. PEARCY.** 1991. The importance of sunflecks for forest understory plants. *BioScience*. 41:760-766.
- CHRISTIAN, K. A., C. R. TRACY, AND C. R. TRACY.** 2006. Evaluating thermoregulation in reptiles: An appropriate null model. *American Naturalist*. 168:421-430.
- CLARK, A. M., P. E. MOLER, E. E. POSSARDT, A. H. SAVITZKY, W. S. BROWN, AND B. W. BOWEN.** 2003. Phylogeography of the Timber Rattlesnake (*Crotalus horridus*) Based on mtDNA Sequences. *Journal of Herpetology*. 37:145-154.
- CLARK, R. W.** 2006. Fixed Videography to Study Predation Behavior of an Ambush Foraging Snake, *Crotalus Horridus*. *Copeia*. 2006:181-187.
- CLARK, R. W., S. TANGCO, AND M. A. BARBOUR.** 2012. Field video recordings reveal factors influencing predatory strike success of free-ranging rattlesnakes (*Crotalus* spp.). *Animal Behaviour*. 84:183-190.
- CONANT, R., AND J. T. COLLINS.** 1998. A field guide to reptiles & amphibians: eastern and central North America. Houghton Mifflin Harcourt.
- CONGDON, J. D., A. E. DUNHAM, AND D. W. TINKLE.** 1982. Energy Budgets and Life Histories of Reptiles, p. 233-271. *In*: *Biology of the Reptiles*. Vol. 13. C. Gans and Pough (eds.). Academic Press, New York.
- CORE, T. R.** 2018. R: A language and environment for statistical computing, p. 2013, R Foundation for Statistical Computing, Vienna, Austria.
- COWLES, R. B., AND C. M. BOGERT.** 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* 83:261-296.

- CUSHMAN, J. H., J. H. LAWTON, AND B. F. MANLY.** 1993. Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia*. 95:30-37.
- DEWALLE, D. R., E. W. BOYER, AND A. R. BUDA.** 2016. Exploring lag times between monthly atmospheric deposition and stream chemistry in Appalachian forests using cross-correlation. *Atmospheric environment*. 146:206-214.
- DUNHAM, A. E., B. W. GRANT, AND K. L. OVERALL.** 1989. Interfaces between Biophysical and Physiological Ecology and the Population Ecology of Terrestrial Vertebrate Ectotherms. *Physiological Zoology*. 62:335-355.
- GARDNER-SANTANA, L. C., AND S. J. BEAUPRE.** 2009. Timber Rattlesnakes (*Crotalus horridus*) exhibit elevated and less variable body temperatures during pregnancy. *Copeia*. 2009:363-368.
- GATES, D. M.** 1980. *Biophysical ecology*. Springer-Verlag, Mineola, NY.
- GIENGER, C., AND D. D. BECK.** 2011. Northern Pacific Rattlesnakes (*Crotalus oreganus*) use thermal and structural cues to choose overwintering hibernacula. *Canadian Journal of Zoology*. 89:1084-1090.
- GIERING, K., I. LAMPRECHT, AND O. MINET.** 1996. Specific heat capacities of human and animal tissues. SPIE.
- GLOYD, H. K.** 1978. *The rattlesnakes, genera Sistrurus and Crotalus: a study in zoogeography and evolution*. Milwaukee: Society for the Study of Amphibians and Reptiles.
- GRANT, B. W.** 1990. Trade-offs in activity time and physiological performance for thermoregulating desert lizards, *Sceloporus merriami*. *Ecology*:2323-2333.
- GRANT, B. W., AND A. E. DUNHAM.** 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology*:167-176.
- GRAVES, B. M., AND D. DUVALL.** 1990. Spring emergence patterns of wandering garter snakes and prairie rattlesnakes in Wyoming. *Journal of Herpetology*:351-356.
- GRIGG, G. C., C. R. DRANE, AND G. P. COURTICE.** 1979. Time constants of heating and cooling in the eastern water dragon, *Physignathus lesueruii* and some generalizations about heating and cooling in reptiles. *Journal of Thermal Biology*. 4:95-103.
- GUNDERSON, A. R., AND M. LEAL.** 2015. Patterns of Thermal Constraint on Ectotherm Activity. *The American Naturalist*. 185:653-664.
- . 2016. A conceptual framework for understanding thermal constraints on ectotherm activity with implications for predicting responses to global change. *Ecology Letters*. 19:111-120.

- HARVEY, D. S., AND P. J. WEATHERHEAD.** 2006. Hibernation site selection by eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) near their northern range limit. *Journal of Herpetology*. 40:66-73.
- . 2010. Habitat selection as the mechanism for thermoregulation in a northern population of massasauga rattlesnakes (*Sistrurus catenatus*). *Ecoscience*. 17:411-419.
- HARVEY, D. S., AND P. J. WEATHERHEAD.** 2011. Thermal ecology of Massasauga Rattlesnakes (*Sistrurus catenatus*) near their northern range limit. *Canadian Journal of Zoology*. 89:60.
- HERTZ, P. E., R. B. HUEY, AND R. STEVENSON.** 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *American Naturalist*:796-818.
- HOEKSTRA, A. A.** 2015. The influence of habitat on body temperature regulation in the timber rattlesnake (*Crotalus horridus*), p. 47. Vol. 1597442. Middle Tennessee State University, Ann Arbor.
- HUEY, R. B.** 1974. Behavioral thermoregulation in lizards: importance of associated costs. *American Association for the Advancement of Science*.
- HUEY, R. B.** 1982. Temperature, Physiology, and the Ecology of Reptiles, p. 25-91. *In: Biology of the Reptilia*. Vol. 12. C. Gans and F. H. Pough (eds.). Academic Press, New York.
- . 1991. Physiological Consequences of Habitat Selection. *The American Naturalist*. 137:S91-S115.
- HUEY, R. B., C. R. PETERSON, S. J. ARNOLD, AND W. P. PORTER.** 1989. Hot Rocks and Not-So-Hot Rocks: Retreat-Site Selection by Garter Snakes and Its Thermal Consequences. *Ecology*. 70:931-944.
- HUEY, R. B., AND E. R. PIANKA.** 1977. Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. *Ecology*:1066-1075.
- HUEY, R. B., AND E. R. PIANKA.** 1981. Ecological consequences of foraging mode. *Ecology*. 62:991-999.
- HUEY, R. B., E. R. PIANKA, AND T. W. SCHOENER.** 1983. Lizard ecology: studies of a model organism. Harvard Univ Pr.
- HUEY, R. B., AND M. SLATKIN.** 1976. Cost and benefits of lizard thermoregulation. *Quarterly Review of Biology*:363-384.
- HUEY, R. B., AND R. STEVENSON.** 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist*. 19:357-366.
- HUTCHISON, B. A., AND D. R. MATT.** 1977. The distribution of solar radiation within a deciduous forest. *Ecological Monographs*. 47:185-207.

- KARSTENSEN, K. A.** 2010. Land-cover change in the Ozark Highlands, 1973-2000. US Geological Survey.
- KOSTEL-HUGHES, F., T. P. YOUNG, AND J. D. WEHR.** 2005. Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation to seed size. *The Journal of the Torrey Botanical Society*. 132:50-61.
- KREITH, F., R. M. MANGLIK, AND M. S. BOHN.** 2012. Principles of heat transfer. Cengage learning.
- LELIEVRE, H., M. LE HÉNANFF, G. BLOUIN-DEMERS, G. NAULLEAU, AND O. LOURDAIS.** 2010. Thermal strategies and energetics in two sympatric colubrid snakes with contrasted exposure. *Journal of Comparative Physiology B*. 180:415-425.
- LEVINE, B. A.** 2013. Genetic structure of the copperhead (*Viperidae: Agkistrodon contortrix mokasen*) at its most northern distribution, p. 78. Vol. 1535348. University of Arkansas, Ann Arbor.
- LI, L., M. LIANG, B. YU, AND S. YANG.** 2014. Analysis of thermal conductivity in living biological tissue with vascular network and convection. *International Journal of Thermal Sciences*. 86:219-226.
- LILLYWHITE, H. B.** 1980. Behavioral thermoregulation in Australian elapid snakes. *Copeia*:452-458.
- . 1987. Temperature, energetics, and physiological ecology. *Smith VII*.
- LIND, C. M., AND S. J. BEAUPRE.** 2015. Male Snakes Allocate Time and Energy according to Individual Energetic Status: Body Condition, Steroid Hormones, and Reproductive Behavior in Timber Rattlesnakes, *Crotalus horridus*. *Physiological and Biochemical Zoology*. 88:624-633.
- LUTTERSCHMIDT, W. I., AND H. K. REINERT.** 2012. Modeling body temperature and thermal inertia of large-bodied reptiles: Support for water-filled biophysical models in radiotelemetric studies. *Journal of Thermal Biology*. 37:282-285.
- MACARTNEY, J. M., AND P. T. GREGORY.** 1988. Reproductive Biology of Female Rattlesnakes (*Crotalus viridis*) in British Columbia. *Copeia*. 1988:47-57.
- MACARTNEY, J. M., K. W. LARSEN, AND P. T. GREGORY.** 1989. Body temperatures and movements of hibernating snakes (*Crotalus* and *Thamnophis*) and thermal gradients of natural hibernacula. *Canadian Journal of Zoology*. 67:108-114.
- MARTIN, W., AND T. TYNING.** 1992. The timber rattlesnake: its distribution and natural history. *Conservation of the Timber Rattlesnake in the Northeast*:13-22.

