DOES DIGESTION AFFECT THERMOREGULATION IN FREE-RANGING TIMBER RATTLESNAKES (*CROTALUS HORRIDUS*)?

by

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ABSTRACT

Increasing body temperature (T_b) during digestion can facilitate localized biochemical reactions and consequently increase passage rate of food through the digestive tract in terrestrial ectotherms. Snakes, particularly infrequent feeders, may benefit from an increase in digestion rate, because they typically feed on relatively large prey, which substantially increases their body mass. There is considerable evidence, particularly from laboratory studies, that postprandial thermophily can be attained through behavioral thermoregulation. However, there are compelling reasons, such as increased predation risk, that some snake species may not choose warmer T_bs during digestion. This study examines thermoregulation, before and after feeding, in free-ranging telemetered timber rattlesnakes (*Crotalus horridus*), an infrequently-feeding snake in central Tennessee. *Crotalus horridus* were observed feeding naturally or offered large food items (laboratory rats weighing 30-50% of snake body mass). Continuous T_bs of 11 *C. horridus* were recorded during feeding events and indicated that limited postprandial thermophily occurred, but is unlikely to be biologically relevant. Additionally, the thermal microhabitats selected by *C. horridus* immediately prior to digestion, during digestion, and after digestion did not differ. This lack of increased thermal selection is counter to the hypothesis of postprandial thermophily, which is generally assumed for most snakes. Because *C. horridus* is an ambush predator, it may sacrifice warmer T_bs to conserve energy and/or to avoid detection. Additionally, the climate in central Tennessee may be adequately warm to facilitate digestion without the need for selection of specific sites.

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CHAPTER 1. INTRODUCTION

Ectothermic vertebrates rely on behavioral thermoregulation as a primary method for maintaining body temperature (T_b), because their metabolism alone does not generate adequate heat. The thermal dependency of biochemical reaction rates (Seebacher and Franklin, 2005) suggests that maintenance of T_b is essential. To regulate T_b, most terrestrial vertebrate ectotherms utilize behaviors such as basking, selecting sites based on temperature (Huey, et al., 1989), and modifying body posture (Peterson, et al., 1993). Physiological functions such as locomotion (Bennett, 1980; Stevenson, et al., 1985; VanDamme, et al., 1991), embryonic development (Charland, 1995; Crane and Greene, 2008; Daut and Andrews, 1993; Gregory, et al., 1999; Mathies, 1997), and digestion (Beck, 1996; Harlow, et al., 1976; Naulleau 1983; Regal, 1966; VanDamme, et al., 1991) depend upon the maintenance of T_b within specific ranges to proceed efficiently, and in many cases they stimulate a thermophilic response. Feeding and digestion in particular have been shown to cause a behavioral increase in temperature in a variety of terrestrial ectotherms. Postprandial thermophily occurs in both amphibians (Gvoždik, 2003; Witters and Sievert, 2001) and reptiles (Angilletta, et al., 2002; Huey, 1982; Gienger, et al., 2013; Stevenson, et al., 1985; Regal, 1966; Sievert, et al., 2005), but reptiles are less thermally constrained than amphibians, as most amphibians limit thermoregulatory behavior when water is absent (Witters and Sievert, 2000).

Reptiles undergo a relatively large increase in body mass after feeding and may experience decreased locomotor performance and increased predation risk (Ford and Shuttlesworth, 1986; Garland and Arnold, 1983). Snakes are potentially more vulnerable to a postprandial reduction in locomotion than other squamate reptiles, because snakes have a much higher relative ingestion capacity (Greene, 1983). Some species can consume prey greater than 100% of their own body mass (Garland and Arnold, 1983; Greene, 1983), and infrequently-feeding snakes experience a substantial increase in localized metabolism after consuming a meal greater than 25% of their body weight (Secor and Diamond, 1997; Secor, 2000). Postprandial thermophily can reduce food passage times (Bedford and Christian, 2000; Greenwald and Kanter, 1979; Henderson, 1970, Naulleau 1983; Toledo, et al., 2003; Wang, et al., 2003); conversely, inadequate T_b maintenance slows digestion and can stimulate regurgitation in snakes (Dorcas, et al., 1997; Naulleau, 1983; Stevenson, et al., 1985). Though postprandial thermophily can be beneficial, there are also potential associated costs. For instance, the energetic demand of thermoregulation can depend on body mass, shape and physiology, habitat composition, and habitat conformation (Peterson, et al., 1993; Seebacher and Franklin, 2004).

Postprandial thermophily in snakes has been well documented in laboratory settings (Dorcas, et al., 1997; Gibson, et al., 1989; Greenwald and Kanter, 1979; Sievert, et al., 2005; Slip and Shine 1988) but relatively few studies have addressed this topic in the field. Wall and Shine (2008) have suggested that results obtained through the use of laboratory thermal gradients “grossly oversimplify” thermal habitat and can misrepresent normal thermoregulatory behavior. Because of the complexity of the available thermal environment, field studies of snake behavior may offer insights into thermoregulatory behavior that laboratory thermal gradients cannot. For example, *Hoplocephalus stephensii*, an arboreal elapid snake found in dense forests in Australia, chooses warmer

T_bs in the laboratory than in the field, but in its normal habitat basks only to raise T_b for short periods after feeding or during gestation, otherwise remaining under cover (Fitzgerald, et al., 2003).

Many of the field experiments examining postprandial thermoregulatory behavior have been limited to active thermoregulators (e. g., *Thamnophis* and *Pantherophis*), which shuttle between thermal environments to maintain T_b within a narrow range (Blouin-Demers and Weatherhead, 2002; Huey, et al., 1989; Peterson, 1987). Less is known about the effects of digestion on the behavior of sit-and-wait predators. Such snakes feed infrequently and generally minimize movement to conserve energy and increase foraging success (Secor, et al. 1994; Wills and Beaupre, 2000). As a consequence, shuttling behavior may be inherently reduced compared to other species. Yet infrequently-feeding snakes undergo a dramatic localized metabolic increase associated with intestinal hypertrophy, peristalsis and increased nutrient transporter activity directly after feeding, much greater than that undergone by snakes that feed more often (Secor and Diamond, 2000; Secor, et al., 1994). Consequently, infrequently-feeding snakes may have different postprandial thermoregulatory behavior than snakes that feed more frequently.

Laboratory-measured metabolic responses in infrequently-feeding snakes suggest that not all species digest food more efficiently at higher temperatures. The cost of digestion and digestive efficiency are temperature-independent in *Python molurus*, but passage time is reduced at warmer temperatures (Wang, 2003), suggesting that postprandial thermophily in snakes may not be as connected to digestive efficiency as reduced passage time (Sievert, et al., 2005). In contrast, passage times in *C. horridus* are similar across temperatures, though this phenomenon may be due to a propensity of large ground-dwelling snakes, particularly pit vipers, to retain fecal material (Beaupre and Zaidan, 2012; Cundall and Greene, 1999). Free-ranging rattlesnakes in the Sonoran Desert, however, experienced a postprandial thermophilic response (increase of ~4 °C), although some individuals had a reduction in T_b directly after feeding, which corresponded to snakes retreating to subsurface refugia, presumably to avoid predation (Beck, 1996). Some of the increase in T_b could be due to heat produced by the digestive process itself. Endogenous heat produced during digestion has been documented in *C. durissus*, but only accounted for a local increase in body surface temperature of 0.9-1.2 °C (Tattersall, et al., 2004). Consequently, it is assumed that the increases in T_b in desert rattlesnakes can be attributed to behavioral thermoregulation.

Because of thermal heterogeneity associated with different geographic regions and environments, postprandial thermophily may not be expected in some situations. *Crotalus horridus*, generally considered to be a forest-dwelling species, exhibits food passage rates (Beaupre and Zaidan, 2012) and specific dynamic action (SDA) that are not strongly dependent on temperature (Zaidan and Beaupre, 2003). This suggests that behavioral increases in T_b during digestion may confer limited benefits. To test this hypothesis, I conducted the following field study to examine the effects of digestion on the thermoregulatory behavior of *C. horridus.* Specifically, I asked: 1) Does *C. horridus* exhibit a post-feeding thermophilic response in the field? and 2) Does *C. horridus* utilize warmer environments during digestion?

CHAPTER 2. MATERIALS AND METHODS

2.1. Field Site

This study was conducted within Flat Rock Cedar Glades and Barrens State Natural Area, a 342-hectare nature preserve in Rutherford County, Tennessee. The preserve and the surrounding area are comprised of a variety of open- and closed-canopy habitats, including fields, pastureland, hardwood (primarily *Carya* and *Quercus* spp.), redcedar forest (*Juniperus virginiana*), and redcedar glades and barrens (characterized by full sunlight, thin soil with bedrock at or near the surface and redcedar trees bordering the periphery (Quarterman, 1989; Ware, 2002). These habitats provided a broad range of thermal microclimates that *C. horridus* utilizes throughout its active seasons (Hoekstra, 2015).

2.2. Study Animal

The timber rattlesnake, *C. horridus* (Family: Crotalidae) is a medium-sized pit viper inhabiting much of the eastern United States (Conant and Collins, 1991). They are primarily active April through October; *C. horridus* emerge in the spring, migrate to foraging and mating sites, and then generally return to their hibernacula with a relatively high degree of philopatry (Clark, et al., 2008; Reinert and Zappalorti, 1988). Within the eastern deciduous forest habitats, such as those in middle Tennessee, their diet mostly consists of small mammals: predominantly mice, voles, and chipmunks, though they will also eat birds, reptiles, and amphibians (Clark, 2002; Reinert, et al., 2011). Large adults can consume prey such as rabbits and squirrels (Clark, 2002).

