

THERMAL ECOLOGY AND BEHAVIORAL ACTIVITY IN
HIBERNATING TIMBER RATTLESNAKES (*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
2013

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ACKNOWLEDGEMENTS

The completion of this thesis would not be possible without first thanking all those who helped me along the way. First and foremost, I would like to thank Dr. Vince Cobb for welcoming me into his lab and giving me the opportunity to conduct research under his guidance. His in-depth knowledge and passion for reptile ecology has provided me with a wealth of information; I have learned so much from the short time we have worked together at MTSU. I would also like to thank both my committee members, Drs. Matt Klukowski and Jeff Walck, for their input and advice throughout all stages of my research. I thank my lab mates and friends, Alyssa and Shane, for their help in the field, data entry, and in-depth discussions of project ideas. I would like to thank my wife, Sarah, for listening to me babble on about my research to no-end, editing countless versions of this thesis, and for encouraging me to pursue my career goals. Last, but certainly not least, I would like to thank my family; a special thanks to my Mom and Dad for their endless support and for their attempts at explaining the importance of my research and conservation to others, regardless of my working with venomous snakes.

ABSTRACT

Hibernation is an important behavioral event in temperate region reptiles for escaping periods of harsh winter environmental temperatures. Snakes are arguably the most thoroughly studied reptilian hibernator; and at high latitudes, snakes may spend a large proportion of the year underground in a state of reduced metabolic activity. Although generally associated with periods of inactivity, some evidence does support limited activity during the winter, both above or below ground. However, observations of such events are difficult due to their typical subterranean refugia. This study examines occurrence of both terrestrial and subterranean activity during winter in the timber rattlesnake, *Crotalus horridus*. Hourly body temperatures (T_{bs}) and small-scale movements of snakes throughout a ~5 month hibernation period in Middle Tennessee were monitored with the purpose of identifying mid-winter activity and potential shuttling behavior in and out of hibernation. Environmental temperatures (air, soil, and den cavities) and snake operative temperatures (using biophysical models) were recorded to help estimate time periods when snakes were at the surface. Snakes returned to the denning areas for ingress (mean \pm SE) on 11 October \pm 3 days, and emerged for egress on 7 April \pm 4.7 days. Visual observations of snakes basking on the surface were rare (0 of 4 snakes in winter 2011 – 2012; 4 of 13 snakes in winter 2012 – 2013), however, hourly sampling of snakes with surgically implanted temperature loggers revealed that 50% (2011 – 2012) and 69% (2012 – 2013) of the snakes emerged to the forest floor surface to

bask two or more times (2011 – 2012: 12 total basking events; 2012 – 2013: 48 total basking events). Mean snake $T_{b,s}$ during winter were $11.9 \pm 1.5^{\circ}\text{C}$ (2011 – 2012) and $11.0 \pm 1.2^{\circ}\text{C}$ (2012 – 2013). A total of 48,223 $T_{b,s}$ were collected over two hibernating periods, with $T_{b,s}$ ranging from $1.1 - 33.7^{\circ}\text{C}$. Snakes made on average 6.1 ± 1.2 movement bouts throughout winter, accumulating a total distance of 146.4 ± 35.5 m. All individuals made small (< 10 m) movements to different micro-hibernation sites throughout winter. If mild winters become more common due to climate change, suitable surface temperatures for snakes emerging from dens during mid-winter warming periods may increase. The energetic costs associated with increasing T_b (via basking) during hibernation have little effect on the total energy budget required to survive winter and therefore the functional significance of mid-winter basking remains to be determined.

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INTRODUCTION

Hibernation is an important behavioral mechanism for many temperate region reptiles to escape harsh environmental temperatures (Gregory, 1982). Reptiles that retreat to underground refugia during winter minimize metabolic activity and the depletion of energy reserves (Bennett & Dawson, 1976; Congdon *et al.*, 1982). To increase the likelihood of winter survival, reptiles must maintain a level of metabolic activity low enough to conserve energy stores (avoiding starvation) and avoid freezing temperatures at the ground surface (Blem & Blem, 1995; Shine & Mason, 2004). Because ectotherms cannot create their own metabolic heat, they must rely on external sources of energy such as solar radiation (Greene, 1997) or changes in activity (Hertz *et al.*, 1993) to regulate body temperatures ($T_{b,s}$). Environmental temperatures play a vital role in the life history and behavior of ectothermic organisms (Huey, 1982; Peterson *et al.*, 1993). Without proper substrate or surface temperatures, reptiles lack the ability to utilize efficient and necessary body functions vital for survival, specifically digestion, locomotion, and defense against predators (Huey & Stevenson, 1979).

When environmental temperatures become unfavorable, reptiles seek temporary refuge underground, in tree stumps or root systems (Gregory, 1982; Kingsbury & Coppola, 2000; Harvey & Weatherhead, 2006), or in a body of water where the temperature fluctuates less dramatically than the air (Costanzo, 1986; Litzgus *et al.*, 1999; Grayson & Dorcas, 2004). Physiological activity may remain relatively normal during short periods of cold weather, and individuals may return to the surface to bask

when favorable conditions become available (Ruby, 1977; Mushinsky *et al.*, 1980). In temperate zones, the ability to locate appropriate refugia becomes essential for reptiles avoiding lethal surface conditions (Gregory, 1982). Long periods of cold winter temperatures, particularly at higher latitudes, may force reptiles to hibernate for as much as 7 – 8 months (Prestt, 1971; Aleksasuk, 1976; Bauwens, 1981; Macartney *et al.*, 1989; Weatherhead, 1989; Brown, 1992; Andersson, 2003; Cobb & Peterson, 2008; Gienger & Beck, 2011). In such regions, it becomes extremely important for reptiles to seek an appropriate hibernaculum to spend the winter to avoid lethal conditions. Low environmental temperatures suppress metabolic activity and locomotor performance (Stevenson *et al.*, 1985; Lillywhite, 1987; Peterson *et al.*, 1993), such as mate searching, prey capture, and predator avoidance.

Hibernaculum structure varies by location and habitat, but in most cases, reptiles at high latitudes will occupy rocky outcrops and bluffs over winter (Gregory, 1982, 1984). These rocky environments allow reptiles to find holes and cracks that lead to underground caverns or ‘dens’, as commonly associated with snake overwintering areas. By moving below the surface (particularly the frost line), snakes are able to maintain relatively stable T_b s above freezing and similar to the surrounding soil and rocks (Lueth, 1941; Sealy, 2002). Hibernation sites vary by species and geographic conditions, but some temperate snake hibernacula (e.g., rattlesnakes) are located on south-facing slopes (Brown, 1982; Reinert & Zappalorti, 1988a; Prior & Weatherhead, 1996; Gienger & Beck, 2011) which generally receive more solar radiation over winter (Hamilton &

Nowak, 2009). Often the den entrance is a vertical or horizontal crack along a rock pile, ledge, or wall (Klauber, 1956; Gregory, 1982), but at times den entrances may be less obvious, such as a small hole at the base of tree roots or a stream bed (Waldron *et al.*, 2006).

Den site selection plays an important role in the survival of hibernating snakes. It is common to find multiple species hibernating communally in a suitable den (Hirth, 1966; Brown & Parker, 1976; Costanzo, 1986; Cobb & Peterson, 2008; Williams *et al.*, 2012). Intraspecific communal hibernation has been documented among multiple genera: *Agkistrodon* (Sanders & Jacob, 1981; Hein & Guyer, 2009), *Coluber* (Brown & Parker, 1976), *Crotalus* (Sexton & Marion 1981; Brown 1982 & 1992; Duvall *et al.*, 1985; Macartney *et al.*, 1989; Martin, 1992), *Pantherophis* (Weatherhead, 1989), and *Thamnophis* (Aleksiuk & Stewart, 1971; Gregory, 1974; Costanzo, 1986). However, individual hibernation also exists (Sexton *et al.*, 1992; Kingsbury & Coppola, 2000; King, 2003; Waldron *et al.*, 2006; Mohr, 2010; Sperry & Weatherhead, 2012), predominantly in lower latitudes and southern populations where only temporary periods of inactivity are necessary (Hein & Guyer, 2009; Waldron *et al.*, 2013). Snakes often have high den site fidelity, and may overwinter at the same den site year after year (Shine & Mason, 2004; Harvey & Weatherhead, 2006; Clark *et al.*, 2008; Cobb & Peterson, 2008; Burger *et al.*, 2012; Shipley *et al.*, 2012). It is thought that offspring and new recruitment often use scent trailing to locate suitable den sites (Costanzo, 1986; Graves *et*

al., 1986; Reinert & Zappalorti, 1988b; Cobb *et al.*, 2005), which may also lead to a high number of communal hibernacula.

Although thermoregulation is a primary component of snake ecology during the active season, there is little evidence of thermoregulatory activity on the surface during the winter (Carpenter, 1953; Jacob & Painter, 1980; Sanders & Jacob 1981; Sexton & Marion, 1981; Costanzo, 1986). The decrease in daily activity in hibernating snakes does not necessarily mean a period of complete inactivity; for example, Cobb and Peterson (2008) documented that subterranean movement occurred for several weeks after snakes retreated underground at a communal rattlesnake hibernaculum in Idaho. It is suspected that individuals may thermoregulate if a subterranean temperature gradient exists (Viitanen, 1967; Aleksuk, 1976). Although snakes were not visibly thermoregulating on the surface, snakes exhibited small shifts (few meters) in location underground throughout winter, which may be in response to varying subterranean temperatures (Cobb & Peterson, 2008).