- MCNAB, B. K., AND W. AUFFENBERG.** 1976. The effect of large body size on the temperature regulation of the komodo dragon, *Varanus komodoensis*. *Comparative Biochemistry and Physiology Part A: Physiology*. 55:345-350.
- MEIRI, S.** 2011. Bergmann's Rule—what's in a name? *Global Ecology and Biogeography*. 20:203-207.
- MICHAEL, P.** 2019. A Conversion Guide: Solar Irradiance and Lux Illuminance., IEEE Dataport.
- MITCHELL, J. W.** 1976. Heat transfer from spheres and other animal forms. *Biophysical Journal*. 16:561-569.
- MUTH, A.** 1977. Thermoregulatory postures and orientation to the sun: a mechanistic evaluation for the zebra-tailed lizard, *Callisaurus draconoides*. *Copeia*:710-720.
- NAKAGAWA, S., AND H. SCHIELZETH.** 2013. A general and simple method for obtaining R<sup>2</sup> from generalized linear mixed-effects models. *Methods in ecology and evolution*. 4:133-142.
- PALADINO, F. V., M. P. O'CONNOR, AND J. R. SPOTILA.** 1990. Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature*. 344:858.
- PARKER, W. S., AND W. S. BROWN.** 1973. Species composition and population changes in two complexes of snake hibernacula in northern Utah. *Herpetologica*:319-326.
- PETERSON, C. R., A. R. GIBSON, AND M. E. DORCAS.** 1993. Snake thermal ecology: the causes and consequences of body-temperature variation, p. 241-314. *In: Snakes, ecology and behavior*. R. A. Seigel and J. T. Collins (eds.). McGraw-Hill, New York.
- PORTER, W. P., AND D. M. GATES.** 1969. Thermodynamic equilibria of animals with environment. *Ecological monographs*. 39:227-244.
- PORTER, W. P., J. W. MITCHELL, W. A. BECKMAN, AND C. B. DEWITT.** 1973. Behavioral implications of mechanistic ecology. *Oecologia*. 13:1-54.
- PORTER, W. P., AND C. TRACY.** 1983. Biophysical analyses of energetics, time-space utilization, and distributional limits. *Lizard ecology: Studies of a model organism*:55-83.
- POUGH, F. H., AND C. GANS.** 1982. Physiology C: Physiologocial Ecology, p. 17-23. *In: Biology of the Reptilia*. Vol. 12. C. Gans (ed.). Academic Press, New York, New York.
- PUTMAN, B. J., AND R. W. CLARK.** 2017. Behavioral thermal tolerances of free-ranging rattlesnakes (*Crotalus oreganus*) during the summer foraging season. *Journal of thermal biology*. 65:8-15.
- R DEVELOPMENT CORE TEAM.** 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.



- REINERT, H. K., AND D. CUNDALL.** 1982. An improved surgical implantation method for radio-tracking snakes. *Copeia*. 1982:702-705.
- ROTH, E. D.** 2003. 'Handedness' in snakes? Lateralization of coiling behaviour in a cottonmouth, *Agkistrodon piscivorus leucostoma*, population. *Animal Behaviour*. 66:337-341.
- SCHANTZ, K. A.** 2009. Characterization of landscape-scale habitat use by timber rattlesnakes (*Crotalus horridus*) within the ridge and valley and highlands regions of New Jersey, p. 195. Vol. 1470007. Rutgers The State University of New Jersey - New Brunswick, Ann Arbor.
- SEARS, M. W.** 2005. Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia*. 143:25-36.
- SEARS, M. W., M. J. ANGILLET, M. S. SCHULER, J. BORCHERT, K. F. DILLIPLANE, M. STEGMAN, T. W. RUSCH, AND W. A. MITCHELL.** 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proceedings of the National Academy of Sciences*.
- SEEBACHER, F., R. M. ELSEY, AND P. L. TROSCLAIR III.** 2003. Body temperature null distributions in reptiles with nonzero heat capacity: seasonal thermoregulation in the American alligator (*Alligator mississippiensis*). *Physiological and Biochemical Zoology*. 76:348-359.
- SEEBACHER, F., AND C. E. FRANKLIN.** 2005. Physiological mechanisms of thermoregulation in reptiles: a review. *Journal of Comparative Physiology B*. 175:533-541.
- SEEBACHER, F., G. C. GRIGG, AND L. BEARD.** 1999. Crocodiles as dinosaurs: behavioural thermoregulation in very large ectotherms leads to high and stable body temperatures. *Journal of Experimental Biology*. 202:77-86.
- SEEBACHER, F., AND R. SHINE.** 2004. Evaluating Thermoregulation in Reptiles: The Fallacy of the Inappropriately Applied Method. *Physiological and Biochemical Zoology*. 77:688-695.
- SHINE, R., P. S. HARLOW, M. J. ELPHICK, M. M. OLSSON, AND R. T. MASON.** 2000. Conflicts between courtship and thermoregulation: The thermal ecology of amorous male garter snakes (*Thamnophis sirtalis parietalis*, Colubridae). *Physiological and Biochemical Zoology*. 73:508-516.
- SHINE, R., AND T. MADSEN.** 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology*. 69:252-269.

- SLIP, D. J., AND R. SHINE.** 1988. Thermophilic response to feeding of the diamond python, *Morelia s. spilota* (Serpentes: Boidae). *Comparative Biochemistry and Physiology Part A: Physiology*. 89:645-650.
- SPOTILA, J. R., P. W. LOMMEN, G. S. BAKKEN, AND D. M. GATES.** 1973. A mathematical model for body temperatures of large reptiles: Implications for dinosaur ecology. *American Naturalist*:391-404.
- SPOTILA, J. R., O. H. SOULE, AND D. M. GATES.** 1972. The biophysical ecology of the alligator: heat energy budgets and climate spaces. *Ecology*:1094-1102.
- STEARNS, S. C., AND S. C. STEARNS.** 1992. *The Evolution of Life Histories*. OUP Oxford.
- STEVENSON, R.** 1985a. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*:102-117.
- STEVENSON, R. D.** 1985b. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*:102-117.
- . 1985c. The Relative Importance of Behavioral and Physiological Adjustments Controlling Body-Temperature in Terrestrial Ectotherms. *American Naturalist*. 126:362-386.
- STICKLEY, S. F., AND J. M. FRATERIGO.** 2021. Understory vegetation contributes to microclimatic buffering of near-surface temperatures in temperate deciduous forests. *Landscape Ecology*. 36:1197-1213.
- TATTERSALL, G. J., V. CADENA, AND M. C. SKINNER.** 2006. Respiratory cooling and thermoregulatory coupling in reptiles. *Respiratory physiology & neurobiology*. 154:302-318.
- TATTERSALL, G. J., W. K. MILSOM, A. S. ABE, S. P. BRITO, AND D. V. ANDRADE.** 2004. The thermogenesis of digestion in rattlesnakes. *Journal of Experimental Biology*. 207:579-585.
- TRACY, C. R.** 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. *Ecological Monographs*. 46:293-326.
- . 1982a. Biophysical modeling in reptilian physiology and ecology. *Biology of the Reptilia*. 12:275-321.
- TRACY, C. R.** 1982b. Biophysical modelling in reptilian physiology and ecology, p. 275-321. *In: Biology of the Reptilia*. Vol. 12. C. Gans, and Pough, F. H. (ed.). Academic Press, New York.
- TURNER, J., AND C. TRACY.** 1985. Body size and the control of heat exchange in alligators. *Journal of thermal biology*. 10:9-11.