Eight *Crotalus horridus* (Table 1) were collected from the study site during 2014 and

2015 using drift fences with funnel traps during periods of ingress and egress from known hibernacula. Tongs were used to capture *C. horridus* if they were found opportunistically during the active season. Captured snakes were transported to Middle Tennessee State University and maintained until surgery (< 24 hours post-capture) in 37.8-L glass aquariums in controlled environmental chambers (27-30 °C) with water supplied *ad libitum*. During this time, each snake was processed by measuring body mass (556.5 ± 89.0 g, mean ± SE), snout-to-vent length (90.6 ± 5.3 cm), and total length (97.2 ± 5.6 cm), identifying sex, assessing general health, and implanting with a subcutaneous passive integrated transponder (PIT) tag.

2.3. Body Temperature Selection

Each rattlesnake was surgically implanted with a temperature data logger to record T_b and a radiotransmitter for the purpose of relocating the snakes in the field. The data loggers (iButton®, model DS1922L, Maxim Integrated, San Jose, California) were accurate to ± 0.5 °C and were programmed to sample snake T_b every 60 minutes over the course of the active season. One of five types of radio transmitter was used, based on each snake's body mass and year of capture. In 2012 and 2013 snakes received a Holohil SI-2 (14.5 g), SB-2T (11 g) or SB-2 (5.2 g) transmitter (Holohil Systems, Ltd., Ontario, Canada). In 2014 and 2015 snakes were implanted with an ATS R1510 (11 g) or R1670 (3.1 g) transmitter (Advanced Telemetry Systems, Inc., Isanti, MN) (Fig. 1). Prior to implantation, the transmitter and data logger were adhered together with a cyanoacrylate adhesive and then dipped in a 1:1 compound of beeswax and paraffin. The coating promoted an immune response which led to connective tissue encapsulation of the transmitter package preventing internal migration (Lutterschmidt, et al. 2012). The transmitter package (2.9 ± 0.2% of snake body mass) was then surgically implanted into the snake's peritoneal cavity with the antenna lying subcutaneously and cranial to the package following procedures similar to Reinert and Cundall (1982). Post-operative snakes were retained for < 48 h in an environmental chamber for monitoring and then released at their capture location. During the active seasons, snakes were tracked via ratiotelemetry every 48-72 hours, and biodegradable flagging was used to mark exact locations within habitats.

2.4. Feeding Observation

Following release and ≥ 30 days post-surgery, snakes were offered a previously-killed laboratory rat (*Rattus norvegicus*) weighing 30 to 50% of snake body mass (Table 2). Snakes were offered rats when they were found in ambush posture as described by Reinert, et al. (2011). If a snake was not visible or inaccessible because of habitat structure, it was not offered a rat at that time. Rats were offered on days that were clear and sunny to potentially facilitate feeding performance. The rats were warmed in the field using portable hand warmers (Grabbers®) and kept in a small insulated container prior to being offered to each snake.

During telemetry relocations, exposed snakes were observed to document any natural feeding events. In all observations except one, the relative size of the food boluses appeared to be comparable to or greater than those of the supplemental rats. In addition to supplemental and natural feedings collected in 2014-15, T_b for 3 natural feeding events occurring in 2012 and 2013 were also analyzed (Table 1).

2.5. Environmental Temperatures

To address whether microhabitats used during digestion differed thermally from those sites used while not digesting, the thermal distribution of pre-digestion and digestion microhabitats were determined using operative snake models. The models consisted of 1.9-cm-diameter by 10-cm long hollow copper pipes spray-painted with Krylon® gray paint primer to simulate the reflectance of timber rattlesnake skin (Peterson, et al., 1993; Wills and Beaupre, 2000). Temperature dataloggers, accurate to ± 1 °C, (iButton® model DS1921G, Maxim Integrated, San Jose, California) were placed inside the pipes and the ends were sealed with rubber stoppers to prevent ambient air temperature from affecting operative temperatures (Fig. 2).

In August of 2015, the digesting and non-digesting microhabitats observed in 2014 and 2015 (N = 16) were modeled simultaneously to control for temporal variation in climate. Each modeled site was defined as the 1-meter square area surrounding the snake at the specified time (pre-digesting or digesting). The site utilized by each snake when it was offered the rat was considered to be the pre-digesting site, and the site where the snake was located 24-48 hours after feeding was considered the digesting site. Six operative snake models were placed in a grid in a 1-m square surrounding the site that the snake occupied for each of the times in question (Fig. 3). Models recorded operative temperatures for at least 48 h on clear, sunny days.

2.6. Behavioral Observation

Notes regarding posture, behavior, body exposure, and habitat features surrounding the snake were recorded at each snake location, similar to the procedure used by Gibson, et al. (2008). Visual estimates were collected on the percentage of the snake that was exposed and snake body posture (coiled, partially coiled, ambush posture (Reinert, et al., 2011), stretched, or active/traveling). Macrohabitat type was recorded as hardwood forest, redcedar forest, cedar glades and barrens, or fields; microhabitat type was noted as open forest floor, brush, vegetation, grass, or rock. Estimates of microhabitat features were also collected on the 1-m diameter around the snake. They included percentage of canopy cover (vegetation > 2 m in height), percentage of rock cover, and percentage of ground vegetation cover (< 1 m in height).

2.7. Statistical Analyses

Body temperatures were divided into three 7-day treatment periods for analysis: pre-digesting—period represented the non-digesting condition prior to feeding (in the case of natural feeding events, the seven days ending on the last day before a food bolus was observed), digesting—period began the day of feeding or the first day a naturally-feeding snake was observed with a food bolus, and post-digesting—period was days 8-14 of digestion.

Snake T_bs (24 h, daytime, and nighttime) were compared across the three treatment periods using repeated-measures analysis of variance (RM ANOVA) with Greenhouse-Geisser adjustment ($\alpha = 0.05$) and Sidak adjustments to the *post hoc* pairwise comparisons. Daytime T_bs were defined as occurring between 10:00 and 18:00 h and nighttime temperatures were defined as 22:00 to 06:00 h. Within individuals, 24-hour, daytime, and nighttime T_bs were also compared using RM ANOVAs with Greenhouse-Geisser adjustments ($\alpha = 0.05$) and Sidak *post hoc* pairwise comparisons.

Body temperatures were collected over multiple months within each active season. To compensate for environmental temperature variation, the differences in T_b between treatment periods for each hour of each feeding event were analyzed. To conduct these tests, the mean T_b of each hour of each day of the pre-digesting period was subtracted from the mean T_b of each hour of each day of the digesting period and then averaged across hours. The same process was repeated to compare the digesting period to the post-digesting period, subtracting the digesting T_b values from the non-digesting values. For example, the mean of all T_bs from 01:00 h Days 8-14 (digestion) minus the mean T_b from 01:00 h Days 1-7 (pre-digestion); 02:00h Days 8-14 minus 02:00h Days 1-7, etc., for each feeding event was calculated. Each difference was then compared to 0 using a two-tailed Student's *t*-test ($\alpha = 0.025$).

Thermal microhabitat distributions (24-h) of pre-digesting and digesting sites were compared using a dependent-samples Student's *t*-test ($\alpha = 0.05$). Daytime and nighttime means, as described above, were also compared using dependent-samples Student's *t*-tests ($\alpha = 0.05$). The number of observations of each body posture, macrohabitat type, and microhabitat type were compared across treatment periods using contingency tables with Pearson Chi-squared tests ($\alpha = 0.05$).

CHAPTER 3. RESULTS

3.1. Thermoregulation

Mean 24-h T_bs for the pre-digestion, digestion, and post-digestion periods were 24.1, 24.5, and 24.6 °C, respectively (Table 3), and were significantly different from each other, $F = 18.921$, $df = 1.873$, $p < 0.001$. The T_bs of the digesting period were significantly greater than the pre-digesting period ($p < 0.001$), but no difference occurred between digesting and post-digesting T_bs ($p = 0.602$) (Fig. 4). The greatest differences in T_b occurred from 22:00 to 08:00 (Fig. 5). In the daytime (10:00 – 18:00 h) snakes chose similar T_bs across the treatment periods, $F = 2.333$, $df = 1.913$, $p = 0.100$. Nighttime (22:00 - 06:00 h) T_b increased by about 0.6 °C to 22.1 °C while digesting, and then to 22.4 °C during post-digestion (Table 3). Nighttime T_bs were significantly higher during digestion ($F = 19.377$, $df = 1.792$, $p = 0.001$), but no difference occurred between digestion and post-digestion ($p = 0.062$). Only 6 of 11 individuals showed an increase in 24-h T_b during digestion, and of those, all had significantly higher T_bs than in the pre-digestion period. Five snakes selected significantly warmer T_bs during the daytime while digesting, and another 5 snakes had higher T_bs during digestion at nighttime (Table 7). The frequency distribution of pooled T_bs for all the feeding events illustrates the overlap of snake T_bs (Fig. 6).