The thermoregulatory movements and behaviors during ingress and egress (beginning and ending hibernation respectively) may extend beyond the retreat and emergence from underground. Behavioral regulation of $T_{b,s}$ has also been documented by movement within the hibernacula (Sexton & Marion, 1981) or basking events near a den entrance (Drda, 1968, Cobb & Peterson, 2008, Sperry & Weatherhead, 2012). While some individuals emerge from their hibernacula to bask on the surface during suitable mid-winter warming events (Carpenter, 1953; Jacob & Painter, 1980; Sanders & Jacob

1981; Sexton & Marion, 1981; Costanzo, 1986), this behavior is rarely documented and remains unstudied. Mid-winter basking events consist primarily of anecdotal observations; the lack of data for this behavior may be attributed to the lack of continuous and frequent sampling at denning sites.

At communal hibernacula, adequate sample sizes can be attained and monitored; whereas it is often difficult to assure that individually hibernating snakes will be exposed to similar environmental conditions. Therefore, a bulk of the literature on snake hibernation occurs on northern populations of communally hibernating snakes (Hirth, 1966; Brown *et al.* 1974; Sexton & Marion 1981; Brown 1982; Macartney *et al.*, 1989; Cobb & Peterson 2008; Harvey & Weatherhead 2009; Sperry & Weatherhead 2012). More southerly populations of snakes (whether hibernation is communal or individual) tend to have shorter periods of inactivity during winter when compared to northern populations (Mushinsky *et al.*, 1980; Hein & Guyer 2009; Sperry & Weatherhead, 2009). The more favorable environmental conditions of southern temperate latitudes, as compared to northern ones, allow more opportunities for mid-winter activity. I hypothesize that the thermal threshold of activity may be breached more often than previously speculated and may lead to intermittent winter basking or increased underground movement that could represent thermoregulatory shuttling.

The objectives of this project were to analyze the thermal ecology and behavioral activity of hibernating snakes in a geographic region that represents an intermediate overwintering climate. Middle Tennessee exhibits a climate that promotes the hibernation

of snakes for approximately 5 months (November – March), usually with several short-term warming events throughout winter. I suspect these warming events may stimulate activity during hibernation and create more variation in body temperatures during the ingress and egress periods. The timber rattlesnake (*Crotalus horridus*) has been chosen as a model because locales exist in Middle Tennessee where this species communally hibernates, thus allowing for a large sample size and a measure of intrapopulation variation.

Throughout this study, four main questions were asked regarding the thermal ecology and behavior of hibernating snakes: 1) Do snakes exhibit thermoregulatory shuttling/movement behaviors above and below ground associated with ingress, egress, and changes in environmental temperatures? 2) Do short mid-winter basking events occur when above ground environmental temperatures become suitable? 3) How do snake T_b s vary between individuals during hibernation? 4) How might an increase in winter activity (basking or above/below ground movements) affect the energy stores, weight loss, or survival of snakes?

METHODS

Study Species

The timber rattlesnake (*Crotalus horridus*) is a large pit viper (Family: Viperidae) whose geographic distribution expands across most of the eastern United States, with historic records as far west as Texas, south to Florida, and as far northeast as Vermont and New Hampshire (Conant & Collins, 1998). Like other temperate snake species, *C. horridus* spends the winter hibernating underground to escape from the harsh temperatures of winter. Communal hibernation is more common in *C. horridus* across northern populations, while individuals in southern populations tend to hibernate singly (Sexton et al., 1992). Timber rattlesnakes can often be found hibernating with other snake species, such as copperheads (*Agkistrodon contortrix*) and rat snakes (*Pantherophis* spp.) (Klauber, 1972; Brown, 1982). In the spring, *C. horridus* emerge from hibernation and disperse into the surrounding woodlands to forage and mate during the active season (Martin, 1992). Gravid females give birth to live young in late August and September to a litter of 7 – 11 neonate snakes (Gibbons, 1972; Fitch, 1985; Brown, 1991; Martin, 1993). Timber rattlesnakes are classic sit-and-wait predators and will stay coiled beside a fallen log or a small game trail waiting for prey (Reinert *et al.*, 1984; Clark, 2004; Reinert *et al.*, 2011).

Study Site

The study site was a 342 ha state natural area in Rutherford County, Tennessee. Data were collected from September – May in 2011/2012 (year 1) and 2012/2013 (year 2). The variety of habitat types available included mixed hardwood forests, redcedar woodlands, old fields, and cedar glades. Cedar glades are unique habitats characterized by open areas with small rock/gravel or pavement substrate and low herbaceous plants surrounded by a redcedar-shrub thicket (Quarterman, 1950). This nature preserve is surrounded by agricultural lands and rural housing which buffer it from the edge of a major city.

Environmental Measurements

Environmental Data

Environmental temperatures (air, soil, and subterranean den) were collected at the denning areas from September – May in 2011 – 2012 and 2012 – 2013. Temperatures were measured at a 1 h sampling rate and using type T and K thermocouple wire and single-channel data loggers (LOGiT®, Sealed Unit Parts Company, New Jersey, USA) housed in water-tight plastic containers (Figure 1). Air temperatures were measured in the shade at a height of 2 m. Soil temperatures were measured at two depths, 5 cm and 20 cm. Den cavity thermocouple wires were placed in cracks or holes (1 – 2 m depths) in which snakes were visually observed crawling in/out of, or suspected of leading to denning areas. Data loggers were programmed to begin recording temperatures in early

October during ingress, when snakes began returning to the denning areas for winter.

Data logging ceased after all snakes had egressed and evacuated the denning area in the spring.

Operative Snake Models

Operative models were used to monitor the availability of surface temperatures for snakes in the denning area (Peterson *et al.*, 1993). Operative snake models were constructed of 2.25 cm diameter copper pipes, cut into 20 cm segments and painted grey with two black bands to approximate the skin reflectance of a *C. horridus* (Peterson *et al.* 1993; Wills & Beaupre, 2000). A miniature data logger (iButton®, 1922L, 3.1 g, Maxim Integrated Products, California, USA) was placed inside each operative model to record temperatures every 1 h throughout hibernation. Both ends of the models were sealed with a black rubber stopper and a golf tee extending inward to keep the iButton® in the center of the model (Figure 2). Twenty-seven operative snake models were placed in varying thermal exposure around the hibernacula. Fifteen operative models were placed in direct sunlight on different substrates (i.e., leaf litter, soil, rock) to measure the warmest available temperatures during the day, which hypothetically would be basking surfaces for snakes. Twelve other models were placed in the shade on the same substrates (i.e., leaf litter, soil, rock) to capture the available surface temperatures not exposed to solar radiation (Figure 2). Operative models were used to define the maximum (T_{eMax}) and minimum (T_{eMin}) environmental temperatures available to snakes but also served as a

reference, along with the other environmental temperatures, for determining times that snakes emerged to the surface during winter.

Snake Capture and Radio Transmitters

During the ingress and egress periods of 2011 and 2012, snakes were captured utilizing drift fences and funnel traps or opportunistic encounters via visual searches. Each snake was anesthetized for transmitter implantation using an isoflurane vapor inhalant delivered through a clear plastic tube. While anesthetized, the following morphological data were collected: snout-to-vent (SVL) and tail length to the nearest 0.1 cm, mass to the nearest 0.1 g, sex of the snake by use of cloacal probe, ventral and subcaudal scale counts, rattle segments, and the snake's overall body condition (Table 1). Each snake also received subcutaneous implantation of a passive integrated transponder (PIT) tag for long-term individual recognition. Miniature data loggers (iButtons®) were attached to the end of the battery pack of the radio transmitter and dipped in a 1:1 ratio of paraffin and beeswax prior to implantation into the peritoneal cavity. Two different Radio transmitter models (SB-2 and SI-2; Holohil Systems, Ottawa, Ontario, Canada) were used depending on the size of the snake and the time of implantation. Snakes received a radio transmitter (with attached iButton®) that did not exceed 5% of their body mass (mean = 2.0%, SE = 0.3, range = 0.7 – 4.7%) using surgical techniques similar to Reinert & Cundall (1982) (Table 2). Each post-operative snake was placed in an environmental chamber (27 – 30°C) for 2 – 3 days to promote undisturbed wound healing before being released at the site of capture. All snake captures

and surgeries were conducted in accordance with IACUC protocol # 12 – 009 (Appendix A).

Radio Telemetry and Movement

Snake relocations were recorded 3 – 4 times per week over the course of hibernation (late-September through mid-May) using a 3-element yagi antenna and handheld receiver (Wildlife Materials International, Inc., Illinois, USA) following release at their capture site. The following data were collected at each snake location: 1) snake movement, behavior, and visibility, 2) micro- and macro- habitat variables, and 3) environmental conditions (e.g., cloud cover, precipitation). Snake locations were initially recorded with a handheld GPS unit (Garmin GPSMAP 76CSx, Garmin International, Inc., Kansas, USA) (accuracy < 5 m) during the beginning of ingress while snakes were making large movements (> 20 m) around the hibernaculum. Once large scale movements ceased, small scale movements (< 20 m) were recorded with a 15 ± 0.1 m measuring tape and a handheld compass for directionality. At each new location, a colored flag (Forestry Suppliers Inc., Jackson, Mississippi, USA) was placed in the substrate for individual recognition of movement patterns.