- UMMENHOFER, C. C., AND G. A. MEEHL.** 2017. Extreme weather and climate events with ecological relevance: a review. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372:20160135.
- VICKERS, M., C. MANICOM, AND L. SCHWARZKOPF.** 2011. Extending the cost-benefit model of thermoregulation: high-temperature environments. *The American Naturalist*. 177:452-461.
- VOGEL, S.** 1996. *Life in moving fluids: the physical biology of flow*. Princeton University Press.
- WANG, T., A. S. ABE, AND M. L. GLASS.** 1998. Effects of temperature on lung and blood gases in the South American rattlesnake *Crotalus durissus terrificus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 121:7-11.
- WILLS, C. A., AND S. J. BEAUPRE.** 2000. An application of randomization for detecting evidence of thermoregulation in timber rattlesnakes (*Crotalus horridus*) from northwest Arkansas. *Physiological and Biochemical Zoology*. 73:325-334.
- WITHERS, P. C.** 1992. *Comparative animal physiology*. Saunders College Pub.
- ZAIDAN III, F., AND D. S. J. BEAUPRE.** 2003. Effects of Body Mass, Meal Size, Fast Length, and Temperature on Specific Dynamic Action in the Timber Rattlesnake (*Crotalus horridus*). *Physiological and Biochemical Zoology*. 76:447-458.
- ZAMORA-CAMACHO, F., S. REGUERA, AND G. MORENO-RUEDA.** 2014. Bergmann's Rule rules body size in an ectotherm: heat conservation in a lizard along a 2200-metre elevational gradient. *Journal of evolutionary biology*. 27:2820-2828.

## Appendix

### Tables and Figures

**Table 1.** Nighttime  $T_e$  summary statistics by position.

Model Position	Min. °C	Max. °C	Mean °C	SD	1 <sup>st</sup> Quartile	Median °C	3 <sup>rd</sup> Quartile	<i>n</i>
Top	-3.5	1.0	-1.3	1.2	-2.5	-1.5	-0.5	504
LC	-2.9	2.0	-0.2	1.2	-1.0	0.0	0.5	504
LH	0.5	4.1	2.5	0.9	2.0	2.5	3.1	504

**Table 2.** Comparison of our full mixed-effects model against a null model for the effects of OTM position on temperature.

Model Name	Nighttime Temperature Models Gaussian Mixed Models	
	Null Model	Full Model
Fixed effects	<i>b</i> [95% CI]	<i>b</i> [95% CI]
Intercept (Top)	0.32 [-0.41 – -1.05]	-1.30 [-2.04 – -0.57]
Model Position (LC)	NA	1.07 [1.00 – 1.15]
Model Position (LH)	NA	3.80 [3.73 – 3.87]
Random effects	VC	VC
Hour	0.39	0.41
Group	0.48	0.49
Residuals	2.93	0.35
$R^2_{GLMM(m)}$	0.000	0.673
$R^2_{GLMM(c)}$	0.229	0.908
AIC	5966.8	2781.5
BIC	5988.1	2813.5

CI, confidence interval; NA, not applicable/available; AIC, Akaike Information Criterion; BIC, Bayesian information criterion; VC, variance components. For full models, the intercept represents Top OTMs. AIC and BIC values were calculated using ML.

**Table 3.** Summary statistics for model position by hour for the subset of data used for the daytime analysis.

Model Position	Min. °C	Max. °C	Mean °C	SD	1 <sup>st</sup> Quartile	Median °C	3 <sup>rd</sup> Quartile	<i>n</i>
Top	23.0	55.0	36.2	8.2	29.1	35.6	42.1	504
LC	21.0	58.0	33.4	8.2	26.5	32.3	39.0	504
LH	15.6	42.5	25.9	5.4	22.0	25.0	29.0	504

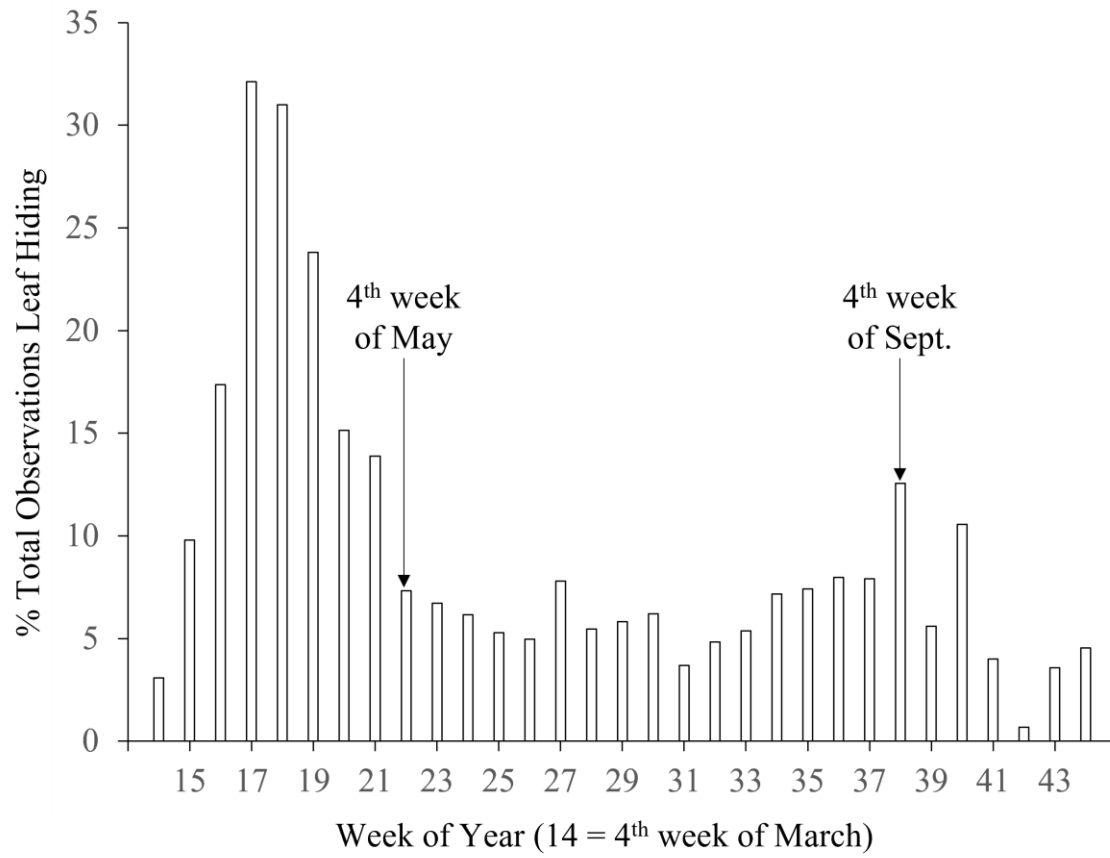
**Table 4.** Model comparison for the effects of OTM position on  $T_e$ .

Model Name	Daytime Temperature Models Gaussian Mixed Models	
	Null Model	Full Model
Fixed effects	<i>b</i> [95% <i>CI</i> ]	<i>b</i> [95% <i>CI</i> ]
Intercept (Top)	31.85 [ 27.25 – 36.44]	36.22 [ 31.61 – 40.84]
Model Position (LC)	NA	-2.82 [-3.35 – -2.28]
Model Position (LH)	NA	-10.32 [-10.85 – -9.78]
Random effects	VC	VC
Hour	29.57	29.66
Group	7.47	7.55
Residuals	37.95	18.84
$R^2_{GLMM(m)}$	0.000	0.253
$R^2_{GLMM(c)}$	0.494	0.749
AIC	9852.7	8806.7
BIC	9873.9	8838.6

CI, confidence interval; NA, not applicable/available; AIC, Akaike Information Criterion; BIC; Bayesian information criterion; VC, variance components. For full models, the intercept represents Top OTMs. AIC and BIC values were calculated using ML.



**Figure 1.** Transition habitat near a known *C. horridus* hibernaculum with a radio-telemetered snake (inset photo) demonstrating leaf hiding behavior (a.). Snakes in the leaf cratered (LC) position (b.) and in the Top position (c.).



**Figure 2.** Percentage of total observations from 19 years of radio-telemetry data (1995 – 2014) where LH behavior was observed in *C. horridus*.

a.

