THE INFLUENCE OF HABITAT ON BODY TEMPERATURE REGULATION IN
THE TIMBER RATTLESNAKE (*CROTALUS HORRIDUS*)

by

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A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of
Master of Science in Biology

Middle Tennessee State University
August 2015

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ACKNOWLEDGMENTS

ABSTRACT

Ectothermic vertebrates are dependent on the availability of environmental temperatures and their ability to behaviorally thermoregulate to maintain a suitable range of body temperatures. In heterogeneous landscapes, such organisms often will encounter a variety of habitat types which impose different environmental conditions for thermoregulation. This study examines the thermal qualities of different habitats within a heterogeneous landscape in Middle Tennessee and infers how this variation may influence body temperature regulation in a population of *Crotalus horridus*. Comparison of four habitats (field, cedar glade, hardwood forest, redcedar forest) indicated considerable variation in environmental temperatures. Nevertheless, *C. horridus* maintained similar body temperatures in all habitats despite the limited availability of suitable temperatures in the field and cedar glade. An index that assesses the effectiveness of thermoregulation was applied to each habitat and indicated high thermal quality in the hardwood forest and redcedar forest compared to the field and cedar glade. These findings imply that *C. horridus* would need to actively thermoregulate more in the field and cedar glade but could rely on thermoconforming behavior in the hardwood forest and redcedar forest. This study illustrates that different habitats may significantly influence the amount of effort a snake such as *C. horridus* may have to expend in order to thermoregulate and maintain appropriate body temperatures.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Environmental temperatures are a primary determinate for body temperature of vertebrate ectotherms (Hertz et al, 1993; Angilletta, 2009). Yet, thermoregulatory behavior allows these organisms to modify their body temperature so that they are not completely at the mercy of the environment (Gates, 1980). Opportunities to regulate body temperature can allow organisms to maximize the performance of behavioral and physiological functions (Angilletta, 2009). Such successful thermoregulation is generally accomplished by selecting suitable habitats (Blouin-Demers and Weatherhead, 2002; Akins et al., 2014), shuttling between microhabitat sites (Grant and Dunham, 1988; Huey et al., 1989; Adolph, 1990; Webb and Shine, 1998), and altering body position (Bauwens et al., 1996; Ayers and Shine, 1997).

The assessment of the thermal quality of habitats, by measuring the available environmental temperatures, is necessary to understand whether or not a limitation occurs in the distribution of body temperatures. While the typical preferred body temperature range of ectothermic vertebrates is relatively narrow (Huey, 1991), habitats that provide adequate temperatures within this range may allow reliance on thermoconforming behavior. Thereby, this behavior reduces energy expenditure and permits physiological and behavioral performance essential for growth (Autumn and DeNardo, 1995; Beaupre, 1995), digestion (Greenwald and Kanter, 1979; Dorcas et al., 1997; Du et al., 2000), and reproduction (Graves and Duvall, 1993; Luiselli and Akani, 2002). Comparing the selected body temperatures to the availability of environmental temperatures allows inference to the extent of thermoregulation or thermoconformity.

Effective thermoregulation of squamates has been widely studied in thermally-variable environments and shown to differ on a temporal scale (Christian et al., 1983; Huey et al., 1989; Shine and Lambeck, 1990), by habitat (Blouin-Demers and Weatherhead, 2002), and with physiological condition (Graves and Duvall, 1993; Gardner-Santana and Beaupre, 2009). For field studies, a common practice is a thermal modeling approach using biophysical models to sample the temperatures of the available environments simultaneously and compare them with the body temperatures of the animals (Peterson, 1987; Bakken, 1992; Hertz et al., 1993; Seebacher and Shine, 2004). While direct comparison of the environmental temperatures to the animal body temperature may be informative (e.g., detecting thermoregulatory behavior), it can also be misleading (Heath, 1964; Hertz et al., 1993). A more rigorous approach has been to calculate the thermal quality of the environment and measure the effectiveness of thermoregulation of a species (Hertz et al., 1993; Christian and Weavers, 1996; Blouin-Demers and Weatherhead, 2001).

Heterogeneous environments can limit the distribution over space and time of an organism if it is exposed to highly variable and extreme temperatures, which are problematic for squamates since they are dependent upon environmental thermal conditions (Porter and Tracy, 1983; Huey, 1991; Beaupre, 1995; Vasconcelos et al., 2012). For example, the introduction of open-canopied areas lead to higher daytime and lower nighttime temperature extremes. The addition of open-canopied areas may benefit actively foraging species (Blouin-Demers and Weatherhead, 2001; Row and Blouin-Demers, 2006), and gravid females that need exposure to higher environmental

temperatures for embryonic development (Lourdais et al., 2004). Also, species using different habitats may be more tolerant of a wider range of environmental temperatures than species that only use a specific habitat type (Ruibal and Philibosian, 1970). Even though heterogeneous habitats have been found to be beneficial for species with opportunities to use different habitats for thermoregulation (Law and Dickman, 1998), there have been few studies that focus on snakes inhabiting an altered and fragmented environment (Blouin-Demers and Weatherhead, 2002; Row and Blouin-Demers, 2006; Foster et al., 2009).

The timber rattlesnake (*Crotalus horridus*) occurs in eastern United States (Brown, 1994). Throughout much of its range, particularly in the northeastern U.S., this species has experienced extirpation and population declines from human interactions and habitat loss (Brown, 1994; Martin et al., 2008). *Crotalus horridus* prefers closed canopied deciduous forests (Reinert and Zappalorti, 1988); however, in western and southern parts of its geographic range, some populations encounter fields and pastures due to agricultural practices (Sealy, 2002; Wittenburg and Beaupre, 2014). Furthermore, *C. horridus* is a sit-and-wait predator remaining at specific hunting sites for several days to a few weeks (Reinert et al., 1984; Clark, 2006), which may influence selection of suitable habitats. Wills and Beaupre (2000) suggest that *C. horridus* is a weak thermoregulator and has a tendency to conform to a wide range of temperatures in a continuous deciduous forest. However, in a fragmented environment *C. horridus* may be forced under physiological pressures to thermoregulate more precisely when exposed to highly variable temperatures in a heterogeneous landscape.

In this study, I address the possible effects and implications of a variable and fragmented environment on the thermoregulation of a *C. horridus* population in Middle Tennessee. I hypothesize that open-canopied habitats will place thermal constraints on *C. horridus* by providing fewer suitable microhabitats than closed-canopied habitats. Also, I hypothesize that *C. horridus* will exhibit more precise thermoregulatory behavior in open canopied habitats and will exhibit a tendency to thermoconform in closed-canopied habitats. To address these questions my approach was to 1) describe how the different habitat types vary in the availability of temperatures, 2) examine the distribution of body temperatures exhibited by *C. horridus*, and 3) assess the thermoregulatory effectiveness of *C. horridus* in different habitats.