Data Analyses

Hibernation length was calculated from the ingress date (first day of hibernation) to the egress date (last day of hibernation). Ingress dates were defined as when an individual had remained underground in the same location for a minimum of 10 days.

Egress dates were defined as when an individual had emerged from the hibernaculum in the spring and was located in a drift fence trap surrounding the denning area, or if they had moved at least 50 m away from the denning area. A two-sample Student's t-test was conducted to test for statistical differences between ingress dates, egress dates, and total length of hibernation between years. Mean T_{bs} from year 1 and year 2 were compared using a Student's t-test to determine statistical differences between years. A one-way repeated measure ANOVA and a Holm-Sidak multiple comparison post-hoc test was run on monthly snake T_{bs} . Basking events were identified by visual observations of individuals on the surface or by analyzing body temperature (T_b) profiles from miniature data loggers. Non-observed basking events were identified if snake T_b increased $\geq 5^\circ\text{C}$ during a heating event. Basking duration times were estimated from the hour of emergence (beginning of elevated T_b) until the individual retreated back underground (marked by a steep decrease in T_b or when the T_b became lower than minimum operative models). A series of Student's t-tests were performed to determine any potential differences between years for environmental temperatures (5 cm soil profiles, 20 cm soil profiles, and 2 m shaded air temperatures). A one-way repeated measure ANOVA and Holm-Sidak multiple comparison post-hoc tests were performed to determine monthly differences in environmental surface temperatures (snake operative models). A series of correlation analyses was performed to determine a correlation value between snake T_b profiles and subterranean environmental temperatures (5 cm soil profile, 20 cm soil profile, and a 2 m denning cavity). Differences in total number of movements were performed using a two-way ANOVA to identify effects of age class or sex, and a

Friedman repeated measure ANOVA on Ranks to test differences in mean number of movements by month. Differences by month for mean distance traveled during hibernation were performed using a one-way repeated measure ANOVA followed by a Holm-Sidak post-hoc test. Yearly differences in mean distance traveled were performed using a Student's t-test. A correlation analysis was performed to determine the correlation value of total distance traveled and total number of movement bouts. A two-way ANOVA was used to analyze effects of age class and sex on total distanced traveled. A significance level of 0.05 was used to determine statistical differences using the program SigmaStat 3.1 (Systat Software Inc., Point Richmond, USA).

RESULTS

Hibernation and Denning

All snakes started to return to their winter denning areas from their respective summer activity areas in late September and throughout October. The date of ingress (mean \pm SE) (year 1: 27 October 2011 \pm 5.5 days; year 2: 8 October 2012 \pm 3.7 days, $t = 2.707$, $P = 0.053$, $df = 4$) and egress (year 1: 23 March 2011 \pm 11.2 days; year 2: 11 April 2012 \pm 4.6 days, $t = -1.588$, $P = 0.210$, $df = 3$) were not statistically different between years, while the total number of days hibernating (year 1: 146 \pm 8.1 days; year 2: 184 \pm 6.6 days) were statistically different between years ($t = -3.571$, $P = 0.016$, $df = 5$) (Table 3). Timber rattlesnakes in this population historically den in a rocky ridge near the north-central boundary of the nature preserve to which all but two individuals (#8 and #10) returned. Snakes that returned to the ridge chose southerly-exposed rocky outcrops and tree root systems which lead to underground cavities and open denning areas (Figure 3). Many individuals possess high den site fidelity and return to the exact locations yearly (V. Cobb, unpublished data) often within 1 m. Individuals #8 and #10 not returning to the communal denning area during ingress 2012 are thought to have hibernated individually or with un-telemetered snakes. Snake 8 hibernated underground at the base of an oak (*Quercus*) tree (DBH \approx 80 cm) and rocks approximately 200 m downhill of the communal den. This individual had been captured initially at the communal hibernaculum during egress in spring 2012, so it appears that some snakes may periodically hibernate in alternate localities. Snake #10 was not initially captured at the

communal den, and did not hibernate near the ridge, but rather underground in a rocky hole in a mixed oak – redcedar forest (1.3 km from communal denning ridge); it is currently unknown if this individual ever hibernates at the communal ridge hibernaculum.

Winter Activity and Movements

The frequent monitoring of not only snake T_b s, but also individual locations via radio telemetry (year 1: 215 relocations of 4 individuals; year 2: 731 relocations of 13 individuals) allowed for close monitoring of small scale movements throughout the hibernation period. No statistical difference was found in the median number of movement bouts between months ($\chi^2_{6,17} = 6.464, P = 0.373$), although a difference between years did exist ($t = -2.231, P = 0.044, df = 13$), likely due to a small sample size ($n = 4$) in during hibernation 2011 - 2012. The total number of movements bouts were not statistically different between age classes ($F_{1,1} = 1.941, P = 0.185$) or sexes ($F_{1,1} = 2.546, P = 0.133$). Individuals moved on average 6.1 ± 1.2 times (Figure 4) accumulating a total distance of 146.4 ± 35.5 m (Figure 5). Individuals that traveled the furthest throughout hibernation did not necessarily move the greatest number of times; the correlation between total distance traveled and total number of movement bouts was weak ($r^2 = 0.1259$) (Figure 6). The mean distance snakes traveled between years ($t = -0.252, P = 0.814, df = 4$) were not statistically different, yet a statistical differences between months did exist ($F_{1,6} = 2.949, P = 0.011$) (Figure 7). In addition, no statistical differences were in the total distance traveled between age classes ($F_{1,1} = 0.453, P = 0.512$) or sexes ($F_{1,1} = 0.0837, P = 0.777$).

Environmental Temperatures

Environmental conditions were similar between years for 5 cm soil profiles ($t = 0.930$, $P = 0.371$, $df = 12$), 20 cm soil profiles ($t = 0.624$, $P = 0.544$, $df = 12$), or 2 m shaded air temperatures ($t = 1.544$, $P = 0.148$, $df = 12$). Operative snake models were similar among years as well ($t = 1.345$, $P = 0.205$, $df = 11$) but were different by month (year: $F_{1,6} = 7.453$, $P = 0.014$), which is not surprising in a geographic area where seasonal variation in winter months can be substantial. During mid-winter emergence events, mean air temperature was variable (mean = $19.3 \pm 0.8^\circ\text{C}$, range = $8.2 - 27.4^\circ\text{C}$) as was operative snake model temperature (mean = $29.3 \pm 1.5^\circ\text{C}$, range = $6.5 - 55.0^\circ\text{C}$), making it difficult to use environmental temperatures as an indicator of when basking events may occur.

Body Temperatures and Basking Events

A total of 48,223 T_b s was recorded from adult ($n = 11$) and sub-adult ($n = 2$) timber rattlesnakes throughout hibernation (Figure 8). Mean snake T_b s between year 1 ($11.9 \pm 1.5^\circ\text{C}$) and year 2 ($11.0 \pm 1.2^\circ\text{C}$) was not statistically different ($t = 0.703$, $P = 0.495$, $df = 12$). The overall mean T_b (11.0°C) in this population of rattlesnakes closely resembled those in previous studies (*C. horridus*, mean $T_b = 10.5^\circ\text{C}$, Brown, 1982; *C. viridis*, mean $T_b = 10.0^\circ\text{C}$, Jacob & Painter, 1980; *C. oreganus*, mean $T_b = 4 - 10^\circ\text{C}$, Cobb & Peterson, 2008). Snake T_b profiles highly correlated ($r^2 = 0.8903$) with den cavities (~2 m) suggesting snakes are hibernating at depths of approximately 2 m

underground (Figure 9). Snake T_b s remained relatively stable while snakes were underground, but periodically individuals emerged to the surface to bask (Figure 10). The minimum T_b experienced by any individual in year 1 (5.9°C) and year 2 (1.1°C) remained above freezing regardless of ambient temperatures periodically dropping below 0°C (Figure 11). Snake T_b s varied over winter by gradually declining in the fall, stabilizing during mid-winter, and increasing again in the spring. Therefore it is not surprising that mean snake T_b s during hibernation were statistically different between months ($F_{7,6} = 102.871, P < 0.001$) but no effect was found for age ($F_{1,1} = 0.013, P = 0.909$) or sex ($F_{1,1} = 0.930, P = 0.339$). Miniature data loggers from one individual in year 1, and three individuals in year 2 could not be retrieved due to premature radio transmitter failure ($n = 3$) or predation ($n = 1$).

Throughout hibernation, 11 of 14 snakes exhibited multiple heating events (i.e., basking) in which the maximum T_b achieved by any individual was 33.7°C (Figure 11). Basking or heating events were recorded (year 1: $n = 12$, year 2: $n = 48$) by analyzing temperature profiles from iButtons® (Figure 10) and visual observations (Figure 12) of surface basking snakes during hibernation. Snakes that exhibited heating or basking events typically emerged from their hibernaculum between 1100 – 1300 h. Snakes were often observed coiled in leaf litter or near rocks in close proximity to the hibernacula entrances. Basking snakes had T_b s of $19.1 \pm 0.4^\circ\text{C}$ and remained on the surface with elevated T_b s for 5.3 ± 0.4 h. Upon emergence, snakes were able to attain a maximum T_b in 2.3 ± 0.5 h, increasing their T_b $13.6 \pm 0.8^\circ\text{C}$ above hibernating T_b . One individual

(#13) increased its T_b by 26.5°C , from a hibernating T_b of 3.6°C to a basking T_b of 30.1°C in as little as 1 – 2 h.