Considerable variation in environmental temperatures during the feeding events occurred over the entirety of the active season each year. To compensate for this variation, the differences in mean T_b (between pre-digesting and digesting conditions, and then digesting and post-digesting conditions) for each hour were calculated within each feeding event and then averaged across all feeding events (Fig. 7). During digestion, T_b increased by 1.0 ± 0.2 °C and then decreased 0.2 ± 0.1 °C in post-digestion. The greatest increase in T_b after feeding occurred at 06:00 to 07:00 h and was 1.4 ± 1.03 °C. The greatest decrease post-digestion was -0.5 ± 0.6 °C, and occurred at 21:00 h (Fig. 7). Overall, digesting snakes increased T_b ~1 °C and post-digesting snakes had T_bs that varied little from those during the pre-digestion period. Hourly differences between treatment periods indicated relatively few instances in the daytime ($n = 10$) when significantly warmer temperatures were more attainable, but more differences occurred at nighttime ($n = 24$). After digestion, there were not many times in either nighttime ($n = 7$) or daytime ($n = 9$) when the hourly difference was significantly less than 0. Regardless of these differences, the total number of significant t-tests ($n = 50$) is low compared to the number of tested hours ($N = 528$). In other words, while mean 24-h T_bs for all the feeding events showed significantly higher temperatures, there were relatively few times where snakes were actually warmer while digesting, when differences between treatment periods were compared on an individual basis.

3.2. Thermal Microhabitat

The snakes in this study did not move to warmer microhabitats after feeding, $t_{(8)} = 0.54$, $p = 0.604$. The mean 24-h operative temperature at the pre-feeding site was 23.5 ± 0.1 °C and was 23.3 ± 0.1 °C at the post-feeding sites. Daytime operative temperatures did not differ significantly ($p = 0.572$) between pre-feeding (29.6 ± 0.2 °C) and post-feeding (28.6 ± 0.1 °C) sites. Nighttime operative temperatures were virtually identical across all hours ($p = 0.930$) for both pre-feeding (19.3 ± 0.04 °C) and post-feeding (19.4

± 0.1 °C) sites (Fig. 8).

3.3 Behavioral Observation

Snakes did exhibit minor behavioral changes after feeding. They did not select different body postures among treatment periods ($\chi^2_{6,\,41} = 8.415$, $p = 0.209$) (Table 4). However, a greater percentage of snakes were partially coiled during the week of digestion than the pre-digestion period, which can be attributed to having a large prey item in the stomach. Snakes utilized different macrohabitat across treatment periods ($\chi^2_{6,\,61} = 14.122$, $p = 0.028$). Before digestion, 43.8% of the snakes were located in closed-canopied habitats (e. g., hardwood, cedar, or mixed forests) and 56.3% in more open-canopied habitat (cedar glades, cedar barrens, fields, or junkyards). During the digestion and post-digestion periods, the proportions of macrohabitat use were similar, but a few individuals moved to closed-canopy macrohabitat from open-canopied macrohabitat (Table 5). Changes in microhabitat use were not statistically significant ($\chi^2_{8,\,74} = 12.773$, $p = 0.120$), but there appeared to be a marginally greater percentage of rock cover in the microhabitats selected by digesting snakes than either pre-or post-digesting, and more snakes tended to choose grassy and rocky areas to carry out digestion (Table 5). Snakes also chose microhabitats with higher percentages of rock, vegetation and canopy cover during digestion (Table 6). These differences in microhabitat use may suggest that these snakes choose areas with dense cover in which to digest. There were, however, relatively few behavioral observations recorded on the snakes in this study, therefore more data on macro- and microhabitat use would be required to discern whether these preferences exist.

CHAPTER 4. DISCUSSION

Digesting *C. horridus* increased mean T_b by only ~0.5-1 °C, indicating a weak postprandial thermophilic response, at best. Snake species that exhibit a thermal response to feeding generally increase T_b to a greater degree than the *C. horridus* in this study. Digesting adult corn snakes, *Pantherophis guttatus,* in a thermal gradient thermoregulate ~3 °C higher than non-fed corn snakes (Greenwald and Kanter, 1979), and juveniles prefer substrate temperatures ~7 °C warmer while digesting (Sievert, et al., 2005). Diamond pythons (*Morelia spilota*) have postprandial T_b*s* ~2.5 °C higher than before feeding (Slip and Shine, 1988). Rattlesnakes in the Sonoran desert choose T_b ~4 °C warmer after feeding than their non-fed counterparts (Beck, 1996). Increasing T_b from 25 to 30 °C has little effect on metabolism and digestive performance in *C. horridus* (Beaupre and Zaidan, 2012; Zaidan and Beaupre, 2003), so the differences in pre- and post-feeding T_b measured in this study are probably insufficient to cause significant change in digestion.

The thermophilic response on the individual level was also inconsistent: only about half of the snakes selected warmer digesting T_bs during either the daytime or the nighttime. For example, one individual, CH 4, fed three times naturally and once supplementally from 2012 to 2014. This rattlesnake increased its T_b after two of the feedings, and decreased T_b after the other two feedings (Fig. 9). Indeed, none of the snakes showed a consistent pattern of T_b change from pre- to post-digesting periods, either within or between individuals (Table 7). These findings may indicate that *C.*

horridus is less motivated by postprandial thermophily than by alternate criteria in choosing suitable digestion sites.

Lack of a postprandial thermophilic response in a snake species is not unprecedented. *Nerodia sipedon* is considered a weak thermoregulator, and it does not exhibit postprandial thermophily in the laboratory or the field, increasing its T_b only ~1 °C while digesting (Brown and Weatherhead, 2000). *Bothrops insularis*, a tropical snake, does not thermoregulate warmer in the field while digesting, although, as a strict forest-dweller, it spends a significant amount of time in closed-canopy habitat (Bovo, et al., 2010). Predators in forested environments limit thermoregulatory movement in favor of concealment, thus conforming to their thermal environment (Bovo, et al., 2012). Because of this behavior, Wills and Beaupre (2000) have suggested that *C. horridus* may also be a weak thermoregulator, which is supported by T_b as well as behavioral observation in the present study. Unlike *Bothrops insularis,* however, *Crotalus horridus* actively thermoregulates during gestation and just prior to ecdysis (Bovo, et al., 2010; Peterson, et al., 1993, Gardner-Santana and Beaupre, 2009). Perhaps gestation and ecdysis place a greater metabolic burden on *C. horridus* than digestion, are longer events, and may simply be more important to fitness.

Reduced locomotor capacity in digesting snakes is common (Crotty and Jayne, 2015; Ford and Shuttlesworth, 1986; Garland and Arnold, 1983; Stevenson, et al., 1985; Willson and Hopkins, 2011), and it is likely reduced in *C. horridus* during digestion. Almost one-third of the digesting *C. horridus* were observed partially coiled, because the large size of the food boluses prevented tight coiling. Such limitation likely extends to defense as well, which is supported by some snakes choosing dense or hidden retreats sites over more exposed areas. A few ($n = 4$) *C. horridus* in this study moved from open-canopied habitats to closed-canopied forests after consuming prey, choosing rocky areas, hollow trees, or underground refugia after feeding. Similar behavior has been observed in digesting rattlesnakes in the Sonoran desert (Beck, 1996). Movements to these sites inherently resulted in lower environmental temperatures, but also provided protective shelter. This further supports the lack of selection for warmer microhabitats during digestion. Desert rattlesnakes in Beck's study (1996) retreated under cover after feeding, but emerged and exhibited thermophilic responses within 3 days of consuming prey. *Crotalus horridus* in the current study did not show evidence of basking > 48 hours after feeding, but modeling multiple thermal microhabitats throughout the active season may have allowed a better description of available environmental temperatures.

Thermophilic responses were weak for both daytime and nighttime. During the daytime, snakes did not raise T_b significantly. The operative temperatures in the microhabitats chosen during digestion, while also non-significant, were actually cooler than those chosen before digestion. *Crotalus horridus* in cooler microhabitats may utilize warm areas within the microhabitats (i.e., small sunlit areas or heat-retaining structures such as rocks) more efficiently during the day to maintain stable T_b regardless of digestive status. Nighttime operative temperatures were relatively low with little variation, ranging from 16 °C to 22 °C across all nighttime hours for all microhabitats. Snakes did manage to attain warmer T_bs at nighttime, but mean T_b only increased ~0.6 °C. This is consistent with the tendency of *C. horridus* to be a somewhat weak thermoregulator (Wills and Beaupre, 2000). *Crotalus horridus* normally occupies microhabitats with dense cover and limits basking to times when the benefits of thermoregulation outweigh the costs (Reinert and Zappalorti, 1988). It is possible that the digesting snakes in this study chose densely-covered microhabitats during the day and chose warmer digesting sites at nighttime, since the costs associated with thermoregulation (time, risk, etc.) are lower. Snakes were located exclusively during daylight hours, therefore their nocturnal behaviors were unknown. Nighttime thermoregulation is not supported through operative temperature modeling, however; there was little variation in nighttime operative temperatures among any of the pre-feeding and post-feeding sites within the same 24-hour period (Fig. 8), though the sites represented a variety of microhabitat types. Elevated T_bs for both daytime and nighttime could be attributed to natural fluctuations in environmental temperature (i.e., weather artifact) or perhaps endogenous heat production from specific dynamic action, but additional experimentation would be required to test these hypotheses.