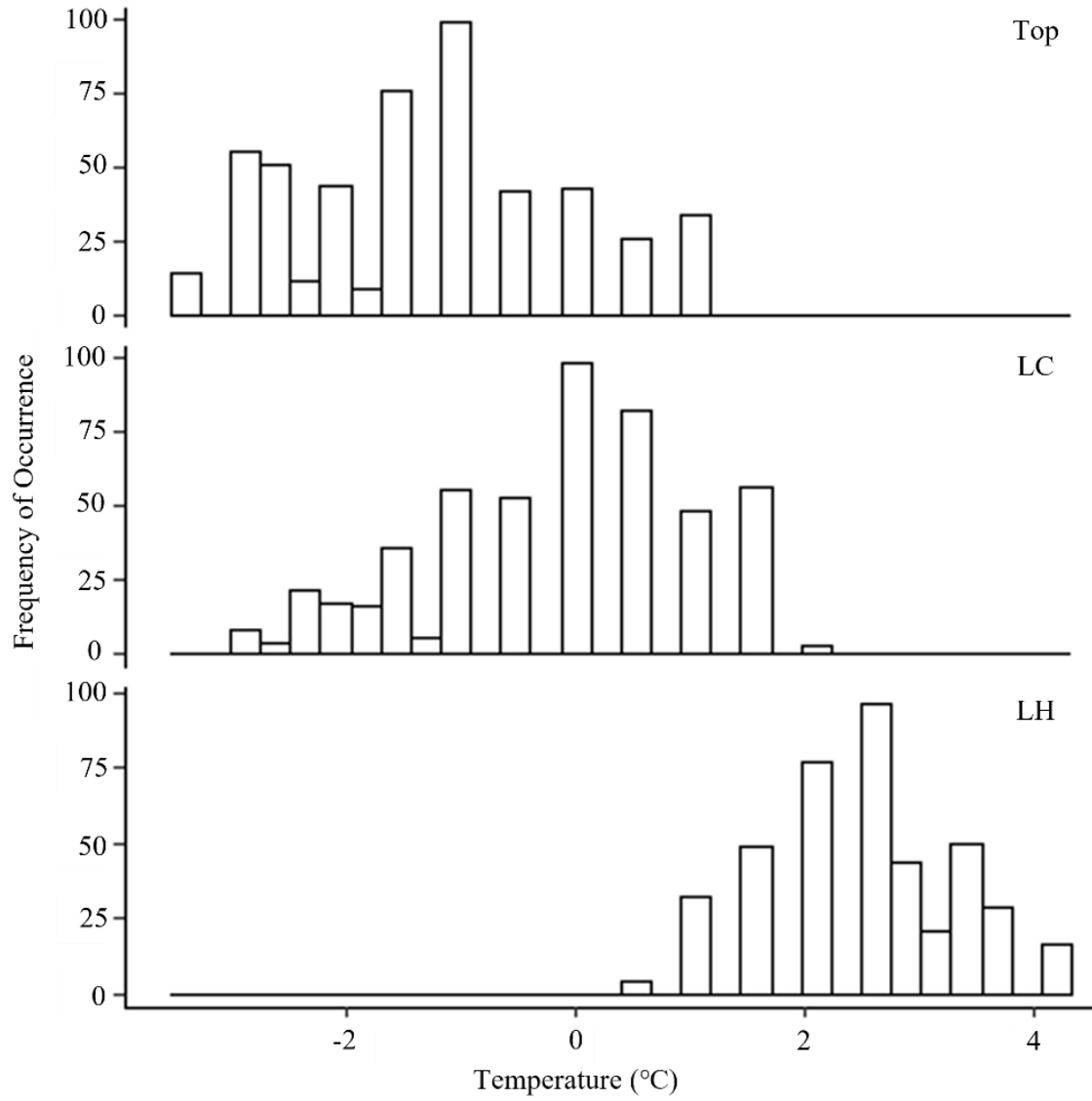


b.

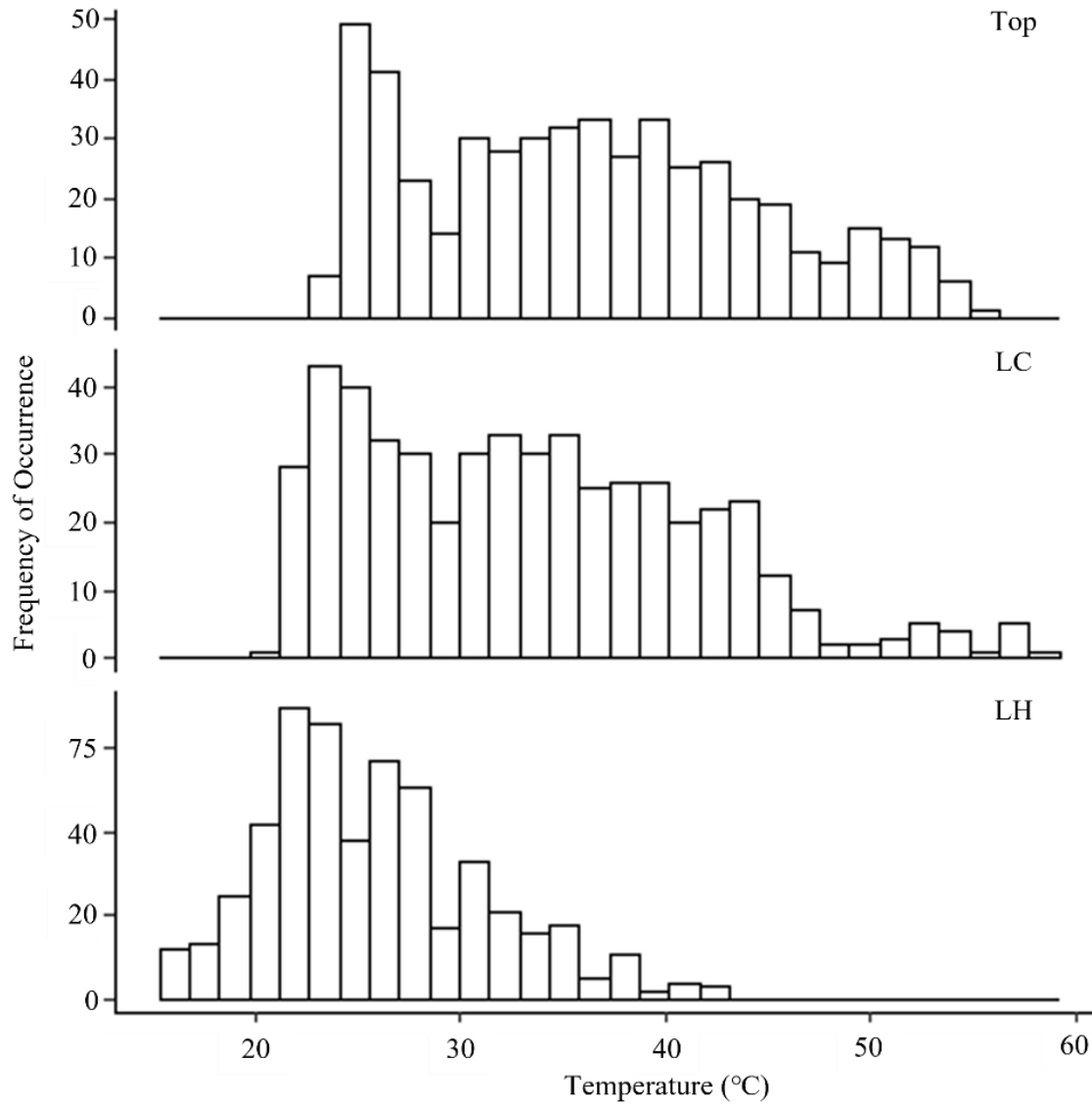


**Figure 3.** Photo of OTM model setup with ibutton<sup>®</sup> secured to a small crafting stick with model clay and positioned in the center of the OTM (a). Typical arrangement of OTMs (i.e., LH, LC, and TOP) situated in the leaf litter in transition habitat near a known hibernaculum (b). The pink flagging identifies the location of the LH model.





**Figure 2.** Nighttime frequency distribution of  $T_{es}$  illustrating the lower variability and higher mean temperature of LH models ( $\bar{x} = 2.5^{\circ}\text{C}$  [SD =  $0.9^{\circ}\text{C}$ ]) versus LC ( $\bar{x} = -0.2^{\circ}\text{C}$  [SD =  $1.2^{\circ}\text{C}$ ]) and Top models  $\bar{x} = -1.3^{\circ}\text{C}$  [SD =  $1.2^{\circ}\text{C}$ ].



**Figure 5.** Daytime frequency distribution of  $T_e$ s illustrating the lower variability and lower mean temperature of LH models ( $\bar{x} = 36.3^\circ\text{C}$  [SD =  $8.2^\circ\text{C}$ ]) versus LC ( $\bar{x} = 33.4^\circ\text{C}$  [SD =  $8.2^\circ\text{C}$ ]) and Top models  $\bar{x} = 36.3^\circ\text{C}$  [SD =  $8.2^\circ\text{C}$ ].

## Conclusion

The constraints imposed by the thermal environment can be an important source of life history variation (Dunham et al., 1989; Beaupre, 1995). Thermoregulating ectotherms are constrained to occupy appropriate thermal microclimates while satisfying other behavioral requirements (Dunham et al., 1989). A trade-off often exists between two activities where the time and energy allocated to one activity often reduces that allocated for another activity such as foraging (Dunham et al., 1989). As a result, environmental thermal constraints can be important in determining life-history phenotypes of terrestrial ectotherms.