DISCUSSION

Hibernation and Denning

Due to the vast geographic distribution of the timber rattlesnake, certain aspects of hibernation (e.g., ingress date, egress date, and length of hibernation) are likely to vary contingent upon climatic variation. Timber rattlesnakes in this study exhibited a hibernation period of approximately 5 months. During snake migration to the hibernacula, it was common to find individuals below ground or under rocks as temperatures decreased. Snakes often shuttled above and below ground prior to the beginning of hibernation. Although not statistically different, ingress and egress dates varied somewhat by year which is likely associated with variations in environmental temperatures. Much of the variation in ingress/egress dates are likely attributed to a small sample size during winter 2011 ($n = 4$). Egress dates were likely delayed in spring 2013 due to a period of cold weather in mid-late March resulting in significantly longer hibernation period in year 2. A majority of snakes (100% in year 1 and 62% in year 2) hibernated at communal den sites with high den site fidelity, as is common in northern populations (Brown, 1982; Brown *et al.*, 1982; Clark *et al.*, 2008), while others hibernated individually (38% in year 2), as commonly observed in southern populations (Waldron *et al.*, 2006; Mohr, 2010). Winter environmental temperatures are not as extreme in Tennessee as they are in the northeastern United States, but hibernation is still necessary for *C. horridus* to survive the winter months.

Winter Activity and Movements

Hibernation is typically considered to be a period of little or no activity (Gilles-Baillien, 1974; Gregory, 1982); therefore, many studies investigating movement and activity of reptiles focus on the “active season” (spring, summer, and fall) (Blouin-Demers & Weatherhead, 2001; Waldron et al., 2006). As snakes retreat underground for winter, researchers often reduce the frequency of telemetry relocations to once every week (Brown, 1982; Cobb & Peterson, 2008) or once per month (Waldron *et al.*, 2006). In this study, snakes were monitored 3 – 4 times per week in my study, which allowed for a detailed examination of snake movements and activity. While a few studies document subterranean movement in the early months of hibernation (Sexton & Hunt, 1980; Macartney *et al.*, 1989; Cobb & Peterson, 2008), Brown (1982) did not document any movement until just before spring emergence. Snakes in my study often made small movements (>10 m) between different micro-sites around the hibernacula during ingress and egress, but also moved throughout hibernation. It is unknown as to the cause for this shuttling behavior, but it is hypothesized that snakes are responding to changes in subterranean thermal gradients (Viitanen, 1974; Brown, 1982; Graves & Duvall, 1990) or following scent trails of conspecifics (Reinert & Zappalorti, 1988b).

While winter movement may be uncommon (Brown, 1982) or at least undocumented in *C. horridus*, winter movement and activity was observed to be fairly common in my study. A majority of the large scale movements (> 100 m) were distributed in a bimodal pattern near ingress and egress as snakes were transitioning from

the active season to hibernation or vice-versa, which most likely contribute to the differences in mean monthly distance traveled (April being responsible for 69% of snakes egressing). Cobb and Peterson (2008) similarly found that underground movements of *C. oregonus* in Idaho were common in the first two months of hibernation as snakes shuttled further away from the hibernaculum entrance. During mid-winter in my study, most movements ranged between 1 – 10 m in length, and were often associated with basking events. Some individuals made lengthy movements early in the hibernation period which increased their overall distance traveled, but remained relatively sedentary through the winter, while others made small but frequent movements.

Body Temperatures and Basking Events

Few studies have documented the T_b s of rattlesnakes throughout hibernation, especially at a high enough sampling rate to detect brief temperature changes. Prior to the miniaturization of high capacity data loggers, T_b s were collected from temperature sensitive radio transmitters. Although accurate, this technique only allows the collection of T_b data at instantaneous times when the researcher is locating the snake via telemetry. Some researchers (e.g., Beaupre & Beaupre, 1994; Brown and Weatherhead, 2000; Cobb & Peterson, 2008) have created automated systems where the receiver continuously records the transmitter signal rates or interpulse intervals, which can later be calibrated to calculate the individual's T_b .

Mid-winter emergence or basking events are not well documented in the literature; often only anecdotal observations exist because mid-winter basking is rarely observed by researchers (Stewart, 1965; Jacob & Painter, 1981; Sanders & Jacob, 1981; Sexton & Marion, 1981). Without rapidly monitoring movements or T_{bs} these behavioral events can be missed or overlooked. Although radio telemetry is useful for documenting behavior, movement, and habitat use, telemetry only provides information from the time of relocation, and can underestimate small scale movements or short term behaviors, such as basking. Combining multiple tools (radio telemetry and high capacity data loggers) allows for a better understanding of complex behaviors (i.e., basking) between telemetry relocations. Monitoring T_{bs} at rapid intervals (every hour) captures heating events regardless of visual observations via telemetry by researchers (Figure 12).

To my knowledge, my study marks the highest frequency of mid-winter basking events known for this species, which may well be a result of rapid T_b sampling. Alternatively, this population is situated geographically where mid-winter warming events are common and may allow snakes to emerge frequently. The importance or benefits of mid-winter basking is unknown, but because it was common among almost all the individuals (11 of 14 exhibited multiple emergence events), it is hypothesized there is some value to be gained from mid-winter emergence.

Although unlikely, it may be possible that snakes are emerging to forage. An increase in snake body mass was documented from two individuals (#5 and #14 gained 5.3% and 0.5%, respectively) from a pre- to post-hibernation measurement (Table 4).

Both individuals were male and exhibited multiple basking events (#5 = 10; #14 = 5), movements (#5 = 10; #14 = 6), and large total distance traveled throughout winter (#5 = 210.7 m; #14 = 302.7 m). The large number of basking events, movements, and distance traveled lead one to believe that these snakes should have indeed lost weight from elevated metabolic activity over winter. Because this was not the case, it is possible that these snakes may have been able to feed sometime between pre- and post- hibernation weight measurements. One would expect that the benefits of emergence would outweigh the costs, otherwise this behavior is counterproductive. Snakes that emerge to bask on the surface are not only exposed to predation, but the increase in T_b elevates metabolic activity, potentially burning through lipid storage necessary for winter survival.

Another possible explanation is that snakes may increase their T_b and metabolic activity in order to increase the immune system response. One individual (#5) tested PCR positive for *Ophidiomyces ophiodiicola* (formerly *Chrysosporium* spp.) after visual inspection of odd facial abnormalities upon returning to the hibernacula in the fall of 2012. This individual exhibited elevated activity (highest number of movements $n = 20$, and basking events $n = 10$, of any individual) throughout hibernation. It is unknown if this behavior is driven by the fungal infection because another individual (#1) tested positive in the spring of 2013 during egress without the same intensity of movements ($n = 5$) or basking events ($n = 2$). Non-infected snakes also exhibited high movement rates and basking events, so it is unclear as to what impact the fungal infection has on the behavior of hibernating snakes; further investigation is warranted. However, the omission of

infected snakes (#1 and #5) from this study does not negate the quantity of mid-winter emergence events and movement bouts by snakes during a period when activity is thought to be minimal.

Energetic Costs of Mid-Winter Basking

Hibernation is not only a mechanism to escape lethal surface temperature, but it also allows for suppressed metabolic activity (Bennett & Dawson, 1976; Congdon *et al.*, 1982). Snake bioenergetics rely heavily on environmental temperatures for thermoregulation, as is illustrated by physiological and behavioral performance curves for such activities as foraging, digesting, and predator avoidance (Stevenson *et al.*, 1985; Lillywhite, 1987; Peterson *et al.*, 1993). While hibernating, suppressed T_b s allow for a reduced metabolic rate. Snake metabolism during hibernation and during extended non-feeding times is fueled from stored glycogen (Zani *et al.*, 2012) and/or lipid reserves (McCue *et al.*, 2012) reserves acquired from active-season foraging. Agugliaro (2011) states that *C. horridus* exhibits a suppression of metabolic activity at low body temperatures (5 – 9°C) which allows for some level of metabolic function, but is much reduced from that of the active season.

Overwintering survivorship could be reduced if a snake expends its energy stores too rapidly by engaging in frequent mid-winter basking. To test how mid-winter basking influences metabolism, equations were used from Zaidan & Beaupre (2003) to estimate baseline metabolic rate $[mL\ CO_2/h = 0.0068W^{0.78}10^{0.03T}]$; where W = snake weight (g), T

= body temperature (°C)] at $T_{bs} > 11^{\circ}\text{C}$, and Agugliaro (2011) to estimate suppressed overwintering metabolic rate [$\text{mL CO}_2/\text{h} = 0.001408W^{0.9297}$; where W = snake weight (g)] at $T_{bs} \leq 11^{\circ}\text{C}$. Data from my study were used to estimate metabolic rates (mean snake weight = 665.8 g, mean hibernation length = 175 d, mean basking duration = 5 h, mean increase in T_b during a basking event = 14°C , and hypothetical T_{bs} ($> 11^{\circ}\text{C}$ for elevated metabolic rate; $\leq 11^{\circ}\text{C}$ for suppressed metabolic rate). Estimated daily metabolic rates for *C. horridus* at my study site were twice as high on days when basking occurred ($2.035 \text{ mL CO}_2/\text{h}$) than when snakes did not emerge to the surface ($0.942 \text{ mL CO}_2/\text{h}$). The total energy expenditure (resting metabolic rate) for a snake that remained in the suppressed metabolic state ($\leq 11^{\circ}\text{C}$, and therefore did not bask) would utilize 128.9 kJ for the entire hibernation period. To meet this hibernation energy budget, a 665.8 g snake would need to consume 24 g of rodent (approximately one deer mouse, *Peromyscus maniculatus*; assuming 8 kJ/g prey wet mass and metabolizable energy content of 80%; Zaidan & Beaupre, 2003) with a total specific dynamic action (SDA; the energetic cost of digestion and its assimilated ingested food) of 24.9 kJ. If snakes emerge from hibernation to bask then additional energy stores begin to be depleted. The estimated total hibernation energy budget for a *C. horridus* basking 10 times throughout winter, the maximum observed in my study, requires 135.1 kJ which could be acquired with one 25 g rodent (SDA = 24.9 kJ). Surface basking of 25 times (once per week for 175 days) requires 144.3 kJ (one 27 g rodent, SDA = 25.9 kJ), and basking 175 times (everyday throughout hibernation) requires 236.5 kJ (one 44 g rodent, SDA = 46.5).