Reluctance to thermoregulate while digesting may also be partially attributed to latitude and/or elevation. Tropical snake species, which do not typically thermoregulate as actively as temperate-zone species (Bovo, et al., 2012; Shine and Madsen, 1996), as well as species that normally maintain higher T_b in general, do not typically demonstrate a thermophilic response to feeding (Bovo, et al., 2010, Hammerson, 1979; Touzeau and Sievert, 1993). While the present study was conducted at only one location in the southeastern United States, the study site offered a wide selection of potential thermal habitats over the course of the active season. In the summer, operative temperatures of open-canopied habitats in this preserve average about 25-29 °C, whereas forested habitats average 21-22 °C, but in both types of habitat temperatures can range from 10 °C to > 65 °C (Hoekstra, 2015). Therefore, there is little thermal constraint on this population of *C. horridus*, and the majority of habitats have temperatures well within the suitable digesting range for most snakes throughout the active season (Dorcas, et al., 1997; Naulleau, 1983; Stevenson, et al., 1985). It may therefore be more advantageous for *C. horridus* in this area to thermoconform during the day rather than incur the costs associated with thermoregulatory behavior. Because of the considerable geographic range of *C. horridus*, thermoregulatory responses to digestion may vary considerably (Beck, 1996; Winne and Keck, 2005); *C. horridus* in open-canopied habitats, where operative temperatures are greater than their preferred temperature range, will thermoregulate to cool themselves (Hoekstra, 2015). Therefore, it is possible that *C. horridus* in cooler climates may choose warmer temperatures while digesting, because a greater percentage of operative temperatures are cooler than temperatures required for digestion to proceed efficiently. However, the digestibility and metabolizable energy of prey is similar for *C. horridus* regardless of geographic origin (Beaupre and Zaidan, 2012), and resting metabolism in this species is similar across its range (Beaupre and Zaidan, 2001), which may make postprandial thermoregulatory behavior similar despite the climate.

A potentially useful measurement not currently in the literature is a T_b setpoint range for *C. horridus.* While limited in its extrapolation to thermoregulation in natural conditions, T_b setpoint values do provide a standard for comparison purposes, particularly for examining the effectiveness of thermoregulation (Hertz, et al., 1993; Blouin-Demers and Weatherhead, 2002). Information on thermoregulatory effectiveness could also be enhanced by determining whether *C. horridus* utilizes thermal microhabitats more efficiently during digestion. This experiment yielded little information on precise thermoregulatory movements within microhabitats. Observational techniques such as video recording could reveal minor changes in thermoregulatory behavior, such as utilization of sunlight flecks within closed-canopy microhabitats, intermittent basking on exposed rock surfaces, and nocturnal microhabitat use (time-lapse videography was attempted in this experiment but was not successful due to limited visibility of *C. horridus* within microhabitats). Because thermoregulation is affected by such a broad range of biotic and abiotic factors, it may be necessary to examine *C. horridus* closely in both laboratory and field settings to gain a complete picture of its postprandial thermoregulatory behavior.

REFERENCES

Angilletta, Jr., M.J., Hill, T., Robson, M.A., 2002. Is physiological performance optimized by thermoregulatory behavior?: a case study of the eastern fence lizard, *Sceloporus undulatus*. J. Therm. Biol. 27, 199-204.

Beaupre, S.J., Zaidan, III, F., 2001. Scaling of CO_2 production in the timber rattlesnake (*Crotalus horridus*), with comments on cost of growth in neonates and comparative patterns. Physiol. Biochem. Zool. 74, 757-768.

Beaupre, S.J., Zaidan, III, F., 2012. Digestive performance in the timber rattlesnake (*Crotalus horridus*) with reference to temperature dependence and bioenergetic cost of growth. J. Herpetol. 46, 637-642.

Beck, D.D., 1996. Effects of feeding on body temperatures of rattlesnakes: a field experiment. Physiol. Zool. 69, 1442-1455.

Bedford, G.S. and Christian, K.A., 2000. Digestive efficiency in some Australian pythons. Copeia 2000, 829-834.

Bennett, A.F., 1980. The thermal dependence of lizard behavior. Anim. Behav. 28, 752-762.

Blouin-Demers, G., Weatherhead, P.J., 2002. An experimental test of the link between foraging, habitat selection, and thermoregulation in black rat snakes *Elaphe obsoleta obsoleta*. J. Anim. Ecol. 70, 1006-1013.

Bovo, R.P., Marques, O.A.V., Andrade, D.V., 2010. Does gestation or feeding affect the body temperature of the golden lancehead, *Bothrops insularis* (Squamata: Viperidae) under field conditions? Zoologia 27, 973-978.

Bovo, R.P., Marques, O.A.V., Andrade, D.V., 2012. When basking is not an option: thermoregulation of a viperid snake endemic to a small island in the south Atlantic of Brazil. Copeia 2012, 408-418.

Brown, G.P., Weatherhead, P.J., 2000. Thermal ecology and sexual size dimorphism in northern water snakes, *Nerodia sipedon*. Ecol. Monogr. 70, 311-330.

Charland, M.B., 1995. Thermal consequences of reptilian viviparity: thermoregulation in gravid and nongravid garter snakes (*Thamnophis*). J. Herpetol. 29, 383-390.

Clark, R.W., 2002. Diet of the timber rattlesnake, *Crotalus horridus*. J. Herpetol. 36, 494-499.

Clark, R.W., Brown, W.S., Stechert, R., Zamudio, K.R., 2008. Integrating individual behaviour and landscape genetics: the population structure of timber rattlesnake hibernacula. Mol. Ecol. 17, 719-730.

Conant, R., Collins, J.T., 1998. A field guide to reptiles and amphibians of eastern and central North America. Houghton Mifflin Company, New York.

Crane, A.L., Greene, B.D., 2008. The effect of reproductive condition on thermoregulation in female *Agkistrodon piscivorous* near the northwestern range limit. Herpetologica 64, 156-167.

Crotty, T.L., Jayne, B.C., 2015. Trade-offs between eating and moving: What happens to the locomotion of slender arboreal snakes when they eat big prey? Biol. J. Linnean Soc. 114, 446-458.

Daut, E.F., Andrews, R.M., 1993. The effect of pregnancy on thermoregulatory behavior of the viviparous lizard *Chalcides ocellatus.* J. Herpetol. 27, 6-13.

Dorcas, M.E., Peterson, C.R., Flint, M.E., 1997. The thermal biology of digestion in rubber boas (*Charina bottae*): physiology, behavior, and environmental constraints. Physiol. Zool. 70, 292-300.

Fitzgerald, M., Shine, R., Lemckert, F., 2003. A reluctant heliotherm: thermal ecology of the arboreal snake *Hoplocephalus stephensii* (Elapidae) in dense forest. J. Therm. Biol. 28, 515-524.

Ford, N.B., Shuttlesworth, G.A., 1986. Effects of variation in food intake on locomotory performance of juvenile garter snakes. Copeia 1986, 999-1001.

Gardner-Santana, L.C., Beaupre, S.J., 2009. Timber rattlesnakes (*Crotalus horridus*) exhibit elevated and less variable body temperatures during pregnancy. Copeia 2009, 363-368.

Garland, T., Arnold, S.J., 1983. Effects of a full stomach on locomotory performance of juvenile garter snakes (*Thamnophis elegans*). Copeia 1983, 1092-1096.

Gibson, A.R., Smucny, D.A., Kollar, J., 1989. The effects of feeding and ecdysis on temperature selection by young garter snakes in a simple thermal mosaic. Can. J. Zool. 67, 19-23.

Gibson, S.E., Walker, Z.J., Kingsbury, B.A., 2008. Microhabitat preferences of the timber rattlesnake (*Crotalus horridus*) in the hardwood forests of Indiana, in: Hayes, W.K., Beaman, K.R., Cardwell, M.D., Bush, S.P. (Eds.), The Biology of Rattlesnakes. Loma Linda University Press, California, pp. 275-286.

Gienger, C.M., Tracy, C.R., Zimmerman, L.C., 2013. Thermal responses to feeding in a secretive and specialized predator (Gila monster, *Heloderma suspectum*). J. Therm. Biol. 38, 143-147.

Greene, H.W., 1983. Dietary correlates of the origin and radiation of snakes. Amer. Zool. 23, 431-441.

Greenwald, O.E., Kanter, M.E., 1979. The effects of temperature and behavioral thermoregulation on digestive efficiency and rate in corn snakes *Elaphe guttata guttata*. Physiol. Zool. 52, 398-408.

Gregory, P.T., Crampton, L.H., Skebo, K.M., 1999. Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: are gravid snakes anorexic? J. Zool. 248, 231-241.

Hammerson, G.A., 1979. Thermal ecology of the striped racer, *Masticophis lateralis*. Herpetologica 35, 267-273.

Harlow, H.J., Hillman, S.S., Hoffman, M., 1976. The effect of temperature on digestive efficiency in the herbivorous lizard, *Dipsosaurus dorsalis*. J. Comp. Physiol. 111, 1-6.

Henderson, R.W., 1970. Feeding behavior, digestion, and water requirements of *Diadophis punctatus arnyi* Kennicot. Herpetologica 26, 520-526.

Hertz, P.E., Huey, R.B., Stevenson, R.D., 1993. Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. Am. Nat. 142, 796-818.

Huey R.B., 1982. Temperature, physiology, and the ecology of reptiles, in: Gans, C., Pough, F.H. (Eds.), Biology of the Reptilia. Academic Press, London, pp. 25-91.

Huey, R.B., Peterson, C.R., Arnold, S.J., Porter, W.P., 1989. Hot rocks and not-so-hot rocks: retreat-site selection by garter snakes and its thermal consequences. Ecology 70, 931-944.

Lutterschmidt, W.I., Smith, A.J., Tivador E.J., Reinhert, H.K., 2012. Diagnostic classification of connective tissue encapsulating transmitters and data acquisition units: evidence for not modifying a classic surgical implantation method. Herpetol. Rev. 43, 381-385.

Mathies, T., Andrews, R.M., 1997. Influence of pregnancy on the thermal biology of the lizard, *Sceloporus jarrovi:* Why do pregnant females exhibit low body temperatures?. Funct. Ecol. 11, 498-507.