MATERIALS AND METHODS

Study Site

The study site was located at Flat Rock Cedar Glades and Barrens State Natural Area located in Rutherford County, Tennessee (35.8571°N, 86.2926°W). This 342 ha preserve consists of four dominant habitats: fields, cedar glades and barrens, hardwood forests, and redcedar forests. Fields have little or no canopy cover with dense ground vegetation and are bordered by hardwood and/or redcedar forests. Cedar glades, another open-canopied habitat, are predominantly found in Tennessee, and consist of shallow soil depth, sparse vegetation, and exposed limestone (Baskin and Baskin, 1999). Small vegetation islands consisting of shrubs and small trees may occur in cedar glades while the perimeter is generally bordered by redcedar trees. Hardwood forests at this site are dominated by oaks (*Quercus* spp.) and hickory (*Carya* spp.) and have a well-established canopy cover with ground vegetation and forest undergrowth varying considerably. Mature redcedar forests (age $\geq$ 10 yr) are characterized by a dense canopy cover with little ground vegetation.

Radio Telemetry and Data Logger Techniques

During March – May 2013 snake emergence from hibernation, I captured 14 *C. horridus* (4 males, 9 non-gravid females, and 1 gravid female) using drift fences and visual searches. Snout-vent length of snakes was 91.3 ± 24.4 cm (mean ± SE) and ranged from 67.0 – 112.5 cm (Table 1). Prior to surgery, each snake was placed in a clear acrylic tube and anesthetized using cotton balls wetted with isoflurane. The forebody of the

snake was placed into the other end of the tube. Immediately upon the loss of muscle tone

and righting response, transmitters (models SI-2 or SB-2, Holohil Systems Ltd., Carp,

Ontario) were surgically implanted into in the body cavity, immediately posterior to the

stomach following similar procedures by Reinert and Cundall (1982). A miniature data

logger (iButton® model DS1921H, Maxim Integrated, San Jose, California) coated with

PlastiDip® had been previously attached to each transmitter using a cyanoacrylate

adhesive. The incision was closed using interrupted simple sutures.

The iButton® was programmed to record snake core body temperature at 30 min

intervals. The transmitter and iButton® logger package was coated with a 1:1 ratio of

beeswax and paraffin to stimulate an immune response in which the unit becomes

encased by connective tissue to prevent movement internally (Lutterschmidt et al., 2012).

Total weight of the transmitter package (14.86 ± 1.28 g) was less than 5% of the body

weight of the snake (541.34 ± 74.41 g). All snakes were released at their point of capture

and located every 48–72 hr from April – October 2013 to note their habitat choice for

comparison with environmental temperatures, and to identify physiological events (e.g.

feeding, ecdysis, gravidity) that could influence body temperature selection.

Environmental Thermal Modeling

Biophysical models were used to measure the thermal quality of each of the four

habitat types found in and around the study site. The models were used to identify the

distribution of operative temperatures available to snakes during their active season in a

specific habitat. Operative temperature models were constructed of hollow copper pipe

25.4 cm in length by 2 cm in diameter spray painted with gray primer and two black

crossbands to approximate the reflectance of *C. horridus* skin (Peterson et al., 1993;

Wills & Beaupre, 2000). The iButtons® (model DS1922L, Maxim Integrated, San Jose,

California) were programmed to record model temperatures at 15 min intervals. A single

iButton® was placed in the center of each operative model, and the ends of the models

were plugged with rubber stoppers. A wooden golf tee was inserted into the center of

each rubber stopper (internal side) to maintain the iButton® in the center of the model.

Each month (June – September 2013) for 5 to 21 days, 18 operative temperature

models were placed along two randomly-selected linear transects at 10 m intervals in

each habitat type (i.e. field, cedar glade, hardwood forest, and redcedar forest). Models

were positioned in alternating patterns of N-S and E-W exposure along each transect. For

cedar glade habitats, one operative model was placed specifically in the shade (i.e. under

rocks) each data collection period. For fields, hardwood forests, and redcedar forests, one

model was placed purposely in the shade (i.e. tall grass or leaf litter) and a second model

was placed in a fully exposed site (i.e. no vegetation or forest canopy opening) during

each data collection period to thoroughly capture available environmental temperatures.

Effectiveness of Thermoregulation Index

To estimate the effectiveness of individuals to thermoregulate in their

environment, I used a thermoregulation index proposed by Hertz et al. (1993). The

equation for the effectiveness of thermoregulation index is $E = 1 - (đ_b/đ_e)$, where E is

the effectiveness of thermoregulation, $đ_b$ represents the average body temperatures (T_b)

that a snake experiences outside its set point range (T_{set}), and d_e represents how closely

the average environmental temperatures (T_e) in a habitat match T_{set}. When E is 1, T_b is

stable relative to T_e suggesting thermoregulation. When E is approaches 0, d_b and d_e

fluctuate indicating that T_b is not selected based on T_e and thermoregulation is not taking

place (Hertz et al., 1993). The thermal set point range (T_{set}) was determined by using the

central 50% of T_bs from the seasonal field T_b data from the current study to estimate a

"preferred" T_b range for *C. horridus*.

Statistical Analyses of Operative and Body Temperatures

Snake T_bs and T_es were grouped by month and sex before statistical analyses.

Snake T_bs were compared using a repeated-measures analyses of variances

(RMANOVAs, $\alpha = 0.05$) with *post hoc* Sidak adjustment to test for differences in 24-hr,

daytime (10:00–16:00 hr), and nighttime (0:00 – 6:00) among months (May – September

2013). Daytime T_bs were compared among individuals found in all four habitats on the

same days using a one-way RMANOVA ($\alpha = 0.05$). Operative temperatures were

compared with two-way RMANOVAs using habitats as the between-subjects factor ($\alpha =$

0.0125) and months as the within-subjects factor ($\alpha = 0.01$) with a *post hoc* Games-

Howell adjustment to test for differences in 24-hr, daytime, and nighttime between May

2014 and June – September 2013. E-indices of snakes found in each habitat were

compared using a one-way RMANOVA ($\alpha = 0.05$) with a *post hoc* Sidak adjustment test

to find differences between habitats. All T_b and T_e data are reported as mean $\pm$ SE.

RESULTS

Body Temperatures

The miniature data loggers recorded 89,278 T_bs in the 14 snakes from May 2013 to September 2013. The number of T_bs was reduced to 49,536 readings because rain and overcast days were omitted from the data set as sunny days offered the best opportunities for thermoregulatory behavior. Male (23.1 ± 0.07°C) and non-gravid female (23.1 ± 0.05°C) T_bs (Table 2) were not significantly different among months (two-way RMANOVA, $F_{(1,8206)} = 0.59$, $p = 0.443$); therefore, T_bs of both sexes were pooled for further analyses. Throughout June, July, and August, snakes were able to maintain stable 24-hour T_bs of approximately 24.0°C (Table 3; $F_{(4,8204)} = 3681.03$, $p < 0.001$) while cooler T_bs occurred in May (20.2 ± 0.07°C) and September (22.1 ± 0.05°C). Snake T_bs were the most variable during the nighttime (0:00 – 6:00 hr) with similar T_bs only occurring in July (21.5 ± 0.04°C) and August (21.6 ± 0.04°C).