In chapter 1, I quantified the conduction pathway of heat flow between *C. horridus* and its immediate environment through its surface (Gates, 1980). I compared the rate of conduction from dorsum to body core and body core to ground to determine if thermal conduction was sufficient to offset the increase in  $T_b$  due to thermal transients. Conduction from the body core to the ground was often higher and more stable compared to  $Q_{cond}$  from dorsum to core, except in rare instances when the dorsum was subjected to high heat loads. A significant relationship exists between the two conduction rates and, conduction from the dorsum to ground was a significant predictor of conduction from the core to the ground after approximately seven to 10 minutes. Solar radiation causes a change in conduction from dorsum to core after 15 min of radiation exposure. Conversely, no coupling between incoming radiation and core to ground conduction was observed. A correlation was not detected between lags of solar radiation exposure and the residuals of each conduction rate (i.e., dorsum to core and core to ground). The two conduction rates were often decoupled suggesting heat storage is a significant factor in maintaining relatively stable  $T_b$ s. Finally, the data indicate extreme thermal transients appear to be uncommon in this system.

The maximum  $T_b$  of  $33.9 \pm 3.3$  °C in our sample was approximately 3 °C cooler than the maximum observed in situ  $T_b$  of 37 °C (Wills and Beaupre, 2000). Heat is continuously being fluxed toward the cooler ground, helping maintain a relatively low body core temperature despite variable and sometimes intense solar irradiation.

Theoretically, the effect of mass on heating and cooling rates suggests heat storage acts as a stabilizing factor for  $T_b$  (Bell, 1980; Slip and Shine, 1988; Seebacher and Shine, 2004). We detected an effect of mass on individual and combined conduction rates ( $F(2, 288) = 57.785$ ,  $p < 0.0001$ ). Anecdotally, conduction from core to ground was often higher than from dorsum to core for snakes less than 1000 g. In snakes with higher masses, the conduction rates appear to reverse with conduction from dorsum to core often higher than conduction from core to ground. Furthermore, we detected significant, yet weak negative correlations between mass and each conduction rate. A negative correlation suggests more energy is required to raise the core temperature in larger snakes indicating a greater tolerance of skin temperature increases. Clark (2006) suggested Timber Rattlesnakes navigate the environment choosing and remaining at potential ambush sites based on the likelihood of encountering potential prey. Timber Rattlesnakes in our population appear to experience relatively few extreme  $T_e$ s that could limit the time available for other activities such as foraging. Our data appears to support the assertion that Timber Rattlesnakes can navigate their environment with minimal thermal constraints (Clark, 2006).

In Chapter 2, I attempted to capture in situ thermoregulatory behavior in *C. horridus* using fixed videography and assess the potential effects on time spent foraging. Twenty video observations capturing  $T_b$  and solar insolation data for 13 snakes over a five-year period were used to assess the effect of transient insolation on  $T_b$ . A voluntary thermal maximum (i.e.,  $VT_{max}$ )

of 34.6 °C was identified for this sample and was exceeded 26% of the total minutes observed (3,005 min). Thus, activity appears to only be thermally constrained between approximately 12:00 and 14:00 hr. Data suggest that  $T_b$  at the time a response occurred, the rate of change in  $T_b$ , and the mean maximum  $T_b$  are significant indicators of whether a snake made a thermal move.

Only the frequency of transient insolation was a significant factor in whether snakes made a thermal movement. The lack of a relationship between solar insolation duration or intensity and thermal movements may be a result of our small sample size ( $n = 20$  of 49 total video recordings). Of the 6,215 min of total observations, 1,941.5 min or 31% of the time resulted in exposure to transient insolation. Subsequently, of the 1,941.5 min of solar transient exposure, 756.5 min or 39 % resulted in a thermal movement. A total of 302 solar transients were recorded for all video observations with 218 associated with non-thermal movements and 84 associated with thermal movements. In a 12 h day (07:00 – 19:00 hr) there are 720 min of potential solar exposure. If we assumed 8% of a typical day involves solar exposure that leads to a thermal movement, approximately 58 min of the available 720 min of potential daytime foraging is lost to thermal movements. Our results suggest snakes are thermally limited during mid-day for less than two hours.

In Chapter 3, I evaluated the effect of position in the leaf litter (i.e., LH, LC, or Top) on *C. horridus* body temperature using OTMs. Timber Rattlesnakes were often observed in the LH position during spring and fall when changes in  $T_a$  over 24-hours can be unpredictable and extreme. In early Spring canopy cover is minimal because mid-and upper-story vegetation have not fully leafed-out (Hutchison and Matt, 1977). The increase in direct solar radiation on OTMs commonly produces temperatures exceeding 58 °C, well above the maximum voluntary  $T_b$  for *C. horridus* ( $\bar{x} = 34.6$  °C,  $SD = 1.3$  °C,  $n = 7$ ).

Data indicated position in the leaf litter had a significant effect on the temperature of *C. horridus*. Models in the LH position generally remained cooler during the day and warmer at night when comparing hourly averages. In general, LH OTM mean hourly temperatures were approximately 10.3 °C (0.3 SE) lower than models in the Top position during the day. During nights when Top  $T_e$ s were below freezing the leaf litter also buffered heat loss for LH models by approximately 3.8 °C (0.2 SE) when compared to Top OTM mean hourly night temperatures.

Reptiles in temperate climates are often behaviorally and physiologically constrained by the thermal environment (Porter et al., 1973; Grant and Dunham, 1988; Beaupre, 2002). Thermal constraints imposed upon the seasonal activity of *C. horridus* could have important biological implications. The annual energy acquired determines life history attributes such as age at sexual maturity, clutch size, and reproductive frequency (Macartney and Gregory, 1988). Extending the active season by even a few weeks could increase foraging opportunities and thus, energy allocated toward production once maintenance costs are met (Congdon et al., 1982).

Our data show that *C. horridus* can reduce the effects of extreme environmental temperatures during Spring emergence using adjustments in position within the leaf litter. Our findings suggest that thermoregulatory strategies may be subtle, requiring detailed knowledge of the mechanisms used to cope with extreme environmental temperature fluctuations. Furthermore, these same mechanisms may provide insight into an organism's ability to cope with the localized effects of climate change and associated impacts to life history.

In conclusion, *C. horridus* in our study population are rarely thermally stressed. During the Summer, the frequency, duration and intensity of solar transients appear to be relatively low in this system rarely initiating a thermoregulatory movement while the ground acts as a heat sink steadily conducting heat from the body core to the ground. During Spring emergence, *C.*

*horridus* take advantage of the insulative effects of the leaf litter to avoid freezing temperatures at night and high temperatures during the day due to increases in direct solar radiation due to less canopy cover.

The results of this study provide insight into the mechanistic coupling between a variable thermal environment and the observed life history traits of *C. horridus* in a temperate deciduous forest. The results of this study and associated behaviors of *C. horridus* highlight potential sources of selection on life history traits. The competing behaviors of thermoregulation and foraging mode in this population of *C. horridus* were assessed and appear to compliment more than contradict each other. The trade-off between thermoregulation and foraging that exists (Dunham et al., 1989; Buckley et al., 2015b) appears to be context specific. Our results suggest geographic variation of environmental tolerance within species (Buckley et al., 2015b) should be considered when considering the effects of climate change.

## References

- ABD LATIF, Z., AND G. A. BLACKBURN.** 2010. The effects of gap size on some microclimate variables during late summer and autumn in a temperate broadleaved deciduous forest. *International Journal of Biometeorology*. 54:119-129.
- ADOLPH, S. C., AND W. P. PORTER.** 1993. Temperature, Activity, and Lizard Life Histories. *The American Naturalist*. 142:273-295.
- AGUGLIARO, J.** 2011. Ecophysiology of hibernation in timber rattlesnakes ( *Crotalus horridus*), p. 277. Vol. Ph.D. University of Arkansas, United States -- Arkansas.
- ALFORD, J. G., AND W. I. LUTTERSCHMIDT.** 2012. Modeling energetic and theoretical costs of thermoregulatory strategy. *Journal of Biological Dynamics*. 6:63-79.
- . 2018. From conceptual to computational: Cost and benefits of lizard thermoregulation revisited. *Journal of Thermal Biology*. 78:174-183.
- ARELLANO, C., AND S. G. PANTULA.** 1995. Testing for trend stationarity versus difference stationarity. *Journal of Time Series Analysis*. 16:147-164.
- AYERS, D., AND R. SHINE.** 1997. Thermal influences on foraging ability: body size, posture and cooling rate of an ambush predator, the python *Morelia spilota*. *Functional Ecology*. 11:342-347.
- BAKDASH, J. Z., AND L. R. MARUSICH.** 2017. Repeated Measures Correlation. *Frontiers in Psychology*. 8.
- BAKKEN, G. S.** 1976. A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data to field ecology. *Journal of Theoretical Biology*. 60:337-384.
- . 1992. Measurement and application of operative and standard operative temperatures in ecology. *American Zoologist*. 32:194-216.
- BAKKEN, G. S., AND D. M. GATES.** 1975. Heat-transfer analysis of animals: some implications for field ecology, physiology, and evolution, p. 255-290. *In: Perspectives of biophysical ecology*. Springer.
- BAKKEN, G. S., W. R. SANTEE, AND D. J. ERSKINE.** 1985. Operative and Standard Operative Temperature: Tools for Thermal Energetics Studies. *American Zoologist*. 25:933-943.
- BARTHOLOMEW, G. A., AND V. A. TUCKER.** 1964. Size, body temperature, thermal conductance, oxygen consumption, and heart rate in Australian varanid lizards. *Physiological zoology*. 37:341-354.