Martin, W.H., 1993. Reproduction of the timber rattlesnake (*Crotalus horridus*) in the Appalachian Mountains. J. Herpetol. 27, 133-143.

Naulleau, G., 1983. The effects of temperature on digestion in *Vipera aspis*. J. Herpetol. 17, 166-170.

Pereira, M.E., Aines, J., Scheckter, J.L., 2002. Tactics of heterothermy in eastern gray squirrels. J. Mammal. 83, 467-477.

Peterson, C.R., 1987. Daily variation in the body temperatures of free-ranging garter snakes. Ecology 68, 160-169.

Peterson, C.R., Gibson, A.R., Dorcas, M.E., 1993. Snake thermal ecology: the causes and consequences of body-temperature regulation. in: Seigel, R.A., Collins J.T. (Eds.), Snakes: Ecology and Behavior. The Blackburn Press, Caldwell, New Jersey. pp. 241-314.

Quarterman, E., 1989. Structure and dynamics of the limestone cedar glade communities in Tennessee. J. Tennessee Acad. Sci. 64, 155-158.

Regal, P.J., 1966. Thermophilic responses following feeding in certain reptiles. Copeia 1966, 588-590.

Reinert, H.K., Cundall, D., 1982. An improved surgical implantation method for radio-tracking snakes. Copeia 1982, 705-708.

Reinert, H.K., Zappalorti, R.T., 1988. Timber rattlesnakes (*Crotalus horridus*) of the pine barrens: their movement patterns and habitat preference. Copeia 1988, 964-978.

Reinert, H.K., MacGregor, G.A., Esch, M., Bushar, L.M, Zappalorti, R.T., 2011. Foraging ecology of timber rattlesnakes, *Crotalus horridus.* Copeia 2011, 430-442.

Secor, S.M., Stein, E.D., Diamond, J., 1994. Rapid upregulation of snake intestine in response to feeding: a new model of intestinal adaptation. Am. J. Physiol. 266, 695-705.

Secor, S.M., Diamond, J., 2000. Evolution of regulatory responses to feeding in snakes. Physiol. Biochem. Zool. 73, 123-141.

Seebacher, F., Franklin, C.E., 2004. Cardiovascular mechanisms during thermoregulation in reptiles. Int. Congr. Ser. 1275, 242-249.

Seebacher, F., Franklin, C.E., 2005. Physiological mechanisms of thermoregulation in reptiles: a review. J. Comp. Physiol. 175B, 533-541.

Shine, R., Madsen, T., 1996. Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. Physiol. Zool. 69, 252-269.

Sievert, L.M., Jones, D.M., Puckett, M.W., 2005. Postprandial thermophily, transit rate, and digestive efficiency of juvenile cornsnakes, *Pantherophis guttatus*. J. Therm. Biol. 30, 354-359.

Slip, D.J., Shine, R., 1988. Thermophilic response to feeding of the diamond python, *Morelia s. spilota* (Serpentes: Boidae). Comp. Biochem. Physiol. 89A, 645-650.

Stevenson, R., Peterson, C.R., Tsuji, J.S., 1985. The thermal dependence of locomotion, tongue flicking, digesting, and oxygen consumption in the wandering garter snake. Physiol. Zool. 58, 46-57.

Tattersall, G.J., Milsom, W.K., Abe, A.S., Brito, S.P., Andrade, D.V., 2004. The thermogenesis of digestion in rattlesnakes. J. Exp. Biol. 207, 579-585.

Toledo, M.F, Abe, A.S., Andrade, D.V., 2003. Temperature and meal size effects on the postprandial metabolism and energetics in a boid snake. Physiol. Biochem. Zool. 76, 240-246.

Touzeau, T., Sievert, L.M., 1993. Postprandial thermophily in rough green snakes (*Opheodrys aestivus*). Copeia 1193, 1174-1176.

VanDamme, R., Bauwens, D., Verheyen, R.F., 1991. The thermal dependence of feeding behavior, food consumption and gut-passage time in the lizard *Lacerta vivipara* Jacquin. Funct. Ecol. 5, 507-517.

Wall, M., Shine, R., 2008. Post-feeding thermophily in lizards (*Lialis burtonis* Gray, Pygopodidae): laboratory studies can provide misleading results. J. Therm. Biol. 33, 274-279.

Wang, T., Zaar, M., Arvedsen, S., Vedel-Smith, C., Overgaard, J., 2003. Effects of temperature on the metabolic response to feeding in *Python molurus*. Comp. Biochem. Physiol. 133A, 519-527.

Ware, S., 2002. Rock outcrop plant communities (glades) in the Ozarks: a synthesis. Southwest. Nat. 47, 585-597.

Wills, C.A., Beaupre, S.J., 2000. An application of randomization for detecting evidence of thermoregulation in timber rattlesnakes (*Crotalus horridus*) from northwest Arkansas. Physiol. Biochem. Zool. 73, 325-334.

Willson, J.D., Hopkins, W.A., 2011. Prey morphology constrains the feeding ecology of an aquatic generalist predator. Ecology 92, 744-754.

Winne, C.T., Keck, M.B., 2005. Intraspecific differences in thermal tolerance of the diamondback watersnake (*Nerodia rhombifer*): effects of ontogeny, latitude, and sex. Comp. Biochem. Physiol. 140A, 141-149.

Witters, L.R., Sievert, L., 2001. Feeding causes thermophily in the woodhouse's toad (*Bufo woodhousii*). J. Therm. Biol. 26, 205-208.

Zaidan, III, F., Beaupre, S.J., 2003. Effects of body mass, meal size, fast length, and temperature on specific dynamic action in the timber rattlesnake (*Crotalus horridus).* Physiol. Biochem. Zool. 76, 447-458.

APPENDICES

APPENDIX A: TABLES

Table 1
Sex, body mass, and snout-to-vent length (SVL) for *Crotalus horridus* used in this study

Year	Snake ID	Sex	Mass (g)	SVL (cm)
2012	5	M	410.0	88.0
2013	10	M	749.1	103.1
	18	M	538.5	93.0
2014	4	F	1050.3	112.0
	11	F	928.7	108.2
	17	M	788.9	108.0
	20	M	255.9	72.0
2015	24	M	201.0	68.5
	25	F	509.6	98.0
	28	M	161.0	59.1
	29	M	528.5	87.0

Table 2
Percent body mass of meals eaten by supplementally-fed *Crotalus horridus*

Year	Snake ID	Date Fed	% Body Mass
2014	4	13-Aug	32.9
	11	3-Jul	47.3
	17	3-Jul	36.9
2015	25	19-May	49.2
	28	6-Jul	54.7

Table 3
Body temperatures (mean ± SE) of *Crotalus horridus* during digestion (dig) treatments

	Period	T_b (°C)
24-hour	Pre-Dig	24.1 ± 0.1
	Digesting	24.5 ± 0.1
	Post-Dig	24.6 ± 0.1
Daytime	Pre-Dig	27.2 ± 0.1
	Digesting	27.5 ± 0.1
	Post-Dig	27.5 ± 0.1
Nighttime	Pre-Dig	21.5 ± 0.1
	Digesting	22.1 ± 0.1
	Post-Dig	22.4 ± 0.1

Table 4
Body postures of *Crotalus horridus* during treatment periods.

	No. of Snake Observations	Percent of Body Exposed (mean ± SE)	Posture	No. of Observations (%)
Pre-Digesting	15	43.6 ± 13.0	Coiled	8 (44.4)
			Ambush	3 (16.7)
			Partial Coil	0
			Active	0
			Stretched	0
			Not Visible	7 (38.9)
Digesting	25	57.9 ± 8.2	Coiled	15 (48.4)
			Ambush	1 (3.2)
			Partial Coil	7 (22.6)
			Active	1 (3.2)
			Stretched	0
			Not Visible	2 (6.5)
			Feeding/ Recently Fed	5 (16.1)
Post-Digesting	15	39.3 ± 10.4	Coiled	7 (70.0)
			Ambush	1 (10.0)
			Partial Coil	2 (20.0)
			Active	0
			Stretched	0
			Not Visible	0

Table 5

Habitat use by *Crotalus horridus* during treatment periods.

	Macrohabitat	No. of Observations (%)	Microhabitat	No. of Observations (%)
Pre-Digesting	Forest	7 (43.8)	Forest floor	5 (23.8)
	Glade/barren	6 (37.5)	Brush/vegetation	9 (42.9)
	Field	0	Grass	2 (9.5)
	Man-made habitat	3 (18.8)	Rocks	1 (4.8)
			Hollow tree	1 (4.8)
			Man-made items	3 (14.3)
Digesting	Forest	13 (48.1)	Forest floor	22 (37.3)
	Glade/barren	9 (33.3)	Brush/vegetation	12 (20.3)
	Field	5 (18.5)	Grass	15 (25.4)
	Man-made habitat	0	Rocks	10 (16.9)
			Hollow tree	0
			Man-made items	0
Post-Digesting	Forest	7 (38.9)	Forest floor	8 (25.0)
	Glade/barren	5 (27.8)	Brush/vegetation	8 (25.0)
	Field	6 (33.3)	Grass	13 (40.6)
	Man-made habitat	0	Rocks	3 (9.3)
			Hollow tree	0
			Man-made items	0

Table 6
Features of microhabitats utilized by *Crotalus horridus* during treatment periods. Values are mean ± SE

	Canopy Cover (% of area covered)	Rock Cover (% of area covered)	Ground Vegetation Cover (% of area covered)
Pre-Digesting	34.7 ± 8.9	12.7 ± 7.1	56.7 ± 8.4
Digesting	42.6 ± 5.7	26.8 ± 7.1	62.4 ± 7.0
Post-Digesting	28.3 ± 7.9	14.7 ± 8.2	60.7 ± 9.1

Table 7
Body temperature (mean ± SD) for *Crotalus horridus* feeding events across pre-digesting, digesting (dig), and post-digesting periods. Table includes data on individuals that fed multiple times.