Although snakes can elevate their T_b s through behavioral thermoregulation (i.e., winter basking on sunny days), high T_b s cannot be elevated for long periods of time because suitable surface temperatures only exist during the warmest period of the day. Although basking events of 5 h length during winter increase the mean daily metabolic rate to twice that of non-basking snakes, the metabolic cost remains low as the suppressed metabolic rate is up to 10x lower than active season metabolic rates (Zaidan & Beaupre, 2003; Agugliaro, 2011). The metabolic cost of basking may have a less effect on the overall energy reserves of *C. horridus* allocated for hibernation than originally thought. The energetic costs associated with mid-winter basking can be offset by acquiring an additional 25 – 44 g of rodent during the active season, which is a fraction of the yearly intake by healthy timber rattlesnakes (1250 – 1550 g per year; Clark, 2006). Other factors hypothesized to contribute to mid-winter emergence (i.e., potential foraging, elevated immune-response, or suitable surface temperatures for activity) may have a larger impact in the occurrence of mid-winter basking. While the potential impact of climate change has been predicted to have detrimental impacts on thermoregulation in some reptile species (Sinervo *et al.*, 2010), the risks of depleting energy stores during hibernation appear to be minimal for this species.

CONCLUSIONS

Temperate reptiles typically have two major activity seasons: the *active* season which includes mate searching, parturition, and foraging, and the *inactive* season which is a period of reduced activity and suppressed metabolic activity. A majority of our overall knowledge of reptile behavior, movement, and general ecology is focused around the active season, when subjects can be observed and monitored. Minimal information is known about the behaviors and activities of reptiles during the dormant or inactive season primarily because they are difficult to study. With the development of new technology (e.g., high capacity data loggers, automated telemetry units) researchers are now able to learn in more detail the daily and seasonal patterns of hibernating reptiles.

With the conclusion of this study, a more detailed view into the activity and behaviors of hibernating snakes is presented to begin filling in the gaps in the literature of snake hibernation. A majority of the literature on snake hibernation has been conducted in the northern regions of this species range. Frequent mid-winter movements and basking events are not well documented in northern populations (Brown, 1982) or even populations at the same latitude (Beaupre, northwest Arkansas; pers. comm.) as this current study. The energetic costs associated with mid-winter basking are low, even with a substantial number of basking events (e.g., once a week), which can be offset by the consumption of just a single 27 g rodent. The depletion of energy reserves is generally accepted as a primary driver for the importance of hibernation, but other factors such as suitable surface temperatures for activity may have a larger impact. Mid-winter basking

does not threaten to deplete significant energy stores, which opens investigations into alternate hypotheses regarding the potential benefit of frequent mid-winter basking.

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Table 1: Morphological characteristics of *Crotalus horridus* measured prior to radio transmitter and data logger implantation; SVL = snout-to-vent length.

	Snake ID	Sex	Age Class	SVL (cm)	Mass (g)
Hibernation 2011 - 2012	*1	Male	Adult	98.0	702.0
	2	Female	Adult	98.0	952.0
	*3	Female	Adult	100.0	715.0
	*4	Female	Adult	114.0	972.0
Hibernation 2012 - 2013	1	Male	Adult	108.5	686.0
	3	Female	Adult	98.3	660.6
	4	Female	Adult	112.5	951.0
	5	Male	Adult	88.0	410.0
	6	Female	Sub-adult	69.8	203.0
	7	Female	Adult	91.5	638.6
	8	Female	Adult	103.0	566.1
	9	Female	Adult	102.5	828.9
	10	Female	Adult	104.0	811.0
	11	Female	Adult	108.0	994.0
	12	Male	Sub-adult	70.5	180.6
	13	Female	Sub-adult	66.8	204.0
	14	Male	Adult	123.5	1370.6
				Mean	97.5
			SE	16.0	77.6

*Three snakes (1, 3, and 4) were monitored during both hibernation periods.

Table 2: Body weights of *Crotalus horridus* and their respective implants of radio transmitters (Holohil Systems Inc.) and dataloggers (iButton®) at time of implantation.

	Snake ID	Snake Weight (g)	Transmitter Type	Transmitter Weight (g)	% of Body Weight
Hibernation 2011 - 2012	*1	702.0	SB-1	7.5	1.1
	2	952.0	SB-1	7.2	0.8
	*3	715.0	SB-1	7.2	1.0
	*4	972.0	SB-2	8.8	0.9
Hibernation 2012 - 2013	1	686.0	SB-2T	8.8	1.3
	3	660.6	SB-2T	8.8	1.3
	4	951.0	SI-2	15.0	1.6
	5	410.0	SI-2	12.9	3.1
	6	203.0	SB-2T	8.9	4.4
	7	638.6	SB-2T	8.5	1.3
	8	566.1	SI-2	13.5	2.4
	9	828.9	SI-2	9.0	1.1
	10	811.0	SI-2	15.0	1.8
	11	994.0	SI-2	15.0	1.5
	12	180.6	SB-2	8.4	4.7
	13	204.0	SB-2	8.5	4.2
	14	1370.6	SB-2	10.0	0.7
		Mean	696.8		10.2
	SE	77.6		0.7	0.3

*Three snakes (1, 3, and 4) were monitored during both hibernation periods.

Table 3: Hibernation characteristics of *Crotalus horridus* during two winters.

	Snake ID	Ingress Date	Egress Date	Hibernation Length (days)	Number of Movement Bouts	Total Distance Traveled (m)	Heating Events	Number of Relocations
Hibernation 2011 - 2012	*1	10/17/11	03/14/12	147	2	33.5	1	57
	2	10/17/11	--	--	1	1.7	--	44
	*3	10/29/11	03/11/12	132	3	42.0	6	55
	*4	11/05/11	04/15/12	160	6	426.0	5	59
Hibernation 2012 - 2013	1	10/06/12	04/16/13	190	5	166.6	2	55
	3	09/28/12	04/17/13	199	7	411.6	3	64
	4	10/07/12	05/14/13	217	7	362.1	2	79
	5	09/19/12	04/06/13	197	20	210.7	10	59
	6	10/28/12	03/16/13	138	3	29.4	2	27
	7	09/29/12	--	--	8	62.7	--	66
	8	10/07/12	04/10/13	183	3	133.3	1	54
	9	10/09/12	--	--	2	2.2	--	46
	10	10/13/12	04/10/13	177	2	13.7	2	51
	11	09/29/12	04/09/13	190	4	66.8	1	59
	12	10/09/12	--	--	15	168.4	10	66
	13	10/13/12	04/06/13	173	10	55.8	10	49
	14	10/23/12	04/16/13	173	6	302.7	5	56
		Mean	11-Oct	7-Apr	175.1	6.1	146.4	4.3
	SE	3.0	4.7	7.0	1.2	35.5	0.9	2.7

*Three snakes (1, 3, and 4) were monitored during both hibernation periods.

Table 4: Change in body mass of *Crotalus horridus* over hibernation with regards to movement and heating events.

	Snake ID	Sex	Hibernation Length (days)	Weight Change (g)	% Change in Body Mass	Number of Movement Bouts	Distance Traveled (m)	Heating Events
Hibernation 2011 - 2012	*1	Male	147	-16.0	-2.3	2	33.5	1
	2	Female	--	--	--	1	1.7	--
	*3	Female	132	-54.4	-7.6	3	42.0	6
	*4	Female	160	-21.0	-2.2	6	426.0	5
Hibernation 2012 - 2013	1	Male	190	--	--	5	166.6	2
	3	Female	199	-3.2	-0.5	7	411.6	3
	4	Female	217	-13.0	-1.4	7	362.1	2
	5	Male	197	20.0	5.3	20	210.7	10
	6	Female	138	-14.8	-7.4	3	29.4	2
	7	Female	--	--	--	8	62.7	--
	8	Female	183	-44.9	-7.0	3	133.3	1
	9	Female	--	--	--	2	2.2	--
	10	Female	177	-61.9	-7.6	2	13.7	2
	11	Female	190	-42.7	-4.3	4	66.8	1
	12	Male	--	--	--	15	168.4	10
	13	Female	173	-19.7	-9.7	10	55.8	10
	14	Male	173	6.2	0.5	6	302.7	5
			Mean	175.1	-22.1	-3.7	6.1	146.4
		SE	7.0	7.1	1.3	1.2	35.5	0.9

*Three snakes (1, 3, and 4) were monitored during both hibernation periods.