- BASSON, C. H., O. LEVY, M. J. ANGILLET, AND S. CLUSELLA-TRULLAS.** 2016. Lizards paid a greater opportunity cost to thermoregulate in a less heterogeneous environment. *Functional Ecology*:n/a-n/a.
- BEATTY, S. W., AND O. D. SHOLES.** 1988. Leaf litter effect on plant species composition of deciduous forest treefall pits. *Canadian Journal of Forest Research*. 18:553-559.
- BEAUPRE, D. S. J., CHAD E. MONTGOMERY.** 2007. The meaning and consequences of foraging mode in snakes. Cambridge University Press.
- BEAUPRE, D. S. J., AND D. J. DUVAL.** 1998. Integrative Biology of the Rattlesnakes: Contributions to biology and evolution. *Bioscience*. 48:531-538.
- BEAUPRE, S. J.** 1995. Effects of Geographically Variable Thermal Environment on Bioenergetics of Mottled Rock Rattlesnakes. *Ecology*. 76:1655-1665.
- BEAUPRE, S. J.** 2002. Modeling time-energy allocation in vipers: individual responses to environmental variation and implications for populations. *Biology of the vipers*:463-481.
- BEAUPRE, S. J.** 2008. Annual variation in time-energy allocation by timber rattlesnakes (*Crotalus horridus*) in relation to food acquisition, p. 111-122. *In: The biology of rattlesnakes*. W. K. Hayes, M. D. Cardwell, K. R. Beaman, and S. P. Bush (eds.). Loma Linda University Press, Loma Linda, California.
- BEAUPRE, S. J., J. AGUGLIARO, J. U. VAN DYKE, AND F. ZAIDAN III.** 2017. Annual Energy Budgets of the Timber Rattlesnake: Advancements, Refinements, and Open Questions, p. 97-108. *In: The Biology of the Rattlesnakes II*. M. J. Dreslik, W. K. Hayes, S. J. Beaupre, and S. P. Mackessy (eds.). ECO Herpetological Publishing and Distribution, Rodeo, New Mexico.
- BEAUPRE, S. J., AND L. E. DOUGLAS.** 2009. Snakes as Indicators and Monitors of Ecosystem Properties.
- BEAUPRE, S. J., AND L. E. DOUGLAS.** 2012. Responses of timber rattlesnakes to fire: lessons from two prescribed burns.
- BEAUPRE, S. J., AND F. ZAIDAN.** 2012. Digestive Performance in the Timber Rattlesnake (*Crotalus horridus*) with Reference to Temperature Dependence and Bioenergetic Cost of Growth. *Journal of Herpetology*. 46:637-642.
- BELL, C. J.** 1980. The Scaling of the Thermal Inertia of Lizards. *Journal of Experimental Biology*. 86:79-85.
- BIRKEBAK, R. C., R. C. BIRKEBAK, AND D. W. WARNER.** 1964. A note on total emittance of animal integuments. *Journal of heat transfer*. 86:287-288.

- BLAND, J. M., AND D. G. ALTMAN.** 1995a. Calculating correlation coefficients with repeated observations: Part 2—Correlation between subjects. *Bmj.* 310:633.
- . 1995b. Statistics notes: Calculating correlation coefficients with repeated observations: Part 1—correlation within subjects. *Bmj.* 310:446.
- BLOUIN-DEMERS, G., K. A. PRIOR, AND P. J. WEATHERHEAD.** 2000a. Patterns of variation in spring emergence by black rat snakes (*Elaphe obsoleta obsoleta*). *Herpetologica*:175-188.
- BLOUIN-DEMERS, G., K. A. PRIOR, AND P. J. WEATHERHEAD.** 2000b. Patterns of Variation in Spring Emergence by Black Rat Snakes (*Elaphe obsoleta obsoleta*). *Herpetologica*. 56:175-188.
- BOGERT, C. M.** 1949. Thermoregulation in Reptiles, A Factor in Evolution. *Evolution.* 3:195-211.
- BORRELL, B. J., T. J. LADUC, AND R. DUDLEY.** 2005. Respiratory cooling in rattlesnakes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology.* 140:471-476.
- BREWSTER, C. L., AND S. J. BEAUPRE.** 2019a. The effect of body posture on available habitat and activity-time in a lizard: Implications for thermal ecology studies. *Journal of Thermal Biology.*
- . 2019b. The effect of body posture on available habitat and activity-time in a lizard: implications for thermal ecology studies. *Journal of thermal biology.* 82:10-17.
- BROWNING, D. M., S. J. BEAUPRE, AND L. DUNCAN.** 2005. Using partitioned Mahalanobis D-2(K) to formulate a GIS-based model of timber rattlesnake hibernacula. *Journal of Wildlife Management.* 69:33-44.
- BRUCKERHOFF, L. A., L. K. KAMEES, A. T. HOLYCROSS, AND C. W. PAINTER.** 2021. Patterns of Survival of a Communally Overwintering Rattlesnake Using an Artificial Hibernaculum. *Ichthyology & Herpetology.* 109:64-74.
- BUCKLEY, L. B., J. C. EHRENBERGER, AND M. J. ANGILLETTA JR.** 2015a. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology.* 29:1038-1047.
- BUCKLEY, L. B., J. C. EHRENBERGER, AND M. J. ANGILLETTA.** 2015b. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Functional Ecology.* 29:1038-1047.
- BUCKLEY, L. B., AND J. G. KINGSOLVER.** 2012. The demographic impacts of shifts in climate means and extremes on alpine butterflies. *Functional Ecology.* 26:969-977.

- BUCKLEY, L. B., AND J. ROUGHGARDEN.** 2005. Effect of species interactions on landscape abundance patterns. *Journal of Animal Ecology*:1182-1194.
- CAMPBELL, G. S., AND J. M. NORMAN.** 2012. An introduction to environmental biophysics. Springer Science & Business Media.
- CANNATELLA, D. C., AND R. O. DE SA.** 1993. *Xenopus laevis* as a model organism. *Systematic Biology*. 42:476-507.
- CAROTHERS, J., H., S. FOX, F., P. MARQUET, A., AND F. JAKSIC, M.** 1997. Thermal characteristics of ten Andean lizards of the genus *Liolaemus* in central Chile. *Revista Chilena de Historia Natural*. 70:297-309.
- CARRUTHERS, N.** 1943. Variations in wind velocity near the ground. *Quarterly Journal of the Royal Meteorological Society*. 69:289-301.
- CHATFIELD, C.** 2003. The analysis of time series: an introduction. Chapman and Hall/CRC.
- CHAZDON, R. L., AND R. W. PEARCY.** 1991. The importance of sunflecks for forest understory plants. *BioScience*. 41:760-766.
- CHRISTIAN, K. A., C. R. TRACY, AND C. R. TRACY.** 2006. Evaluating thermoregulation in reptiles: An appropriate null model. *American Naturalist*. 168:421-430.
- CLARK, A. M., P. E. MOLER, E. E. POSSARDT, A. H. SAVITZKY, W. S. BROWN, AND B. W. BOWEN.** 2003. Phylogeography of the Timber Rattlesnake (*Crotalus horridus*) Based on mtDNA Sequences. *Journal of Herpetology*. 37:145-154.
- CLARK, R. W.** 2006. Fixed Videography to Study Predation Behavior of an Ambush Foraging Snake, *Crotalus Horridus*. *Copeia*. 2006:181-187.
- CLARK, R. W., S. TANGCO, AND M. A. BARBOUR.** 2012. Field video recordings reveal factors influencing predatory strike success of free-ranging rattlesnakes (*Crotalus* spp.). *Animal Behaviour*. 84:183-190.
- CONANT, R., AND J. T. COLLINS.** 1998. A field guide to reptiles & amphibians: eastern and central North America. Houghton Mifflin Harcourt.
- CONGDON, J. D., A. E. DUNHAM, AND D. W. TINKLE.** 1982. Energy Budgets and Life Histories of Reptiles, p. 233-271. *In: Biology of the Reptiles*. Vol. 13. C. Gans and Pough (eds.). Academic Press, New York.
- CORE, T. R.** 2018. R: A language and environment for statistical computing, p. 2013, R Foundation for Statistical Computing, Vienna, Austria.
- COWLES, R. B., AND C. M. BOGERT.** 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* 83:261-296.