Year	Subject No.	Period	24-hour	Daytime	Nighttime
2012	4	Pre	25.8 ± 2.5	27.4 ± 2.7	24.5 ± 1.4
		Dig	26.6 ± 3.0*	29.3 ± 2.3*	24.4 ± 1.6
		Post	27.2 ± 3.2	30.0 ± 2.4	24.8 ± 1.2
	5	Pre	25.3 ± 5.2	29.2 ± 3.5	21.4 ± 3.7
		Dig	28.0 ± 3.6*	30.2 ± 2.4	25.8 ± 3.1*
		Post	24.4 ± 2.4**	26.1 ± 2.6**	23.0 ± 1.3**
2013	4	Pre	24.6 ± 3.5	27.5 ± 2.9	22.3 ± 1.8
		Dig	24.0 ± 4.6	28.3 ± 4.0	20.9 ± 2.0
		Post	23.8 ± 4.1	27.0 ± 3.6**	21.5 ± 2.6
	5	Pre	25.2 ± 4.0	28.9 ± 2.7	22.2 ± 2.1
		Dig	24.3 ± 5.2	29.3 ± 3.6	20.5 ± 2.4
		Post	22.5 ± 4.2**	25.9 ± 3.3**	20.1 ± 3.1
	10	Pre	22.7 ± 6.7	27.9 ± 4.6	18.5 ± 5.0
		Dig	24.2 ± 3.9*	26.5 ± 4.3	22.3 ± 1.7*
		Post	26.1 ± 4.7	30.3 ± 3.1	22.5 ± 2.6
	11	Pre	22.5 ± 2.8	24.9 ± 2.4	20.7 ± 1.4
		Dig	23.7 ± 2.6*	25.9 ± 2.6*	22.1 ± 1.0*
		Post	24.6 ± 1.7	25.5 ± 2.0	24.0 ± 1.0
	17	Pre	24.8 ± 3.8	28.3 ± 3.3	21.9 ± 1.3
		Dig	25.8 ± 3.7*	28.6 ± 3.1	23.7 ± 2.3*
		Post	23.0 ± 3.0**	24.6 ± 3.8**	21.7 ± 1.3*
	17	Pre	25.8 ± 2.9	28.1 ± 3.0	24.0 ± 1.1
		Dig	23.5 ± 4.0	27.1 ± 3.4	20.8 ± 1.8
		Post	23.9 ± 2.5	25.7 ± 2.6**	22.3 ± 1.2
	18	Pre	21.7 ± 6.3	28.4 ± 3.6	16.6 ± 2.7
		Dig	23.0 ± 3.7*	26.1 ± 3.1	20.4 ± 2.3*
		Post	24.8 ± 3.8	28.4 ± 2.8	22.0 ± 1.8
	18	Pre	23.5 ± 3.4	26.5 ± 2.7	21.2 ± 1.8
		Dig	25.1 ± 3.7*	28.5 ± 3.0*	22.4 ± 1.4*
		Post	24.9 ± 2.7	27.4 ± 2.6**	23.0 ± 0.8

Table 7 (cont.)
Body temperature (mean ± SD) for *Crotalus horridus* feeding events across pre-digesting, digesting (dig), and post-digesting periods. Table includes data on individuals that fed multiple times.

2014	4	Pre	25.7 ± 2.4	27.0 ± 2.5	24.7 ± 1.9
		Dig	23.0 ± 4.1	25.9 ± 3.8	20.7 ± 3.0
		Post	25.2 ± 2.2	27.2 ± 2.0	23.8 ± 0.9
	4 (2nd feeding)	Pre	23.0 ± 4.1	25.9 ± 3.8	20.7 ± 3.0
		Dig	25.2 ± 2.2	27.2 ± 2.0	23.7 ±0.9
		Post	24.7 ± 2.8	26.9 ± 2.7	23.0 ± 1.2
	11	Pre	24.9 ± 2.6	26.9 ± 2.5	23.3 ± 1.3
		Dig	24.7 ± 2.7	25.1 ± 2.9	24.4 ± 2.3*
		Post	25.3 ± 2.6	26.1 ± 3.4	24.7 ± 1.5
	17	Pre	25.1 ± 3.8	28.0 ± 3.2	22.4 ± 1.8
		Dig	24.3 ± 5.2	28.6 ± 4.1	20.8 ± 2.9
		Post	24.9 ± 4.6	28.5 ± 4.0	21.9 ± 2.4
	20	Pre	24.2 ± 2.3	26.2 ± 2.2	22.6 ± 0.8
		Dig	21.4 ± 4.5	25.7 ± 3.5	18.1 ± 2.0
		Post	20.0 ± 5.1**	24.9 ± 2.4	16.3 ± 2.7**
2015	24	Pre	25.5 ± 2.4	27.9 ± 1.9	23.5 ± 1.0
		Dig	24.8 ± 3.7	28.2 ± 3.2	22.0 ± 1.7
		Post	24.3 ± 2.8**	27.1 ± 2.3**	22.3 ± 1.3
	25	Pre	19.1 ± 4.2	21.5 ± 4.1	17.1 ± 3.4
		Dig	22.1 ± 5.0*	26.4 ± 3.8*	18.3 ± 3.1
		Post	22.4 ± 4.3	25.8 ± 4.6	19.6 ± 1.5
	28	Pre	23.3 ± 2.1	25.1 ± 1.7	21.8 ± 1.3
		Dig	27.4 ± 2.8*	30.3 ± 1.8*	25.1 ± 1.4*
		Post	26.5 ± 2.5**	28.6 ± 2.2**	25.0 ± 1.3
	29	Pre	27.5 ± 3.5	31.3 ± 1.8	24.4 ± 1.1
		Dig	27.2 ± 3.0	29.9 ± 2.1	24.9 ± 1.7
		Post	27.0 ± 2.9	29.5 ± 2.2	25.0 ± 1.9

* Digesting mean is significantly greater than Pre-digesting mean when compared using Sidak *post hoc* analysis (α = 0.05)
**Post-digesting mean is significantly lower than Digesting mean when compared using Sidak *post hoc* analysis (α = 0.05)

APPENDIX B: FIGURES

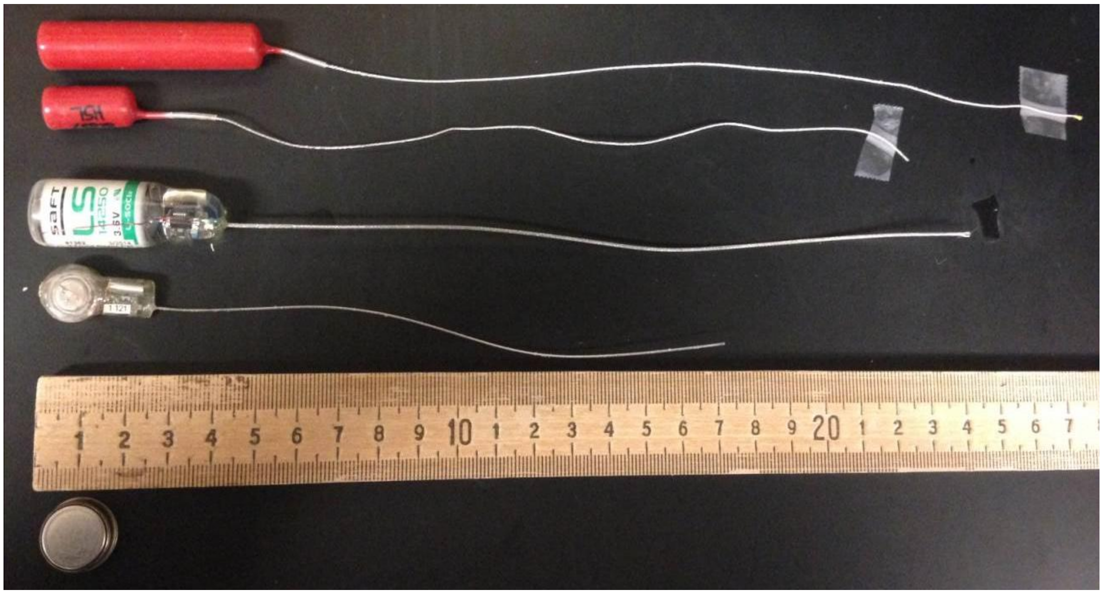

Fig. 1. Relative size of Holohil® radio transmitters (above meter stick, red), ATS® transmitters (above meter stick, clear) and iButton® temperature data logger (below meter stick). The large transmitters were implanted in large snakes and the small in small snakes. All snakes received an iButton®.