A



B



Figure 1: A = Environmental temperatures (soil, air, and den cavities) were measured using single-channel thermocouple wire and data loggers (LOGiT®) housed in water-tight containers; B = Data logger *in situ* at hibernaculum den cavity (~2 m depth).

A



B



Figure 2: A = Operative snake models were created from 20 cm copper pipes painted grey with two black bands. Rubber stoppers with golf tees were placed on each end to keep the iButton® in the center of the model; B = Operative snake model *in situ* placed around the denning area to estimate potential thermal minimum and maximum temperatures for snakes on the surface.



Figure 3: Communal snake hibernacula habitat along a rocky ridge.

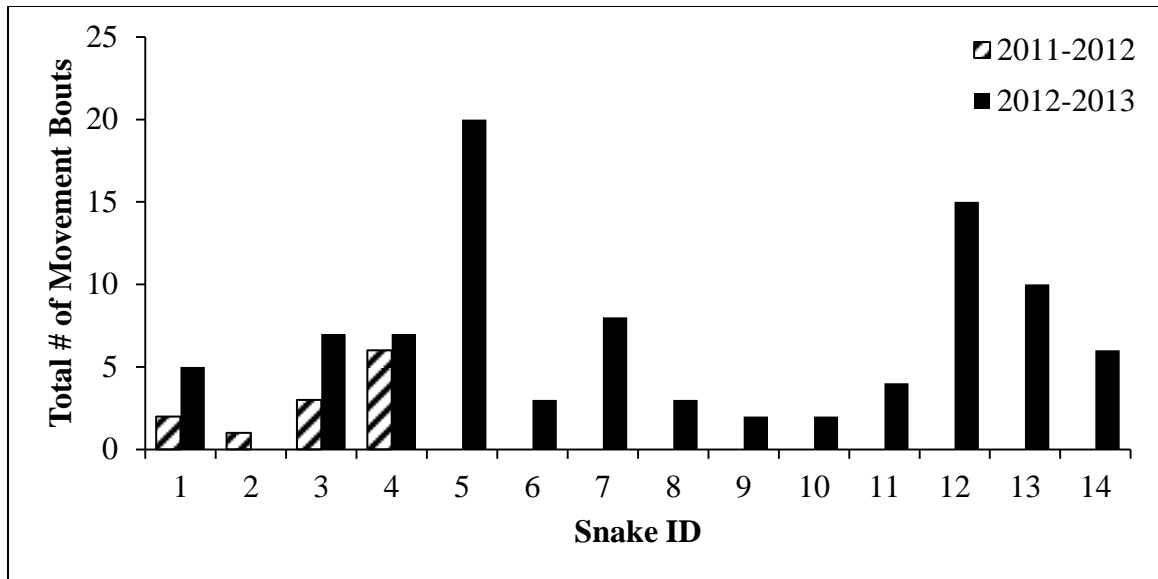


Figure 4: The total number of movement bouts (≥ 0.5 m) made by four *Crotalus horridus* during hibernation in 2011 – 2012, and 13 *Crotalus horridus* in 2012 – 2013. Three snakes (1, 3, and 4) were monitored during both hibernation periods.

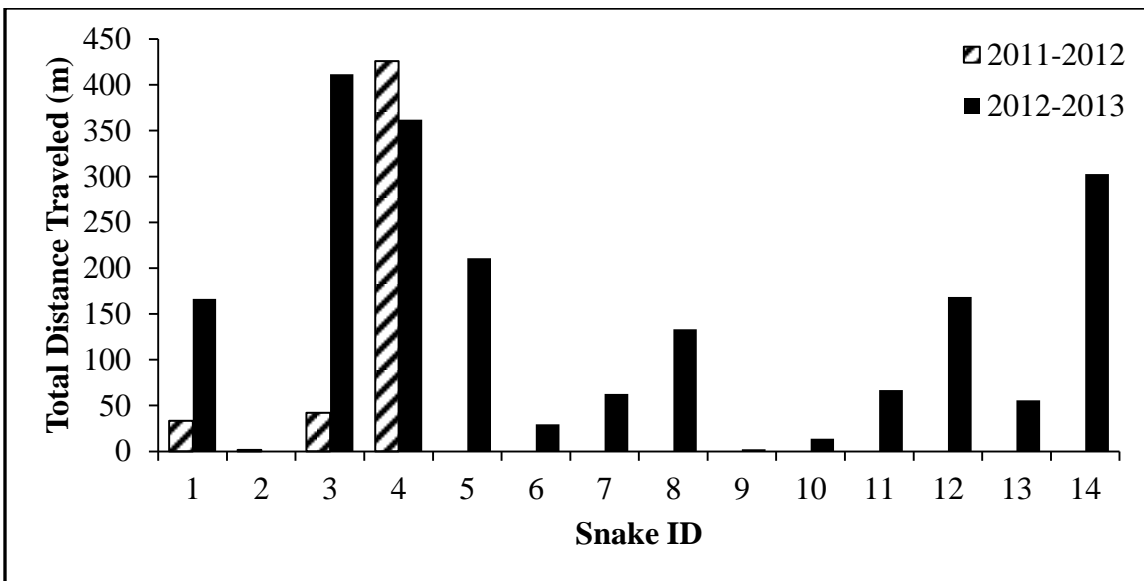


Figure 5: The total distance traveled (the sum of all movements) above and below ground made by four *Crotalus horridus* during hibernation in 2011 – 2012, and 13 *Crotalus horridus* in 2012 – 2013. Three snakes (1, 3, and 4) were monitored during both hibernation periods.

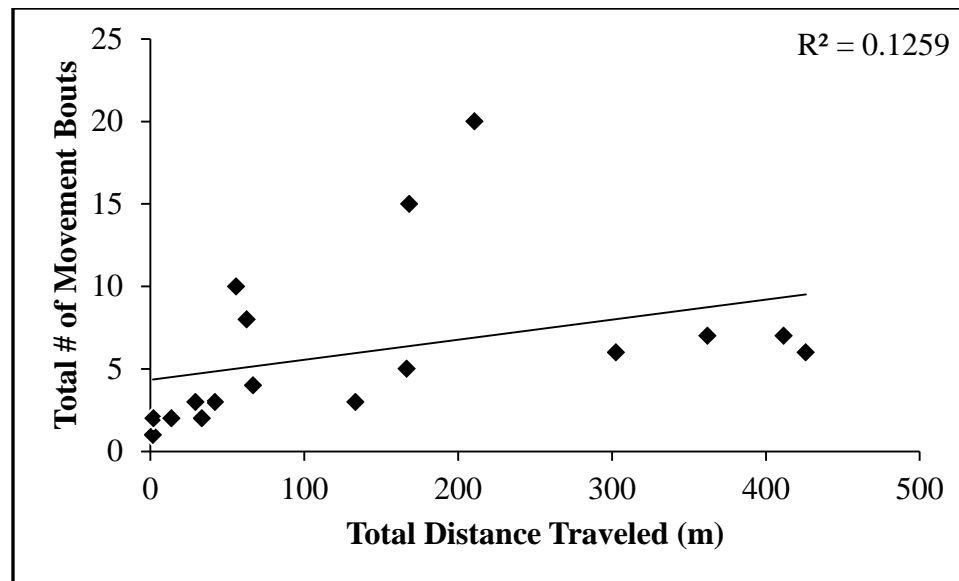


Figure 6: Correlation between total distance traveled (sum of all movements) and the total number of movement bouts (changes in location) for 17 *Crotalus horridus* across two hibernation periods (2011 – 2012 and 2012 – 2013). Three snakes (1, 3, and 4) were included in both hibernation periods.

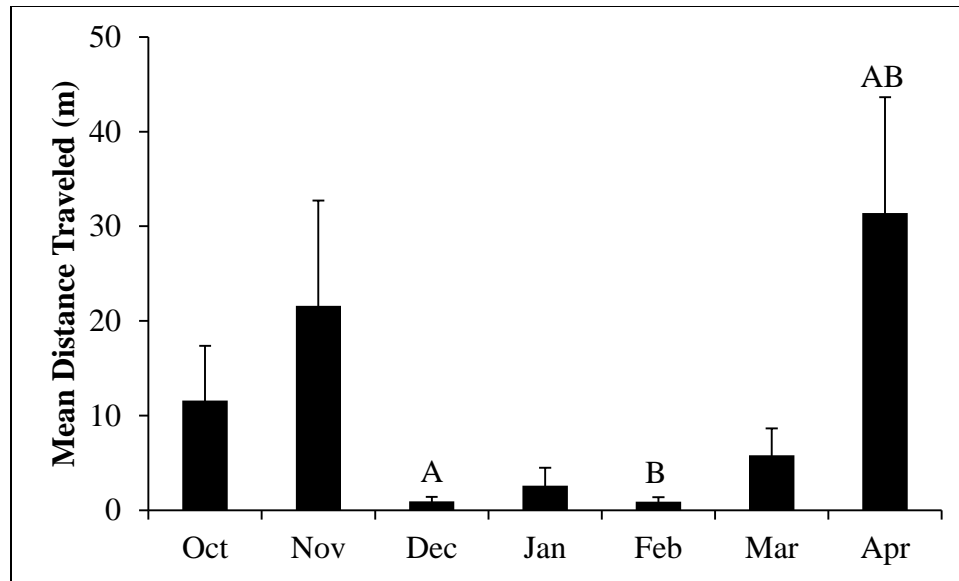


Figure 7: The mean distance traveled per month of *Crotalus horridus* during hibernation in 2011 – 2012 and 2012 – 2013. Letters mark statistical differences ($P < 0.05$). Error bars represent \pm SE.