The thermal set point range (T_{set}) was determined to be 24.2 – 30.1°C using the central 50% of field T_bs in this study. The distribution of snake T_bs were divided into four time periods: night (0:00 – 6:00), heating (6:30 – 9:30), day (10:00 – 16:00), and cooling (16:30 – 23:30). The distribution of snake T_bs over the entire active season on clear days exhibited a unimodal distribution (Figure 1). Snakes selected daytime T_bs of 26.7 ± 0.04°C (range = 9.2 – 38.2°C). During the evening cooling period, snakes selected 24.6 ± 0.03°C (range = 10.2 – 35.2°C). Snakes were able to choose T_bs within T_{set} 29.1% of the time from 10:00 – 23:30 hr throughout their active season. However, snakes were not able to maintain T_bs within T_{set} at nighttime (19.7 ± 0.03°C, range = 2.6 – 27.6°C) and

during the heating period (19.5 ± 0.04°C, range = 2.1 − 33.2°C) as 39.7% of selected

body temperatures were cooler than T_{set}. Body temperatures within T_{set} accounted for

only 31.1% of the selected T_bs during the active season with 60.3% falling below T_{set}.

Telemetry relocation data were used to compare daytime T_bs of individuals

between each habitat (Figure 2). Snake T_bs were similar ($F_{(3,5.78)}$ = 1.124, p = 0.346)

between field (27.6 ± 0.39°C), cedar glade (27.4 ± 0.68°C), hardwood forest (26.4 ±

0.48°C), and redcedar forest (26.8°C ± 0.55). Snakes were able to select T_bs within T_{set}

63.8% of the time in hardwood forest and 55.6% of the time in redcedar forest during

daytime hours with snakes selected T_bs less often within T_{set} in field (52.6%) and cedar

glade (53.5%) habitat. Snakes found in open-canopied habitats selected above T_{set} more

often (field = 30.3% and cedar glade = 34.1%) than in closed-canopied habitat (hardwood

forest = 12.6% and redcedar forest = 21.7%) indicating that open-canopied habitats may

expose snakes to temperatures above T_{set} more often than closed-canopied habitats.

Operative Environmental Temperatures

The operative snake models recorded 439,914 temperatures (T_e) in May 2014 and

June 2013 to October 2013. In May 2013, only T_es in the field habitat were measured, but

in May 2014 all habitats were modeled. Field T_es between May 2013 and May 2014 were

not significantly different between years using an independent samples t-test ($t_{(11728.81)}$ =

0.370, p = 0.771); therefore, T_e data collected in May 2014 were used for all habitats in

statistical comparisons. The number of T_es was reduced to 140,544 because rain and

overcast days were omitted from the data set.

The thermal quality between habitats was different throughout the season (Figure 3). Operative model temperatures (T_es) were warmest in cedar glade ($28.5 \pm 0.05°C$) and field ($25.2 \pm 0.07°C$) habitats and coolest in hardwood forest ($21.8 \pm 0.03°C$) and redcedar forest ($21.6 \pm 0.03°C$) habitats. Habitats were significantly different among months ($F_{(4,23417)} = 6879.22$, $p < 0.001$) for 24-hr T_es with the exception of field and cedar glade in May in which the mean T_e only differed by $0.7°C$ (Table 4). Hardwood forest had the least variability among months throughout the season having no differences in T_e among May, June, and August. Daytime T_es were highest in field and cedar glade habitats remaining above T_{set} from May to September (Table 5). T_es were lowest in hardwood forest and redcedar forest habitats remaining within T_{set} except May T_e in hardwood forest was above T_{set} and September T_e in redcedar forest was below T_{set}. Cedar glade had similar T_es in May and August. Hardwood forest had the least variability among the habitats during daytime hours. Nighttime T_es were more similar among habitats than 24-hr and daytime hours and remained below T_{set} across all habitats throughout the season.

Open-canopied habitats had a greater breadth in T_e distributions and most temperatures remained above T_{set} compared to the closed-canopied habitats (Figure 4). Seasonal daytime T_e in field ($39.2 \pm 0.08°C$) and cedar glade ($44.0 \pm 0.07°C$) habitats were above T_{set} and and warmer than the T_e in the hardwood forest ($28.2 \pm 0.04°C$) and redcedar forest ($26.9 \pm 0.05°C$), which remained within the T_{set} range. Cedar glades had the lowest thermal quality of the four habitats, with the majority of T_es only being available above T_{set} during daytime hours (Figure 5). Hardwood forests had the highest

thermal quality with the majority of T_es being available within T_{set}. Redcedar forests had

a high availability of T_es within T_{set}, but also provided a high number of T_es below T_{set}

making them more thermally unfavorable than hardwood forests.

Thermoregulatory Effectiveness

Calculated E-indices for the habitats were as follows: field – 0.29 ($N = 12$ d),

cedar glade – 0.43 ($N = 10$ d), hardwood forest – 0.09 ($N = 11$ d), and redcedar forest –

0.04 ($N = 6$ d). E-indices between open-canopied habitats and closed-canopied habitats

were significantly different ($F_{(3,15.48)} = 10.86$, $p < 0.001$). Both field and cedar glade E-

indices were significantly different from the hardwood forest ($p = 0.021$ and 0.005,

respectively) and redcedar forest ($p = 0.013$ and 0.002, respectively). Additionally, E-

indices were similar between field and cedar glade habitats ($p = 0.150$) and between

hardwood forest and redcedar forest habitats ($p = 0.694$). The E-indices indicate that

snakes were able to alter their T_b selection based on their selected habitat (Figure 6). The

calculated deviations of operative temperatures (d_e) and deviations of snake body

temperature (d_b) for each habitat indicate that more precise thermoregulation was

necessary for snakes using field and cedar glade habitats than snakes using hardwood

forest and redcedar forest habitats (Figure 7). In the closed-canopied habitats, snakes

were able to rely on thermoconforming behavior. The d_e values for open-canopied

habitats were much higher compared to closed-canopied habitats with d_es in closed-

canopied habitats being lower and closer to T_{set}. The d_b values were similar across all

four habitats despite differences in habitat d_es demonstrating snakes needing to

behaviorally thermoregulate to maintain the same T_bs.

DISCUSSION

Snakes maintained similar mean T_bs in all four habitats despite encountering open-canopied habitats that exposed them to upper extreme T_es during daytime hours. Operative temperature models in open-canopied habitats exhibited fewer microhabitat sites that were within the thermal set point range of *C. horridus* as compared with a large number of suitable microhabitat sites in closed-canopied habitats. Calculations of the index of thermoregulatory effectiveness in each habitat revealed that *C. horridus* had strong tendencies to be thermoregulators in open-canopied habitats and strong tendencies to be thermoconformers in closed-canopied habitats.

Body temperatures of *C. horridus* in this study were similar to those reported in previous studies for this species. Non-gravid females had T_bs similar to non-gravid females in the Gardner-Santana and Beaupre (2009) study (~ 24.2°C). However, the range in T_bs for non-gravid females in the present study spanned 20°C in July and August (15.7 – 35.7°C) while the non-gravid females in the Gardner-Santana and Beaupre (2009) study spanned only 9.2°C (19.6 – 28.8°C). While both studies occurred at similar latitudes, the differences in the two studies could be due to 1) a more rapid T_b sampling rate in this study or 2) habitat fragmentation leading to a potentially broader range of T_es in the present study. Additionally, daily T_bs of snakes in the present study (mean 26.7°C) were similar to those reported in Brown et al. (1982) for *C. horridus* (mean 26.9°C).

Crotalus horridus in the Middle Tennessee population encountered a variety of habitats that differed in thermal quality. Hardwood forest was the most common habitat (66.7%) at the study site offering the highest availability of temperatures within T_{set}.