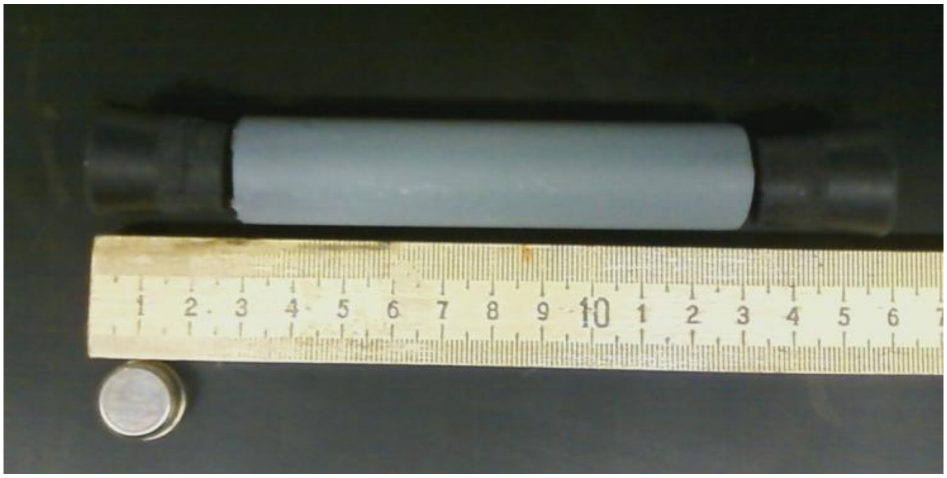

Fig. 2. Operative snake model. Each model contained an iButton® (bottom left).

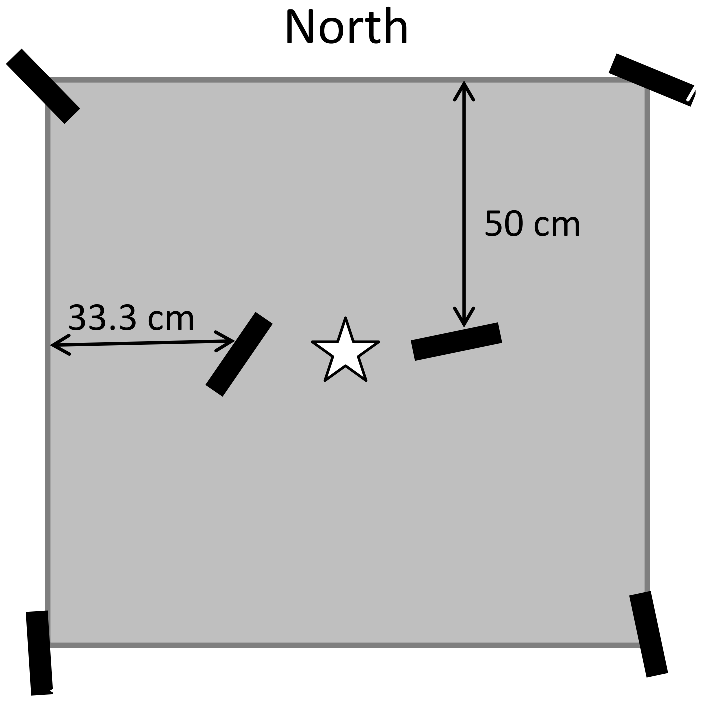

Fig. 3. Operative snake model grid. Each pre-feeding and post-feeding site (*N* = 16) was modeled using a grid of six operative snake models. Snake position is represented by the star, and the black rectangles indicate the placement of the models.

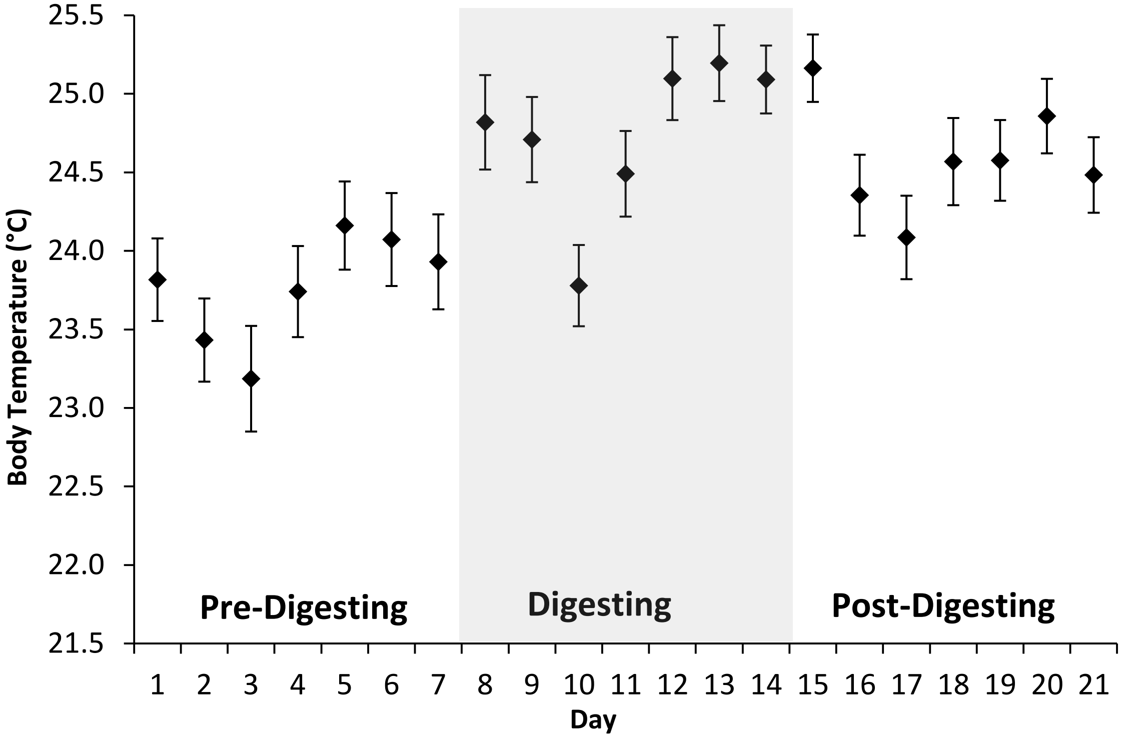

Fig. 4. Daily body temperature (± SE) for all feeding events. The T_b readings for all events were pooled for each 24-hour period and averaged.

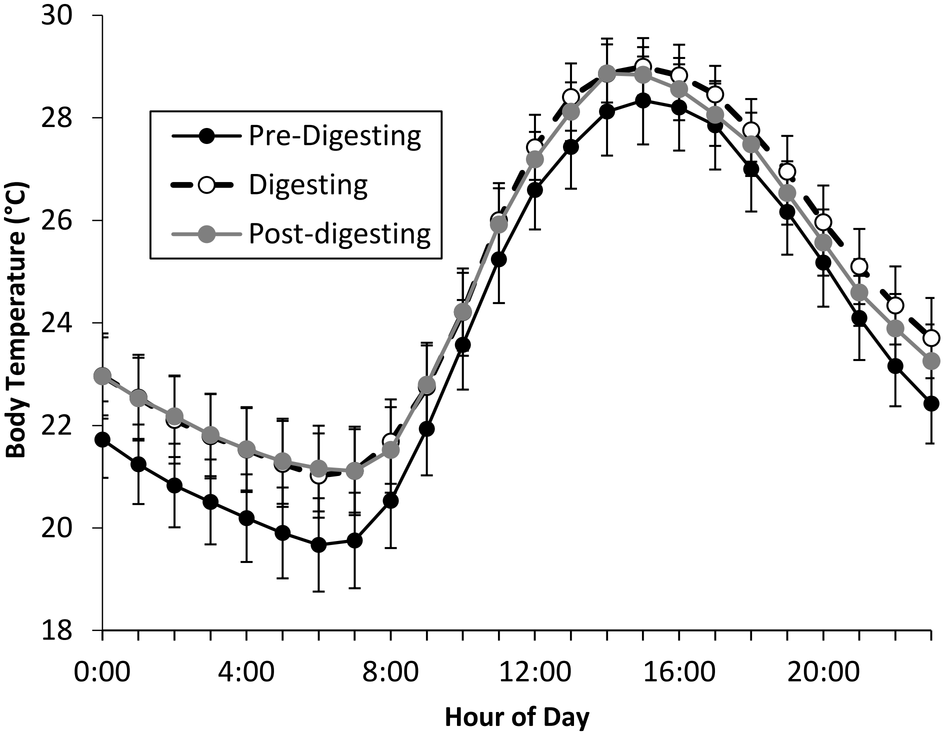

Fig. 5. Hourly body temperatures (mean ± SE) for each treatment period for all feeding events pooled hourly and averaged.

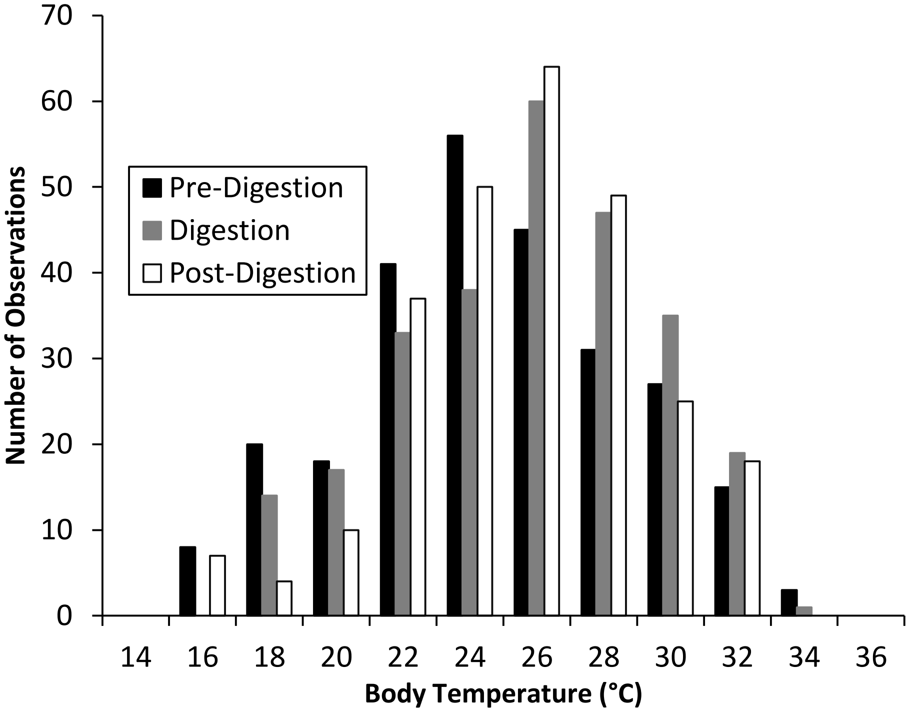

Fig. 6. Frequency distribution of snake body temperatures pooled for all feeding events (*N* = 759).