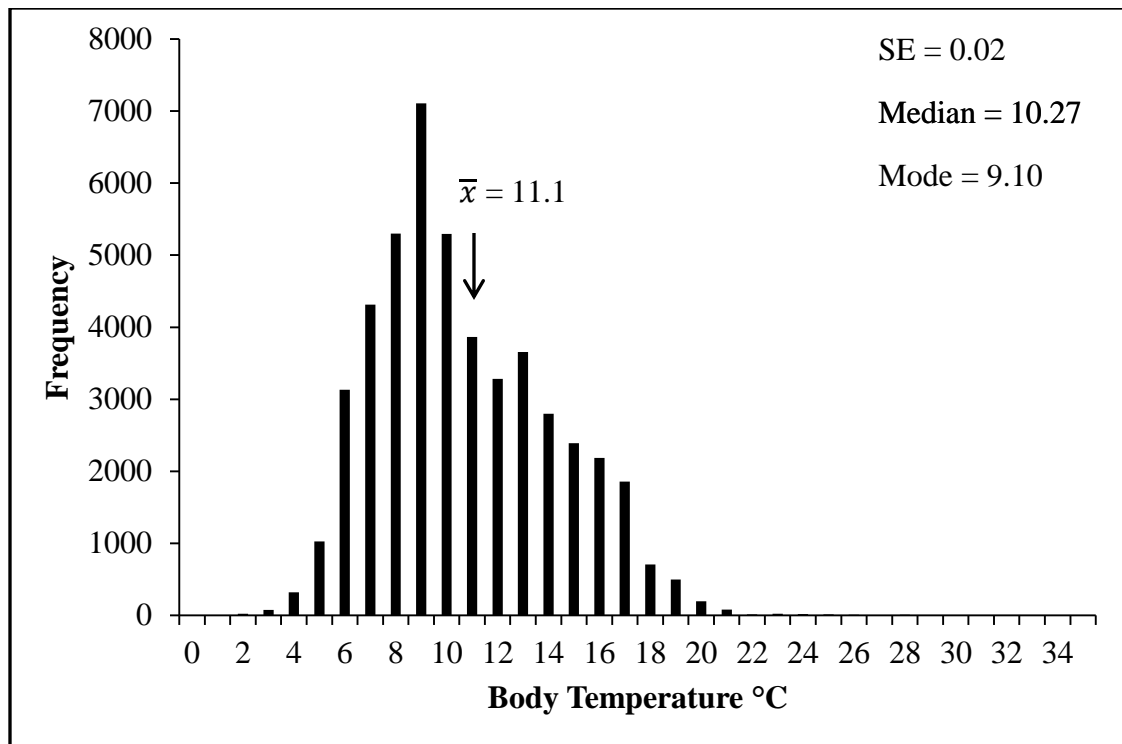


Figure 8: A frequency distribution of body temperatures ($n = 48,223$) for 13 hibernating *Crotalus horridus* during winter 2011 – 2012 and 2012 – 2013. Three snakes were monitored during both hibernation periods.

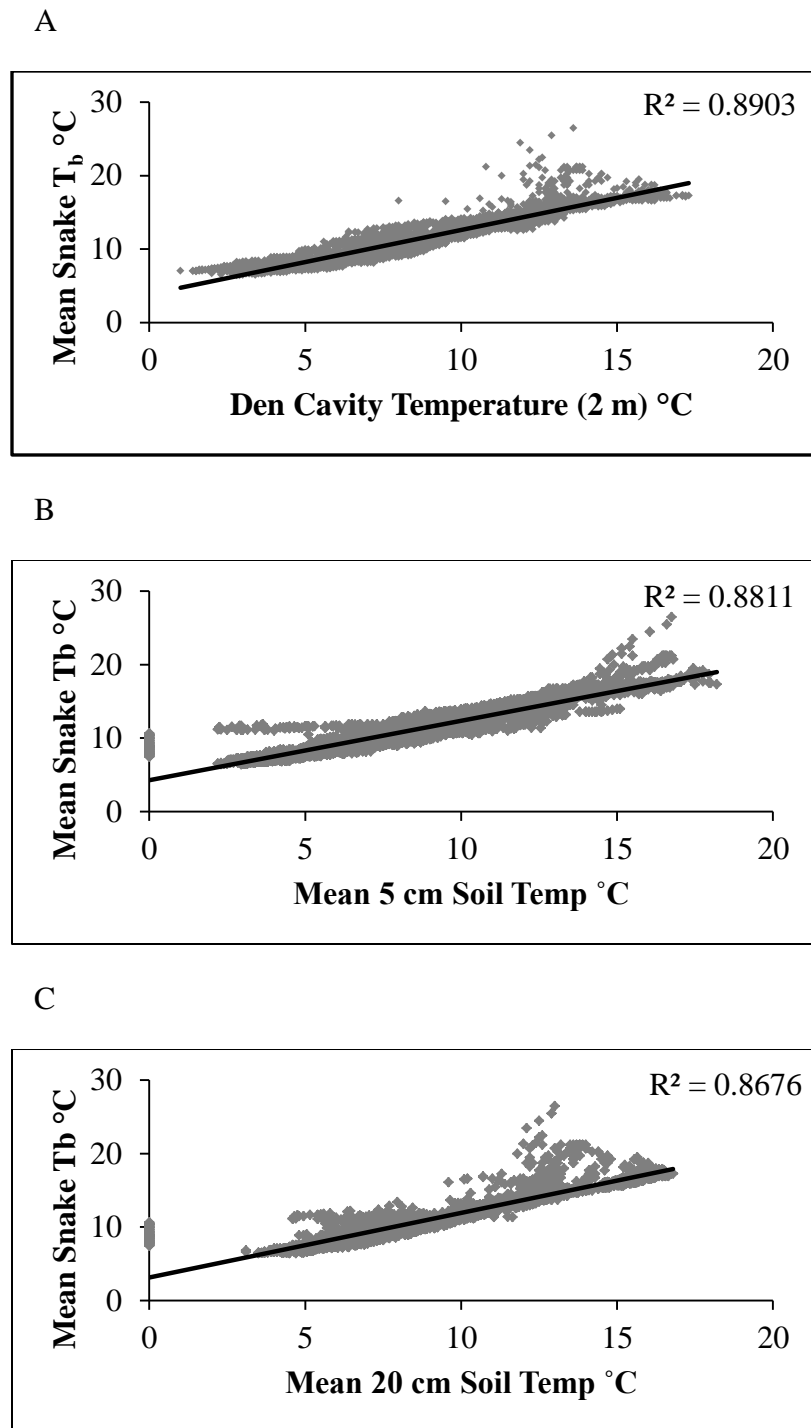


Figure 9: Correlation of mean snake body temperature (10 *Crotalus horridus* in 2012 – 2013) and environmental temperatures; A = a den cavity/rock crevasse (depth ~ 2 m); B = 5 cm soil profile; C = 20 cm soil profile.