- CUSHMAN, J. H., J. H. LAWTON, AND B. F. MANLY.** 1993. Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia*. 95:30-37.
- DEWALLE, D. R., E. W. BOYER, AND A. R. BUDA.** 2016. Exploring lag times between monthly atmospheric deposition and stream chemistry in Appalachian forests using cross-correlation. *Atmospheric environment*. 146:206-214.
- DUNHAM, A. E., B. W. GRANT, AND K. L. OVERALL.** 1989. Interfaces between Biophysical and Physiological Ecology and the Population Ecology of Terrestrial Vertebrate Ectotherms. *Physiological Zoology*. 62:335-355.
- GARDNER-SANTANA, L. C., AND S. J. BEAUPRE.** 2009. Timber Rattlesnakes (*Crotalus horridus*) exhibit elevated and less variable body temperatures during pregnancy. *Copeia*. 2009:363-368.
- GATES, D. M.** 1980. *Biophysical ecology*. Springer-Verlag, Mineola, NY.
- GIENGER, C., AND D. D. BECK.** 2011. Northern Pacific Rattlesnakes (*Crotalus oreganus*) use thermal and structural cues to choose overwintering hibernacula. *Canadian journal of zoology*. 89:1084-1090.
- GIERING, K., I. LAMPRECHT, AND O. MINET.** 1996. Specific heat capacities of human and animal tissues. SPIE.
- GLOYD, H. K.** 1978. The rattlesnakes, genera *Sistrurus* and *Crotalus*: a study in zoogeography and evolution. Milwaukee: Society for the Study of Amphibians and Reptiles.
- GRANT, B. W.** 1990. Trade-offs in activity time and physiological performance for thermoregulating desert lizards, *Sceloporus merriami*. *Ecology*:2323-2333.
- GRANT, B. W., AND A. E. DUNHAM.** 1988. Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami*. *Ecology*:167-176.
- GRAVES, B. M., AND D. DUVALL.** 1990. Spring emergence patterns of wandering garter snakes and prairie rattlesnakes in Wyoming. *Journal of Herpetology*:351-356.
- GRIGG, G. C., C. R. DRANE, AND G. P. COURTICE.** 1979. Time constants of heating and cooling in the eastern water dragon, *Physignathus lesueruii* and some generalizations about heating and cooling in reptiles. *Journal of Thermal Biology*. 4:95-103.
- GUNDERSON, A. R., AND M. LEAL.** 2015. Patterns of Thermal Constraint on Ectotherm Activity. *The American Naturalist*. 185:653-664.
- . 2016. A conceptual framework for understanding thermal constraints on ectotherm activity with implications for predicting responses to global change. *Ecology Letters*. 19:111-120.

- HARVEY, D. S., AND P. J. WEATHERHEAD.** 2006. Hibernation site selection by eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) near their northern range limit. *Journal of Herpetology*. 40:66-73.
- HERTZ, P. E., R. B. HUEY, AND R. STEVENSON.** 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *American Naturalist*:796-818.
- HUEY, R. B.** 1974. Behavioral thermoregulation in lizards: importance of associated costs. *American Association for the Advancement of Science*.
- HUEY, R. B.** 1982. Temperature, Physiology, and the Ecology of Reptiles, p. 25-91. *In: Biology of the Reptilia*. Vol. 12. C. Gans and F. H. Pough (eds.). Academic Press, New York.
- . 1991. Physiological Consequences of Habitat Selection. *The American Naturalist*. 137:S91-S115.
- HUEY, R. B., C. R. PETERSON, S. J. ARNOLD, AND W. P. PORTER.** 1989. Hot Rocks and Not-So-Hot Rocks: Retreat-Site Selection by Garter Snakes and Its Thermal Consequences. *Ecology*. 70:931-944.
- HUEY, R. B., AND E. R. PIANKA.** 1977. Seasonal variation in thermoregulatory behavior and body temperature of diurnal Kalahari lizards. *Ecology*:1066-1075.
- HUEY, R. B., AND E. R. PIANKA.** 1981. Ecological consequences of foraging mode. *Ecology*. 62:991-999.
- HUEY, R. B., E. R. PIANKA, AND T. W. SCHOENER.** 1983. Lizard ecology: studies of a model organism. Harvard Univ Pr.
- HUEY, R. B., AND M. SLATKIN.** 1976. Cost and benefits of lizard thermoregulation. *Quarterly Review of Biology*:363-384.
- HUEY, R. B., AND R. STEVENSON.** 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. *American Zoologist*. 19:357-366.
- HUTCHISON, B. A., AND D. R. MATT.** 1977. The distribution of solar radiation within a deciduous forest. *Ecological Monographs*. 47:185-207.
- KARSTENSEN, K. A.** 2010. Land-cover change in the Ozark Highlands, 1973-2000. US Geological Survey.
- KOSTEL-HUGHES, F., T. P. YOUNG, AND J. D. WEHR.** 2005. Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation to seed size. *The Journal of the Torrey Botanical Society*. 132:50-61.
- KREITH, F., R. M. MANGLIK, AND M. S. BOHN.** 2012. Principles of heat transfer. Cengage learning.

- LELIEVRE, H., M. LE HÉNANFF, G. BLOUIN-DEMERS, G. NAULLEAU, AND O. LOURDAIS.** 2010. Thermal strategies and energetics in two sympatric colubrid snakes with contrasted exposure. *Journal of Comparative Physiology B*. 180:415-425.
- LEVINE, B. A.** 2013. Genetic structure of the copperhead (*Viperidae: Agkistrodon contortrix mokasen*) at its most northern distribution, p. 78. Vol. 1535348. University of Arkansas, Ann Arbor.
- LI, L., M. LIANG, B. YU, AND S. YANG.** 2014. Analysis of thermal conductivity in living biological tissue with vascular network and convection. *International Journal of Thermal Sciences*. 86:219-226.
- LILLYWHITE, H. B.** 1980. Behavioral thermoregulation in Australian elapid snakes. *Copeia*:452-458.
- . 1987. Temperature, energetics, and physiological ecology. *Smith VII*.
- LIND, C. M., AND S. J. BEAUPRE.** 2015. Male Snakes Allocate Time and Energy according to Individual Energetic Status: Body Condition, Steroid Hormones, and Reproductive Behavior in Timber Rattlesnakes, *Crotalus horridus*. *Physiological and Biochemical Zoology*. 88:624-633.
- LUTTERSCHMIDT, W. I., AND H. K. REINERT.** 2012. Modeling body temperature and thermal inertia of large-bodied reptiles: Support for water-filled biophysical models in radiotelemetric studies. *Journal of Thermal Biology*. 37:282-285.
- MACARTNEY, J. M., AND P. T. GREGORY.** 1988. Reproductive Biology of Female Rattlesnakes (*Crotalus viridis*) in British Columbia. *Copeia*. 1988:47-57.
- MACARTNEY, J. M., K. W. LARSEN, AND P. T. GREGORY.** 1989. Body temperatures and movements of hibernating snakes (*Crotalus* and *Thamnophis*) and thermal gradients of natural hibernacula. *Canadian Journal of Zoology*. 67:108-114.
- MARTIN, W., AND T. TYNING.** 1992. The timber rattlesnake: its distribution and natural history. *Conservation of the Timber Rattlesnake in the Northeast*:13-22.
- MCNAB, B. K., AND W. AUFFENBERG.** 1976. The effect of large body size on the temperature regulation of the komodo dragon, *Varanus komodoensis*. *Comparative Biochemistry and Physiology Part A: Physiology*. 55:345-350.
- MEIRI, S.** 2011. Bergmann's Rule—what's in a name? *Global Ecology and Biogeography*. 20:203-207.
- MICHAEL, P.** 2019. A Conversion Guide: Solar Irradiance and Lux Illuminance., IEEE Dataport.
- MITCHELL, J. W.** 1976. Heat transfer from spheres and other animal forms. *Biophysical Journal*. 16:561-569.