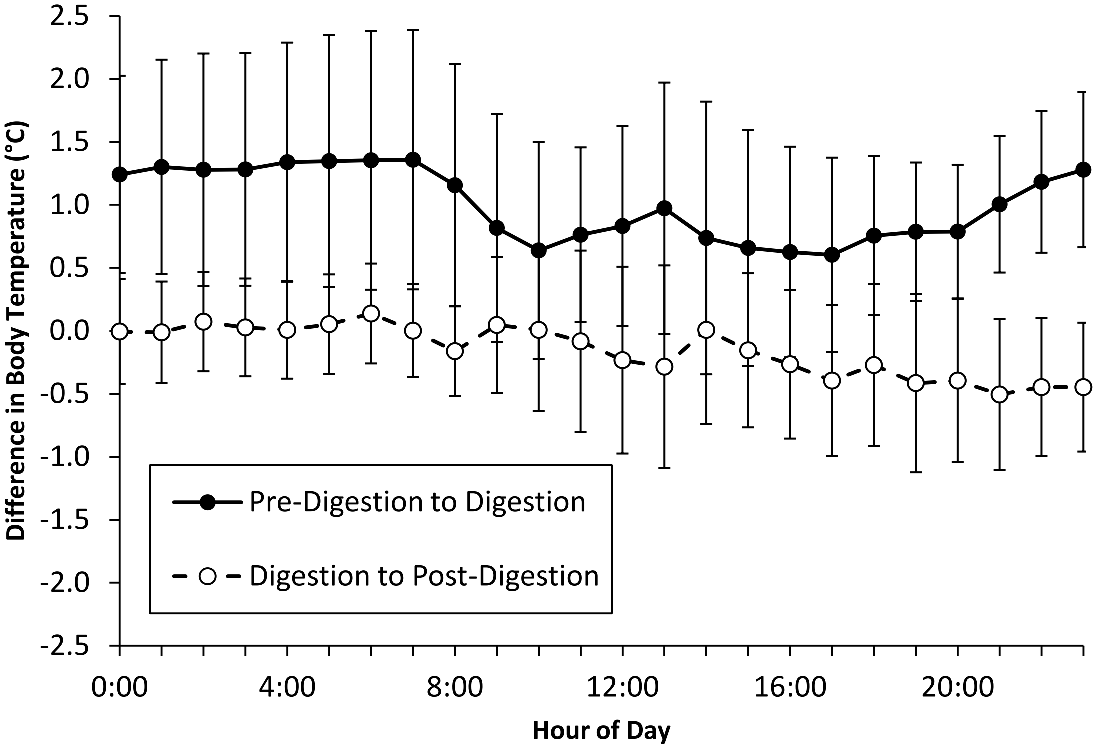

Fig. 7. Differences in body temperature (± SE) between periods. Differences between treatment periods were calculated for each hour, and then averaged across all events.

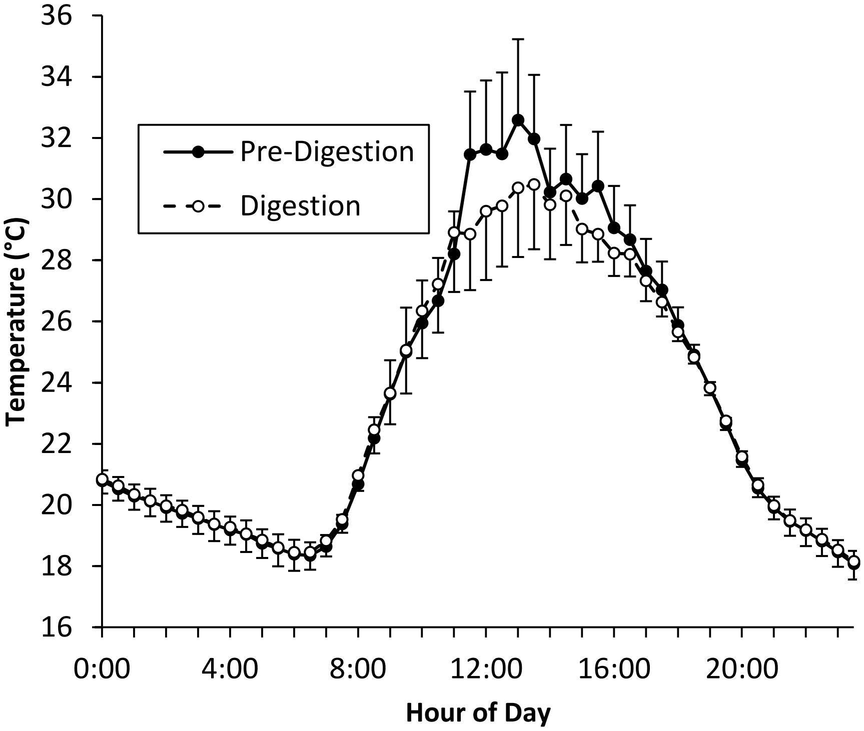

Fig. 8. Hourly operative temperatures (mean ± SE) of pre-digesting and digesting sites.

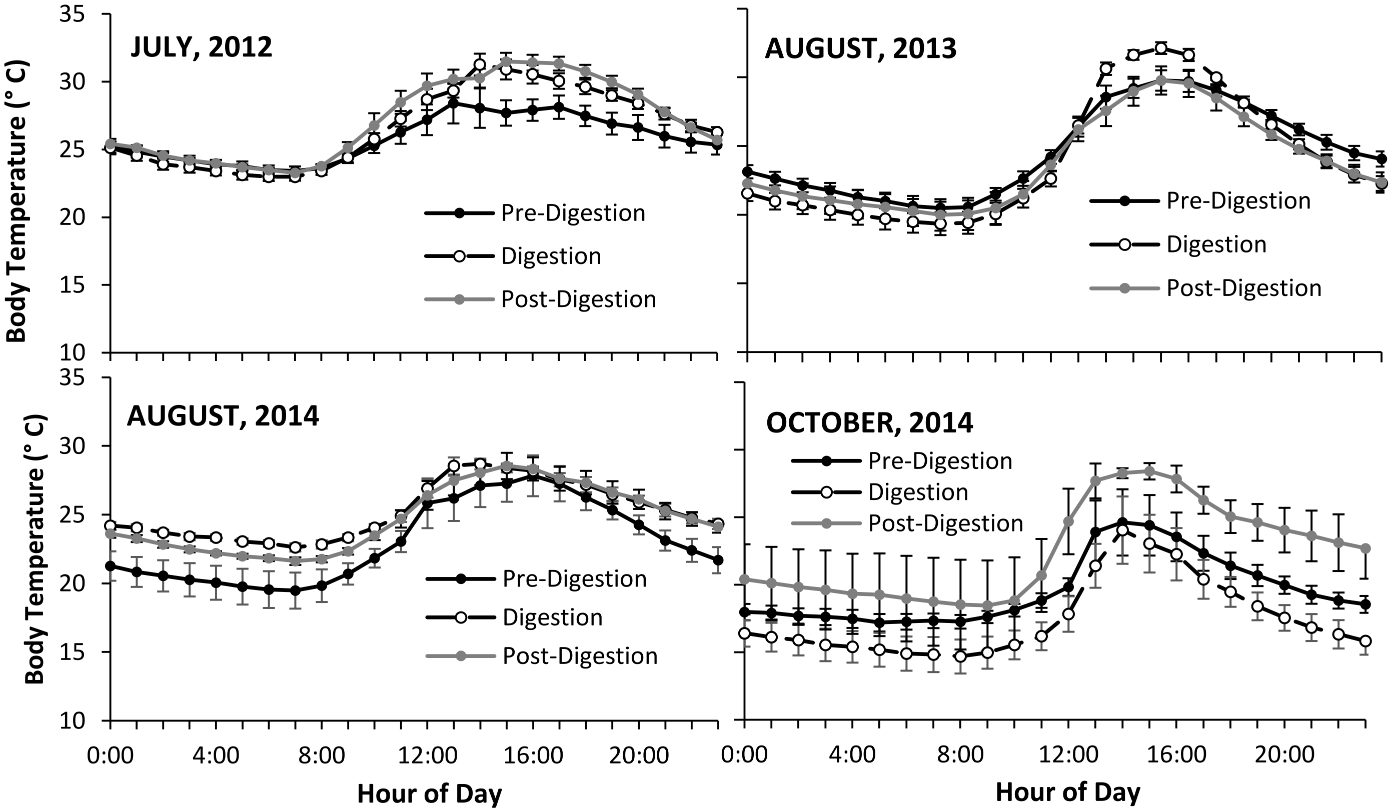

Fig. 9. Mean body temperatures (mean ± SE) of CH4 by feeding event.

APPENDIX C: IACUC APPROVAL LETTER

MIDDLE
TENNESSEE
STATE UNIVERSITY

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