A

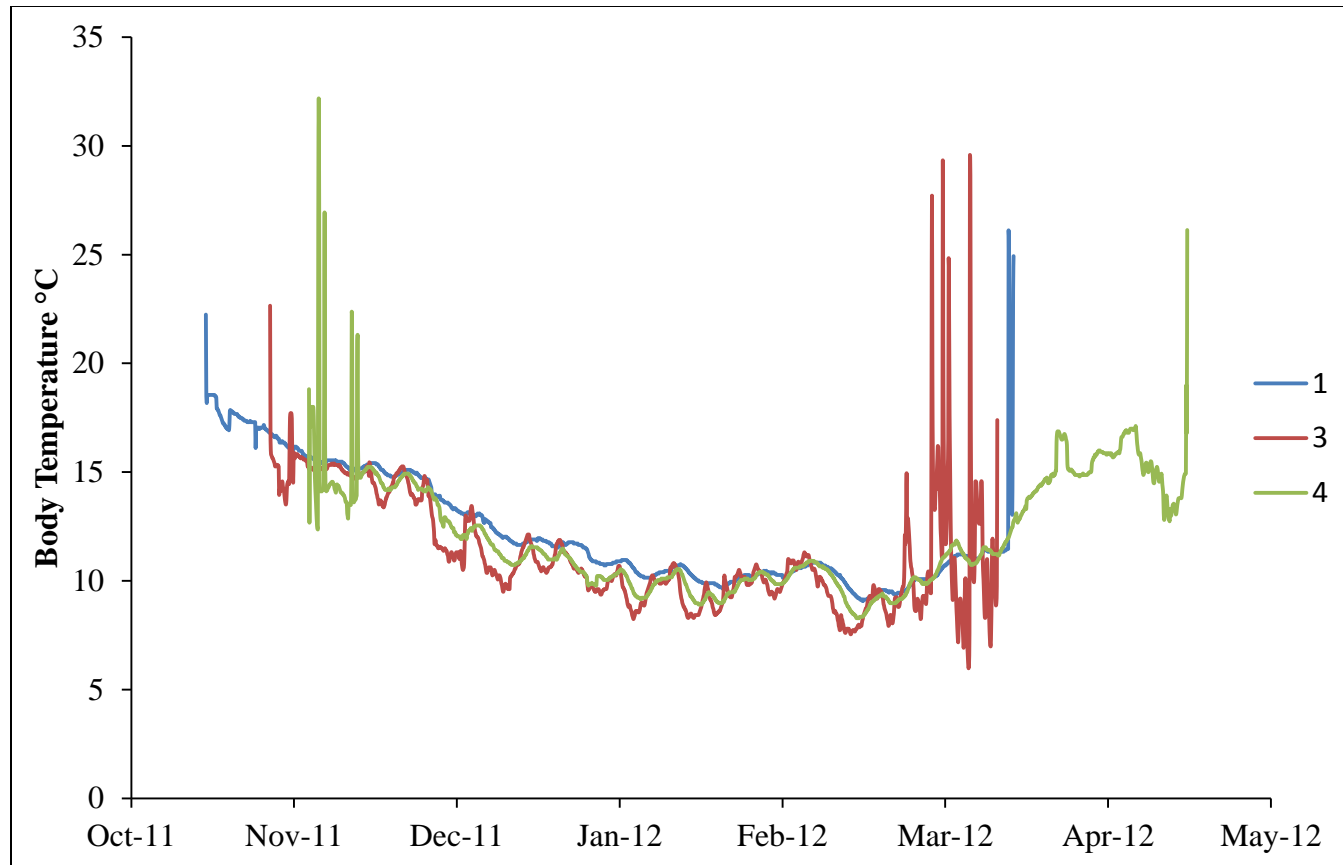


B



Figure 10: A = Snake # 12 coiled in sunlight on 12/23/2012 after emerging to the surface to bask. B = Mid-winter arboreal basking behavior of snake # 5 on 01/29/2013.

A



B

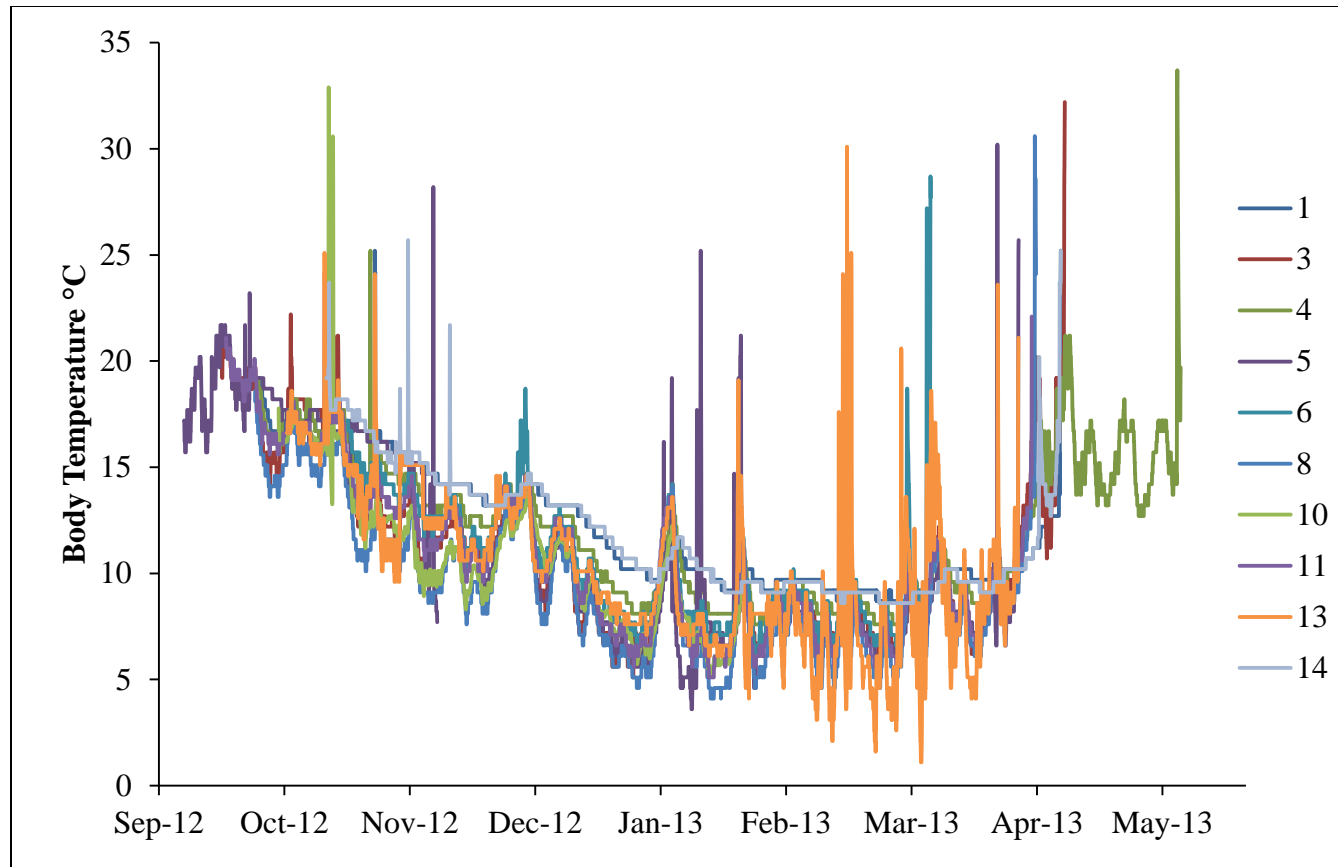


Figure 11: Body temperature profiles of 3 *Crotalus horridus* during hibernation 2011 – 2012 (A) and 10 *Crotalus horridus* during hibernation 2012 – 2013 (B). Peaks in body temperature represent basking/emergence events to which elevated body temperatures could be attained.

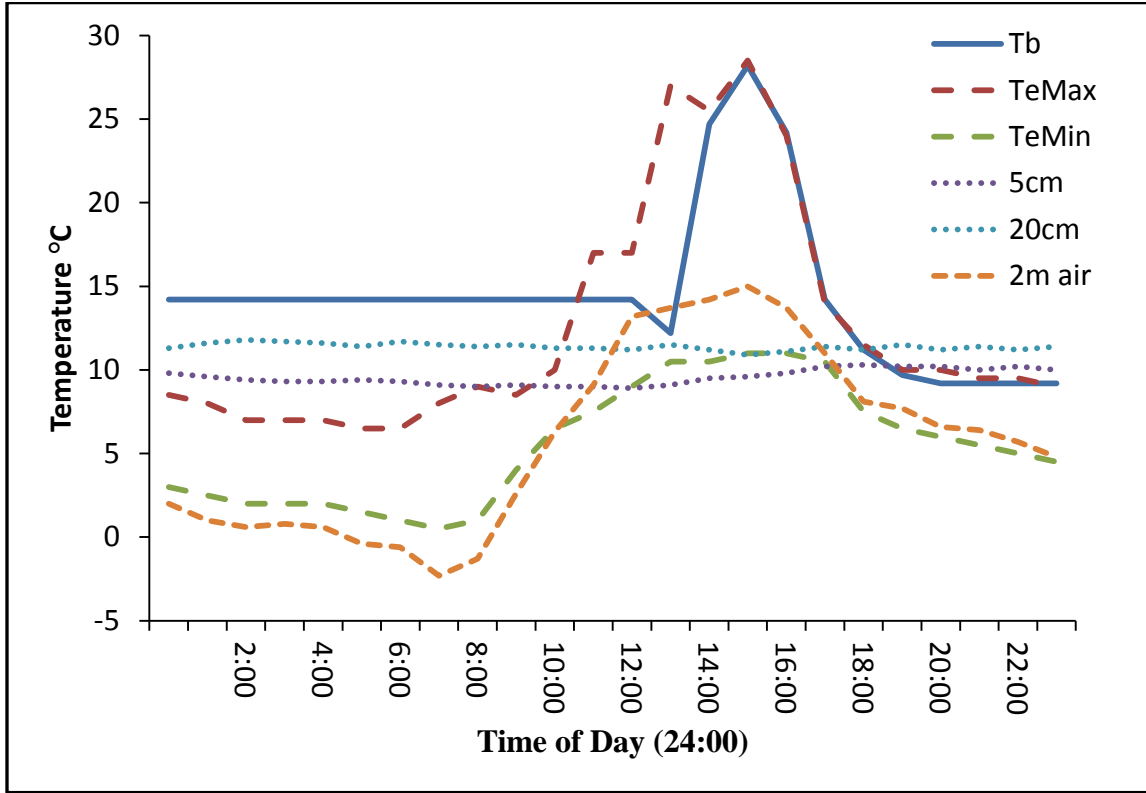


Figure 12: A snake basking event recorded from implanted miniature data loggers (snake T_b) and environmental temperatures from single channel data loggers. (T_{eMax} = thermal environmental maximum from exposed surface operative snake models; T_{eMin} = thermal environmental minimum from shaded (not-exposed) surface operative snake models; 5 cm = soil temperature at 5 cm depth; 20 cm = soil temperature at 20 cm depth; 2 m air = air temperature at shaded 2 m height).

APPENDIX

APPENDIX A: IACUC Approval Letter



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,

A handwritten signature in cursive script that reads "Emily Born".

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