However, the hardwood forest habitat was discontinuous, separated by field and cedar glade habitats with few opportunities for snakes to use either of the forested habitats in some areas of the field site. Throughout the active season, snakes moved between habitat types as part of their annual migratory pattern and were found in both open-canopied habitats even when forested habitats were available to them. Even though predicted thermoregulatory strategies were different based on the habitat being used (open-canopied or closed-canopied), snakes maintained nearly identical T_bs despite risk of being exposed to upper extreme temperatures in the open-canopied environments during daytime hours. The same T_b patterns across all habitats indicate that snakes were actively seeking suitable microhabitats for more precise thermoregulation in open-canopied habitats.

The calculated E-indices further support for more active thermoregulatory behavior in the open-canopied than closed-canopied habitats. Thermoregulatory strategy differences between the habitats appeared to be dictated by the presence of a forest canopy with little emphasis on the specific habitat. Higher d_es occurring in open-canopied habitat necessitates for snakes to be stronger thermoregulators in open habitats than snakes in closed-canopied habitat. The large availability of operative temperatures in closed-canopied habitats allowed snakes to be less selective in microhabitat selection (at least regarding temperature) and therefore may have more of a thermoconforming strategy since d_es were closer to T_{set}. Such variation in thermoregulatory strategy could have implications for other activities such as foraging and reproduction.

Extreme upper T_es may restrict movement of *C. horridus* during daytime hours within open-canopied habitats. Seasonal T_es exceeded T_{set} at 11:00 hr in field and cedar glade habitats and did not cool down to T_es within or below T_{set} until 17:00 hr. Seasonal T_es in closed-canopied habitats stayed within T_{set} between 10:00 – 16:00 hr in hardwood forest and 12:00 – 16:00 hr in redcedar forest. Clark (2006) found that *C. horridus* generally abandoned ambush sites from 19:00 – 22:00 hr. Based on the T_b and T_e patterns in this study, T_es in open-canopied areas will begin to cool down falling within or below T_{set} during those hours allowing movement between habitats and within open-canopied habitats without risking exposure to T_es above T_{set}. The thermal availability within open-canopied habitats may only allow movement during nighttime hours.

Open-canopied habitats could vary the movements associated with mate finding for male *C. horridus*. Male rattlesnakes typically have larger home ranges than females due to mate searching behavior (Reinert and Zappalorti, 1988; King and Duvall, 1990; Brown, 1994; Waldron et al., 2006; Anderson, 2010). Females can be difficult to find since they are widely distributed in an unpredictable pattern and may require males to move more often and travel larger distances to find mates. Encountering more open-canopied habitats may restrict daytime movements and increase search time within those habitats for females. Restriction of daytime movement within closed-canopied habitats based on available T_es is unlikely and could be used by males during mate searching. Open-canopied habitats may restrict movement during daytime hours and could reduce opportunities for male *C. horridus* to mate search in those habitats. Field and cedar glade habitats cause separation of forested habitats in a majority of the foraging areas used by

snakes at this study site which could further prevent males from taking advantage of using the more thermally suitable habitats during daytime hours to find females for mating.

Crotalus horridus is historically known to have occurred predominately in continuous deciduous forest habitat (Brown, 1994). The T_e and T_b patterns in this study showed that hardwood forest habitat had the highest thermal quality of the four habitat types providing the most suitable environment to maximize the life history characteristics of *C. horridus*. Increased human activity and introduction of open-canopied habitats causing fragmentation between forested habitats could have conservation implications. Anthropogenic impacts such as roads can cause barriers and restrict movement between populations reducing genetic diversity, increasing susceptibility to diseases, and decreasing population sizes of *C. horridus* (Clark et al., 2010; Clark et al., 2011). The *C. horridus* population in this study does not only encounter challenges imposed by open-canopied habitats fragmenting forested habitat, but major roads further isolate the population approximately 2 km from the main denning site in all directions. It is unclear as to how anthropogenic activities influence the dynamics of this population; however, due to increased development and urbanization in many regions better understanding the effects of altered habitats on snake populations is important for any conservation efforts.

In summary, this study illustrates that the thermoregulatory behavior of *C. horridus* may vary with the thermal qualities in different habitats. Even though populations of snakes in closed-canopied habitats in warm climate regions could potentially reduce energy expenditures by allowing snakes to thermoconform within

forested habitats, the opposite may be true for populations in cool climates. With the

latter, open canopies may be more of a necessity, particularly for gravid females (Brown

et al., 1982; Gardner-Santana and Beaupre, 2009).

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APPENDICES

APPENDIX A: TABLES

Table 1. Body mass (without transmitter package),
snout-vent length (SVL), tail length (TL) and sex
(M = male, F = female, G = gravid female)
for 14 *Crotalus horridus* used in this study.

Snake ID	Sex	Mass (g)	SVL (cm)	TL (cm)
1	M	851.1	108.5	8.0
3*	F	604.6	98.3	6.2
4	F	891.6	112.5	6.4
5	M	400.0	88.0	7.4
6	F	185.2	69.8	4.2
8	F	595.1	103.0	6.0
10	F	749.1	103.1	7.2
11	F	951.3	108.0	6.0
13*	F	184.3	68.0	4.4
15	F	196.5	68.0	4.1
16	F	177.1	67.0	3.8
17	M	750.3	98.2	7.4
18	M	538.5	93.0	7.1
19	G	504.0	92.5	5.5
Mean		541.3	91.3	6.0
SE		74.4	24.4	1.6

*lost during active season due to mortality

Table 2. Body temperatures (mean ± SE) of male (N = 16,080 T_bs) and non-gravid female (N = 30,624 T_bs) snakes from May to September 2013 for 24 hr, daytime (10:00 – 16:00), and nighttime (0:00 – 6:00).

Sex	Time Period	Month	T_b (°C)	Range (°C)
Male (N = 4)	24 hr	May	20.1 ± 0.11	7.6 – 34.6
		June	24.7 ± 0.07	14.7 – 34.2
		July	24.4 ± 0.07	16.6 – 35.6
		August	24.4 ± 0.07	15.2 – 38.2
		September	22.1 ± 0.08	11.2 – 38.2
	Daytime	May	24.8 ± 0.21	9.6 – 34.6
		June	28.2 ± 0.11	19.6 – 34.2
		July	27.8 ± 0.10	19.1 – 35.6
		August	27.9 ± 0.12	18.2 – 38.2
		September	25.9 ± 0.14	16.1 – 38.2
	Nighttime	May	16.0 ± 0.12	7.6 – 25.6
		June	21.0 ± 0.07	14.7 – 26.1
		July	21.3 ± 0.07	16.6 – 25.7
		August	21.2 ± 0.06	15.2 – 25.6
		September	18.4 ± 0.09	11.7 – 24.6
Female (N = 9)	24 hr	May	20.2 ± 0.07	2.1 – 35.7
		June	24.2 ± 0.05	14.1 – 35.7
		July	24.3 ± 0.05	16.2 – 35.7
		August	24.4 ± 0.05	15.7 – 35.1
		September	22.3 ± 0.05	12.6 – 34.7
	Daytime	May	25.7 ± 0.12	9.2 – 35.7
		June	27.7 ± 0.08	18.1 – 35.7
		July	27.1 ± 0.08	19.2 – 35.7
		August	27.0 ± 0.09	17.6 – 35.1
		September	25.1 ± 0.10	15.2 – 34.7
	Nighttime	May	15.4 ± 0.08	2.6 – 25.6
		June	20.8 ± 0.05	14.6 – 26.7
		July	21.5 ± 0.05	16.2 – 26.7
		August	21.8 ± 0.04	16.2 – 27.6
		September	19.4 ± 0.06	13.1 – 25.2

Table 3. Post-hoc Sidak comparisons of monthly 24-hr mean body temperatures.