- MUTH, A.** 1977. Thermoregulatory postures and orientation to the sun: a mechanistic evaluation for the zebra-tailed lizard, *Callisaurus draconoides*. *Copeia*:710-720.
- NAKAGAWA, S., AND H. SCHIELZETH.** 2013. A general and simple method for obtaining R<sup>2</sup> from generalized linear mixed-effects models. *Methods in ecology and evolution*. 4:133-142.
- PALADINO, F. V., M. P. O'CONNOR, AND J. R. SPOTILA.** 1990. Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature*. 344:858.
- PARKER, W. S., AND W. S. BROWN.** 1973. Species composition and population changes in two complexes of snake hibernacula in northern Utah. *Herpetologica*:319-326.
- PETERSON, C. R., A. R. GIBSON, AND M. E. DORCAS.** 1993. Snake thermal ecology: the causes and consequences of body-temperature variation, p. 241-314. *In*: Snakes, ecology and behavior. R. A. Seigel and J. T. Collins (eds.). McGraw-Hill, New York.
- PORTER, W. P., AND D. M. GATES.** 1969. Thermodynamic equilibria of animals with environment. *Ecological monographs*. 39:227-244.
- PORTER, W. P., J. W. MITCHELL, W. A. BECKMAN, AND C. B. DEWITT.** 1973. Behavioral implications of mechanistic ecology. *Oecologia*. 13:1-54.
- PORTER, W. P., AND C. TRACY.** 1983. Biophysical analyses of energetics, time-space utilization, and distributional limits. *Lizard ecology: Studies of a model organism*:55-83.
- POUGH, F. H., AND C. GANS.** 1982. Physiology C: Physiologocial Ecology, p. 17-23. *In*: Biology of the Reptilia. Vol. 12. C. Gans (ed.). Academic Press, New York, New York.
- PUTMAN, B. J., AND R. W. CLARK.** 2017. Behavioral thermal tolerances of free-ranging rattlesnakes (*Crotalus oreganus*) during the summer foraging season. *Journal of thermal biology*. 65:8-15.
- R DEVELOPMENT CORE TEAM.** 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- REINERT, H. K., AND D. CUNDALL.** 1982. An improved surgical implantation method for radio-tracking snakes. *Copeia*. 1982:702-705.
- ROTH, E. D.** 2003. 'Handedness' in snakes? Lateralization of coiling behaviour in a cottonmouth, *Agkistrodon piscivorus leucostoma*, population. *Animal Behaviour*. 66:337-341.
- SCHANTZ, K. A.** 2009. Characterization of landscape-scale habitat use by timber rattlesnakes (*Crotalus horridus*) within the ridge and valley and highlands regions of New Jersey, p. 195. Vol. 1470007. Rutgers The State University of New Jersey - New Brunswick, Ann Arbor.

- SEARS, M. W.** 2005. Geographic variation in the life history of the sagebrush lizard: the role of thermal constraints on activity. *Oecologia*. 143:25-36.
- SEARS, M. W., M. J. ANGILLET, M. S. SCHULER, J. BORCHERT, K. F. DILLIPLANE, M. STEGMAN, T. W. RUSCH, AND W. A. MITCHELL.** 2016. Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proceedings of the National Academy of Sciences*.
- SEEBACHER, F., R. M. ELSEY, AND P. L. TROSCLAIR III.** 2003. Body temperature null distributions in reptiles with nonzero heat capacity: seasonal thermoregulation in the American alligator (*Alligator mississippiensis*). *Physiological and Biochemical Zoology*. 76:348-359.
- SEEBACHER, F., AND C. E. FRANKLIN.** 2005. Physiological mechanisms of thermoregulation in reptiles: a review. *Journal of Comparative Physiology B*. 175:533-541.
- SEEBACHER, F., G. C. GRIGG, AND L. BEARD.** 1999. Crocodiles as dinosaurs: behavioural thermoregulation in very large ectotherms leads to high and stable body temperatures. *Journal of Experimental Biology*. 202:77-86.
- SEEBACHER, F., AND R. SHINE.** 2004. Evaluating Thermoregulation in Reptiles: The Fallacy of the Inappropriately Applied Method. *Physiological and Biochemical Zoology*. 77:688-695.
- SHINE, R., P. S. HARLOW, M. J. ELPHICK, M. M. OLSSON, AND R. T. MASON.** 2000. Conflicts between courtship and thermoregulation: The thermal ecology of amorous male garter snakes (*Thamnophis sirtalis parietalis*, Colubridae). *Physiological and Biochemical Zoology*. 73:508-516.
- SHINE, R., AND T. MADSEN.** 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiological Zoology*. 69:252-269.
- SLIP, D. J., AND R. SHINE.** 1988. Thermophilic response to feeding of the diamond python, *Morelia s. spilota* (Serpentes: Boidae). *Comparative Biochemistry and Physiology Part A: Physiology*. 89:645-650.
- SPOTILA, J. R., P. W. LOMMEN, G. S. BAKKEN, AND D. M. GATES.** 1973. A mathematical model for body temperatures of large reptiles: Implications for dinosaur ecology. *American Naturalist*:391-404.
- SPOTILA, J. R., O. H. SOULE, AND D. M. GATES.** 1972. The biophysical ecology of the alligator: heat energy budgets and climate spaces. *Ecology*:1094-1102.
- STEARNS, S. C., AND S. C. STEARNS.** 1992. *The Evolution of Life Histories*. OUP Oxford.



- STEVENSON, R.** 1985a. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*:102-117.
- STEVENSON, R. D.** 1985b. Body size and limits to the daily range of body temperature in terrestrial ectotherms. *American Naturalist*:102-117.
- . 1985c. The Relative Importance of Behavioral and Physiological Adjustments Controlling Body-Temperature in Terrestrial Ectotherms. *American Naturalist*. 126:362-386.
- STICKLEY, S. F., AND J. M. FRATERRIGO.** 2021. Understory vegetation contributes to microclimatic buffering of near-surface temperatures in temperate deciduous forests. *Landscape Ecology*. 36:1197-1213.
- TATTERSALL, G. J., V. CADENA, AND M. C. SKINNER.** 2006. Respiratory cooling and thermoregulatory coupling in reptiles. *Respiratory physiology & neurobiology*. 154:302-318.
- TATTERSALL, G. J., W. K. MILSOM, A. S. ABE, S. P. BRITO, AND D. V. ANDRADE.** 2004. The thermogenesis of digestion in rattlesnakes. *Journal of Experimental Biology*. 207:579-585.
- TRACY, C. R.** 1976. A model of the dynamic exchanges of water and energy between a terrestrial amphibian and its environment. *Ecological Monographs*. 46:293-326.
- . 1982a. Biophysical modeling in reptilian physiology and ecology. *Biology of the Reptilia*. 12:275-321.
- TRACY, C. R.** 1982b. Biophysical modelling in reptilian physiology and ecology, p. 275-321. *In: Biology of the Reptilia*. Vol. 12. C. Gans, and Pough, F. H. (ed.). Academic Press, New York.
- TURNER, J., AND C. TRACY.** 1985. Body size and the control of heat exchange in alligators. *Journal of thermal biology*. 10:9-11.
- UMMENHOFER, C. C., AND G. A. MEEHL.** 2017. Extreme weather and climate events with ecological relevance: a review. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 372:20160135.
- VICKERS, M., C. MANICOM, AND L. SCHWARZKOPF.** 2011. Extending the cost-benefit model of thermoregulation: high-temperature environments. *The American Naturalist*. 177:452-461.
- VOGEL, S.** 1996. *Life in moving fluids: the physical biology of flow*. Princeton University Press.
- WANG, T., A. S. ABE, AND M. L. GLASS.** 1998. Effects of temperature on lung and blood gases in the South American rattlesnake *Crotalus durissus terrificus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 121:7-11.

- WILLS, C. A., AND S. J. BEAUPRE.** 2000. An application of randomization for detecting evidence of thermoregulation in timber rattlesnakes (*Crotalus horridus*) from northwest Arkansas. *Physiological and Biochemical Zoology*. 73:325-334.
- WITHERS, P. C.** 1992. *Comparative animal physiology*. Saunders College Pub.
- ZAIDAN III, F., AND D. S. J. BEAUPRE.** 2003. Effects of Body Mass, Meal Size, Fast Length, and Temperature on Specific Dynamic Action in the Timber Rattlesnake (*Crotalus horridus*). *Physiological and Biochemical Zoology*. 76:447-458.
- ZAMORA-CAMACHO, F., S. REGUERA, AND G. MORENO-RUEDA.** 2014. Bergmann's Rule rules body size in an ectotherm: heat conservation in a lizard along a 2200-metre elevational gradient. *Journal of evolutionary biology*. 27:2820-2828.

## Appendix

### IACUC Approval Letter



UNIVERSITY OF  
ARKANSAS

*Office of Research Compliance*

To: Steve Beaupre  
From: Jeff Wolchok  
Date: December 3, 2019  
Subject: IACUC Approval  
Expiration Date: December 2, 2022

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol #17038, *Behavioral, Physiological and thermoregulatory monitoring of the top predators in the Ozark Ecosystem*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond November 12, 2021 you may submit a modification to extend the project up to three years, or submit a new protocol. The IACUC may not approve a study for more than three years at a time.

The following individuals are approved to work on this study: Steve Beaupre, Larry Kamees, Bannon Gallaher, and Max Carnes-Mason, Jason Ortega and Caylie Funk. Please submit personnel additions to this protocol via the modification form prior to their starting work.

The IACUC appreciates your cooperation in complying with University and federal guidelines involving the care and use of animals.

JCW/jgr

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