Month	Month	P-value
May	June	0.001
	July	0.001
	August	0.001
	September	0.001
June	July	0.999
	August	0.981
	September	0.001
July	August	0.888
	September	0.001
August	September	0.001

alpha level = 0.05

Table 4. Monthly 24-hr operative temperature (mean ± SE) in each habitat from May 2014 and June to September 2013. Same letter denotes no difference.

Habitat	Month	T_e (°C)	Range (°C)
Field	May	25.7 ± 0.16^a	4.6 – 66.0
	June	27.0 ± 0.13	9.5 – 62.5
	July	27.7 ± 0.11	13.5 – 64.0
	August	23.6 ± 0.10	10.5 – 55.0
	September	21.7 ± 0.11	8.5 – 54.0
Cedar Glade	May	25.0 ± 0.14^a	7.5 – 58.0
	June	29.0 ± 0.11	10.5 – 59.0
	July	30.0 ± 0.10	14.0 – 57.5
	August	28.0 ± 0.09	12.0 – 61.0
	September	24.7 ± 0.09	11.0 – 54.5
Hardwood Forest	May	21.7 ± 0.15^{bc}	8.5 – 58.5
	June	21.6 ± 0.12^b	13.5 – 45.0
	July	24.0 ± 0.10	15.5 – 57.0
	August	21.8 ± 0.09^c	14.5 – 48.0
	September	19.8 ± 0.10	12.5 – 47.0
Redcedar Forest	May	21.0 ± 0.17	12.0 – 57.0
	June	23.4 ± 0.14	16.0 – 54.0
	July	22.6 ± 0.12	15.5 – 49.0
	August	21.3 ± 0.11	13.0 – 58.5
	September	19.0 ± 0.11	13.0 – 40.5

[a]alpha level = 0.0125
[bc]alpha level = 0.01

Table 5. Daytime (10:00 – 16:00 hr) and nighttime (0:00 – 6:00 hr) monthly
operative temperature (mean ± SE) in each habitat from May 2014
and June to September 2013. Same letter denotes no difference.

Habitat	Month	Daytime (°C)		Nighttime (°C)	
		T_e	Range	T_e	Range
Field	May	46.6 ± 0.21	10.0 – 66.0	10.5 ± 0.06	4.6 – 17.0
	June	43.1 ± 0.17	22.0 – 62.5	16.7 ± 0.08	9.5 – 23.0
	July	40.6 ± 0.17	22.5 – 64.0	18.8 ± 0.05	13.5 – 22.0
	August	33.6 ± 0.17	21.0 – 55.0	17.2 ± 0.06	10.5 – 22.0
	September	32.7 ± 0.15	17.5 – 54.0	16.0 ± 0.06[c]	8.5 – 29.5
Cedar	May	42.0 ± 0.18[a]	14.5 – 58.0	13.0 ± 0.05	7.5 – 36.5
Glade	June	45.7 ± 0.15	23.5 – 59.0	17.5 ± 0.07	10.5 – 24.0
	July	43.0 ± 0.15	25.0 – 57.5	20.3 ± 0.05	14.0 – 25.0
	August	41.7 ± 0.15[a]	20.0 – 61.0	18.9 ± 0.06[d]	12.0 – 23.5
	September	40.9 ± 0.13	19.5 – 54.5	16.1 ± 0.05[c]	11.0 – 20.0
Hardwood	May	33.0 ± 0.19	19.5 – 58.5	13.5 ± 0.06	8.5 – 17.5
Forest	June	25.7 ± 0.16[b]	19.5 – 45.0	18.2 ± 0.07	13.5 – 22.6
	July	29.2 ± 0.16	22.0 – 57.0	19.8 ± 0.05	15.5 – 22.7
	August	25.8 ± 0.15[b]	19.0 – 48.0	18.9 ± 0.06[d]	14.5 – 22.1
	September	25.8 ± 0.14[b]	18.5 – 47.0	16.0 ± 0.05[c]	12.5 – 19.1
Redcedar	May	28.1 ± 0.23	18.0 – 57.0	15.1 ± 0.07	12.0 – 17.5
Forest	June	27.1 ± 0.19	20.5 – 54.0	20.8 ± 0.08	16.0 – 24.5
	July	26.2 ± 0.19	20.0 – 49.0	19.6 ± 0.06	15.5 – 22.5
	August	24.7 ± 0.18	17.5 – 58.5	18.7 ± 0.07[d]	13.0 – 22.5
	September	23.1 ± 0.16	17.0 – 40.5	16.3 ± 0.06	13.0 – 18.5

[ab]alpha level = 0.01
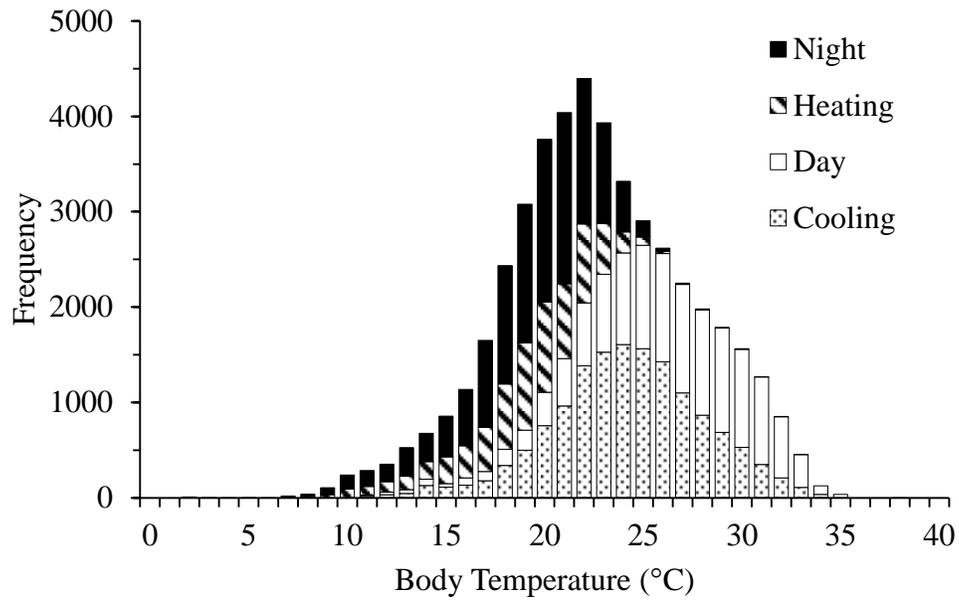[cd]alpha level = 0.0125

APPENDIX B: FIGURES

Figure 1. Seasonal body temperatures exhibited by *C. horridus* for nighttime (0:00 – 6:00 hr), heating period (6:30 – 9:30 hr), daytime (10:00 – 16:00 hr), and cooling period (16:30 – 23:30 hr) during their active season (May 2014 and June to September 2013).
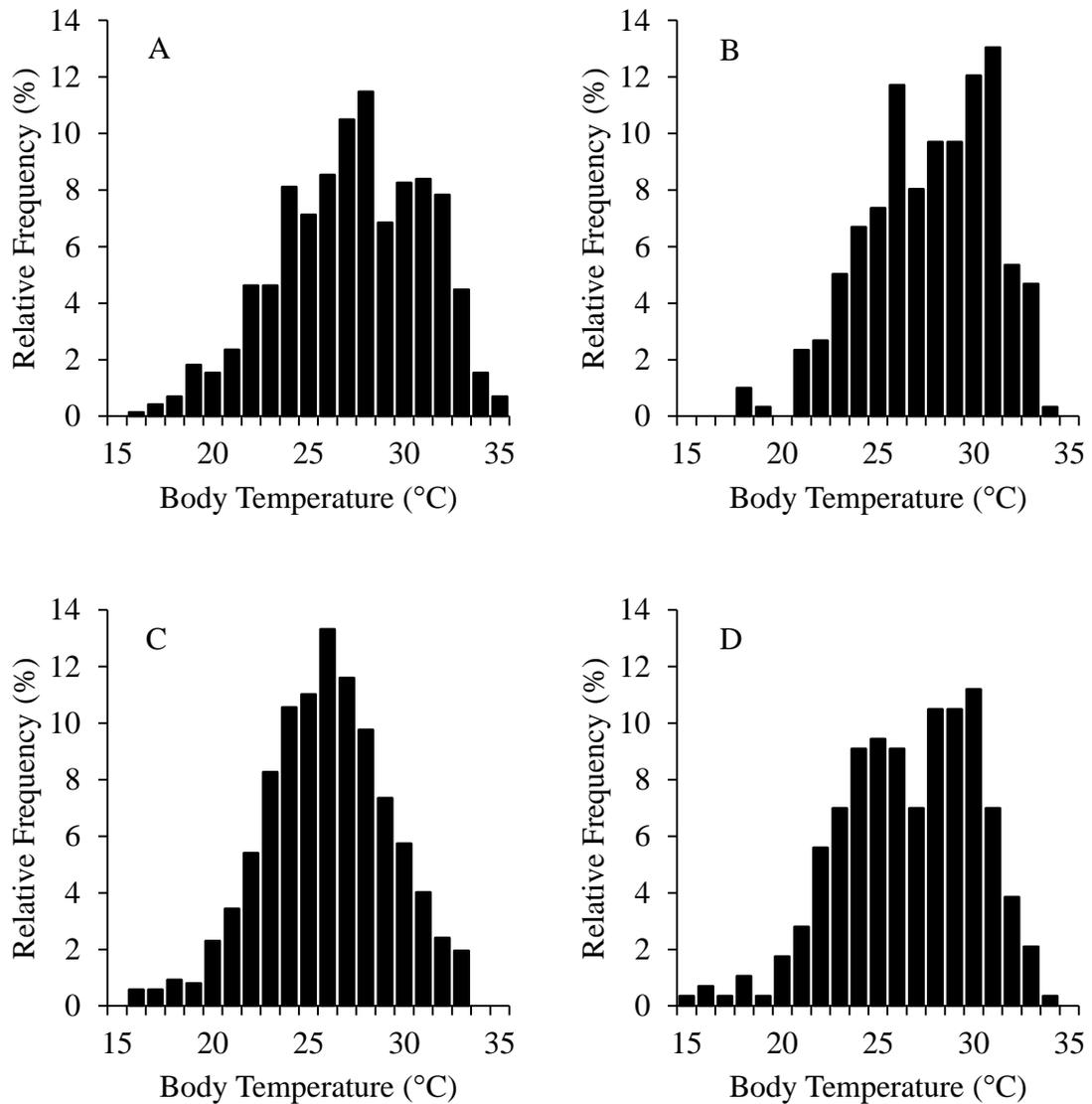
Figure 2. Daytime (10:00 – 16:00 hr) snake body temperatures found within each habitat (N = 18 d). Habitat categories are A) Field (N = 715 T_bs), B) Cedar Glade (N = 299 T_bs), C) Hardwood Forest (N = 871 T_bs), and D) Redcedar Forest (N = 286 T_bs).
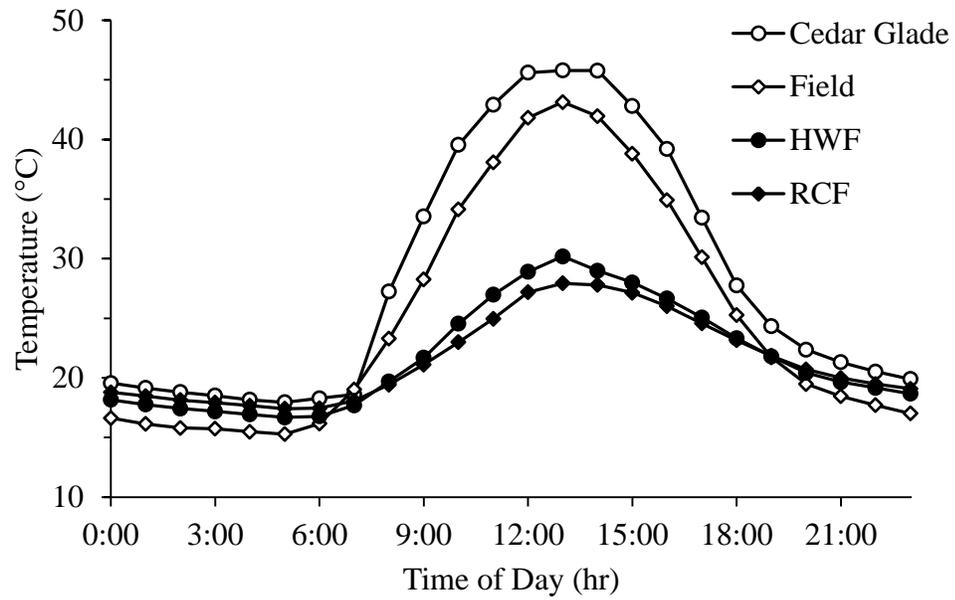
Figure 3. Seasonal mean operative temperatures sampled hourly in all four habitats in May 2014 and June to September 2013 (HWF = hardwood forest; RCF = redcedar forest).
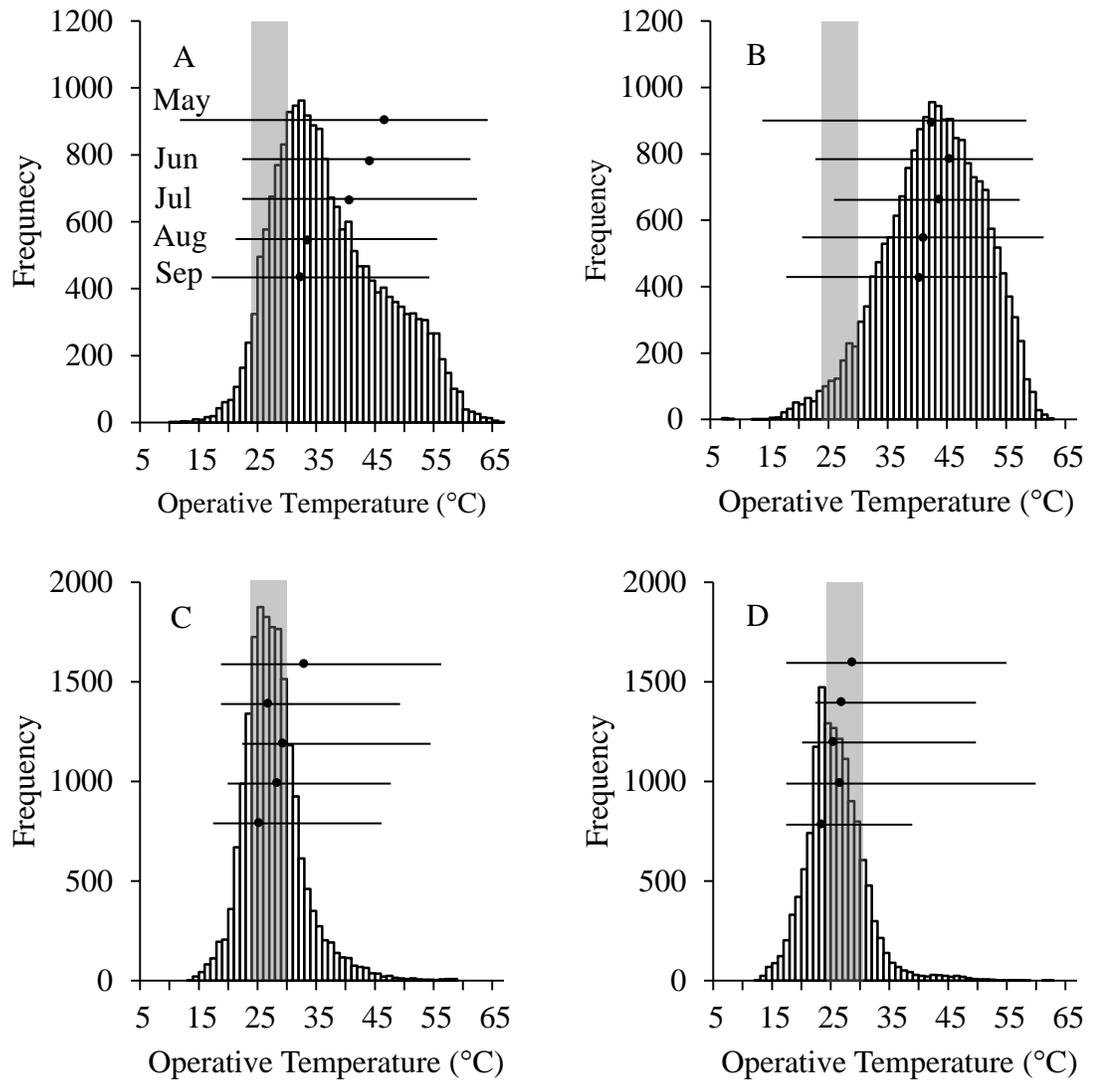
Figure 4. Operative temperatures (means ± SE) in each habitat from May 2014 and June to September 2013 during daytime (10:00 – 16:00) hours. A) Field (N = 14,475), B) Cedar Glade (N = 14,650), C) Hardwood Forest (N = 14,550), and D) Redcedar Forest (N = 9,675). Monthly daytime mean indicated by black circle with range. Shaded area represents thermal set point range.
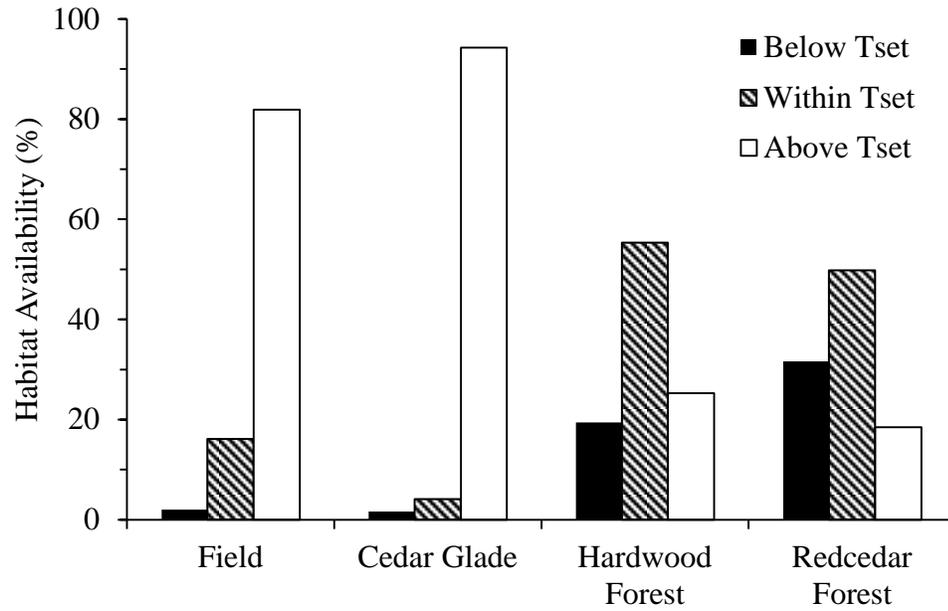
Figure 5. Habitat availability below, within, and above the thermal set point range (T_{set}) from May 2014 and June to September 2013 during daytime (10:00 – 16:00 hr) in each habitat type.
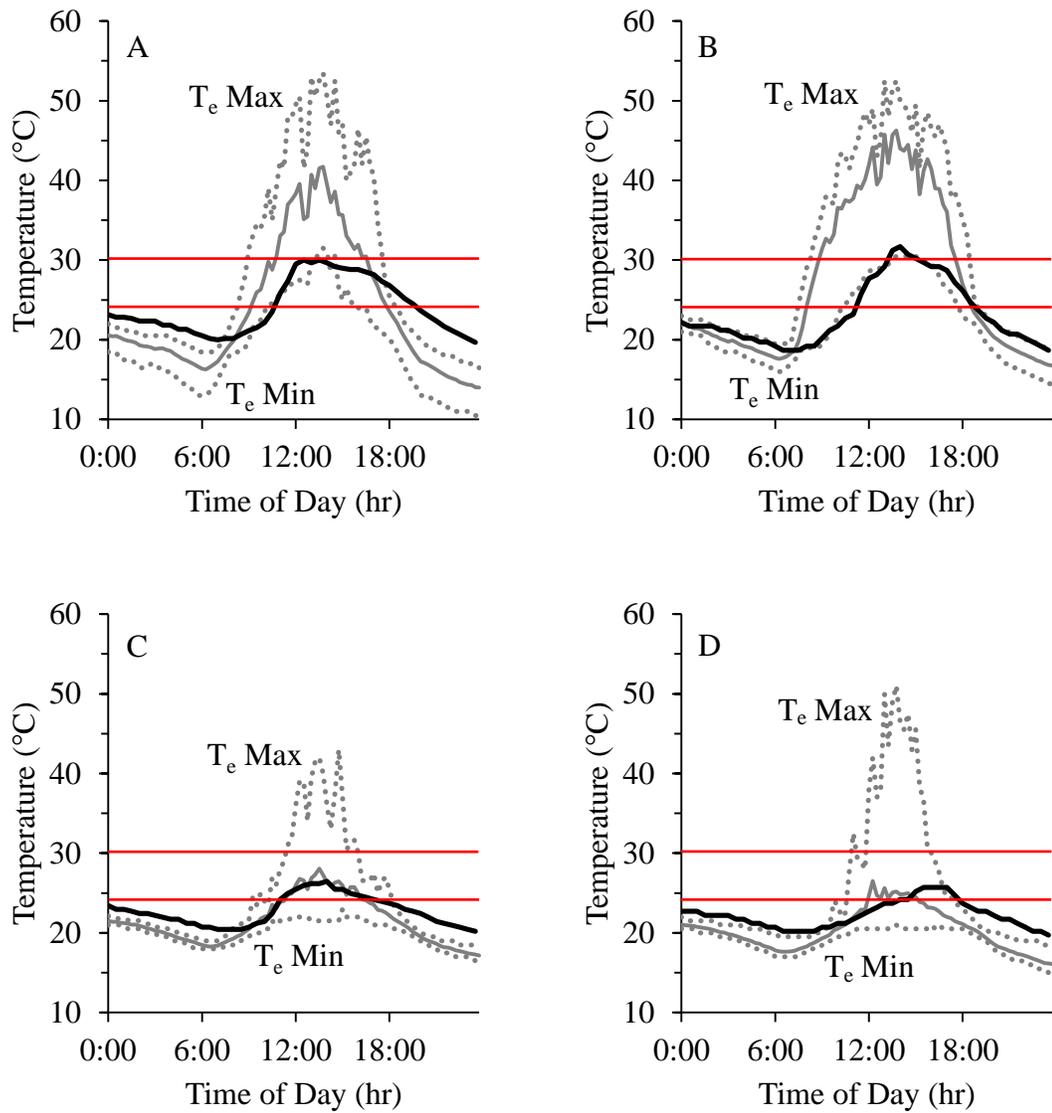
Figure 6. Daily mean body temperature (solid black line) and operative model temperature (solid grey line) patterns of snakes found in each habitat: A) Field, B) Cedar Glade, C) Hardwood Forest, and D) Redcedar Forest on August 14, 2013. Thermal set point range is indicated by the parallel thin solid lines.
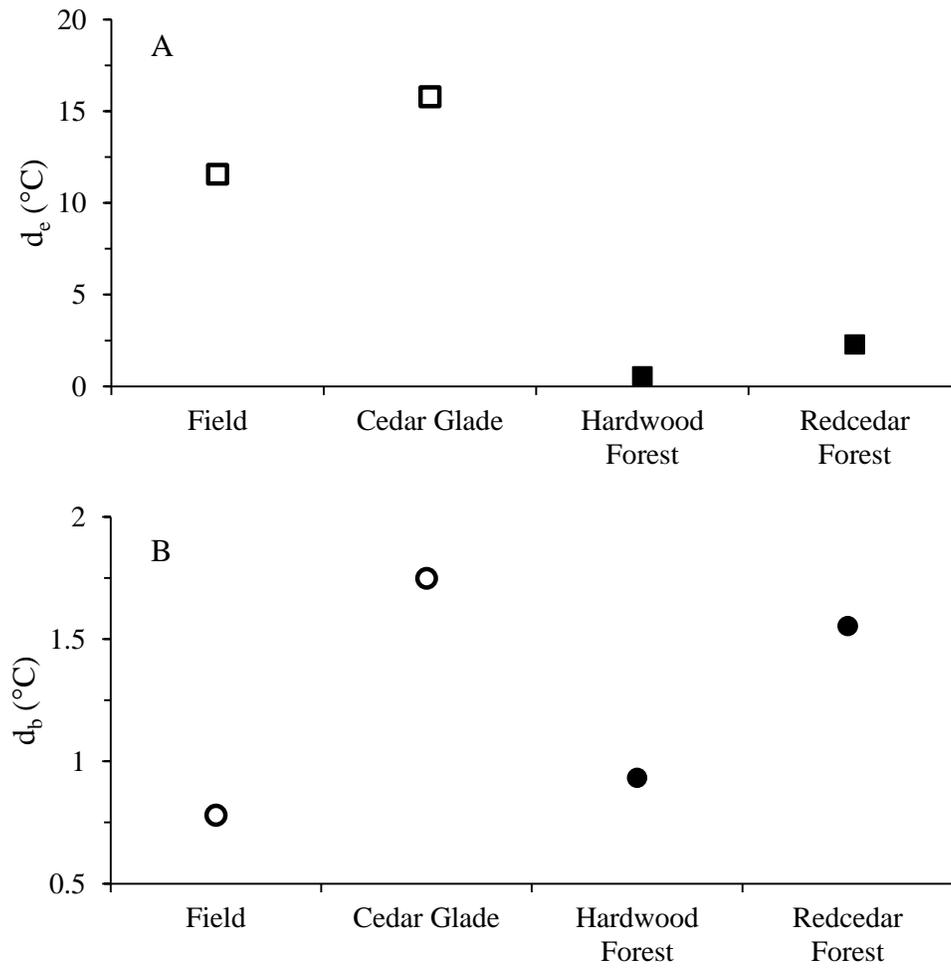
Figure 7. A) The deviation of mean operative model temperatures from the thermal set point range for each habitat. B) The deviation of mean snake body temperatures from the thermal set point range for each habitat.

APPENDIX C: IACUC APPROVAL FORM

May 2, 2012

Investigator(s) Name: Vincent Cobb
Investigator(s) Email: vincent.cobb@mtsu.edu
Department of Biology

Protocol Title: "Timber Rattlesnake Ecology"
Protocol Number: 12-009

Dear Investigator,

The MTSU Institutional Animal Use and Care Committee has reviewed your research proposal identified above and has approved your research under the PHS definition of animal, pending the compliance office receives a copy of your TWRA permit before you begin.

Approval is granted for three (3) years. **Please note you will need to file a Progress Report annually regarding the status of your study in order to keep the study active.**

According to MTSU Policy, an investigator is defined as anyone who has contact with animals for research purposes. Anyone meeting this definition needs to be listed on the protocol and needs to provide a certificate of training to the Office of Compliance. If you add investigators to an approved project, please forward an updated list of investigators and their certificates of training to the Office of Compliance before they begin to work on the project.

Any change to the protocol must be submitted to the IACUC before implementing this change. Any unanticipated harms to subjects or adverse events must be reported to the Office of Compliance at (615) 494-8918.

You will need to submit an end-of-project report to the Office of Compliance upon completion of your research. Complete research means that you have finished collecting data and you are ready to submit your thesis and/or publish your findings. Should you not finish your research within the three (3) year period, you must submit a Progress Report and request a continuation prior to the expiration date. Please allow time for review and requested revisions. Your study expires **May 2, 2015**.

Also, all research materials must be retained by the PI or faculty advisor (if the PI is a student) for at least three (3) years after study completion. Should you have any questions or need additional information, please do not hesitate to contact me.

Sincerely,

Emily Born

Emily Born
Compliance Officer
615-494-8918
emily.born@mtsu.edu