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Authors: Marinho, Patrícia S., Ortega Chinchilla, Jesús E., Braz, Henrique B., and Almeida-Santos, Selma M.

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Effects of pregnancy on the body temperature of the South American rattlesnake, *Crotalus durissus*, in southeastern Brazil

Patrícia S. Marinho^{1,3}, Jesús E. Ortega Chinchilla^{2,4}, Henrique B. Braz³, Selma M. Almeida-Santos^{3,*}

¹ Programa de Pós-graduação em Anatomia dos Animais Domésticos e Silvestres, Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Avenida Orlando Marques de Paiva, 87, Cidade Universitária, São Paulo, SP, 05508-000, Brazil.

² Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14, 321, Cidade Universitária, São Paulo, SP, 05508-090, Brazil.

³ Laboratório de Ecologia e Evolução, Instituto Butantan, Avenida Vital Brazil, 1500, São Paulo, SP, 05503-900, Brazil.

⁴ Laboratorio de Fisiología, Genómica y Transcriptómica adscrito al grupo CINBIN, Escuela de Biología, Universidad Industrial de Santander, Carrera 27 – Calle 9, Ciudad Universitaria, Bucaramanga, Colombia.

* Corresponding author. Email: selma.santos@butantan.gov.br

Abstract. Changes in the body temperature (T_b) of reproductive females are well documented in squamate reptiles. However, the direction of these changes varies among species. Pregnant females may exhibit a lower or higher (and less variable) T_b than nonpregnant females. In some species, pregnancy has no detectable effect on female T_b . In this study, we compared the T_b of female rattlesnakes (*Crotalus durissus*) in different reproductive statuses (pregnant and nonpregnant) to investigate whether pregnancy influences T_b . We measured the T_b of female rattlesnakes kept in a semi-natural outdoor enclosure during summer and spring 2016 and summer 2017. Pregnant females selected a higher mean T_b than nonpregnant females. Moreover, we found significant differences in T_b among seasons. Body temperatures in summer 2017 were higher than in other seasons; however, reproductive status did not influence T_b variance. Therefore, our results agree with the hypothesis that females change their thermoregulatory behavior during pregnancy. Female rattlesnakes may increase their T_b during pregnancy to increase offspring fitness or to maximize their lifetime fitness.

Keywords. Daily variation; Pregnancy; Reproduction; Seasonal variation; Thermoregulation; Viviparity.

INTRODUCTION

Changes in body temperature (T_b) as a function of female reproductive status are well documented in viviparous reptiles (Beuchat, 1986; Charland and Gregory, 1990; Charland, 1995; Mathies and Andrews, 1997; Chiaraviglio, 2006; Gardner-Santana and Beaupre, 2009). Relative stability and changes in T_b during pregnancy are important mechanisms for the proper development of the embryos (Schwarzkopf and Shine, 1991; Brown and Weatherhead, 2000). This is true for both temperate (e.g., Brown and Weatherhead, 2000; Ladyman et al., 2003; Gao et al., 2010) and some tropical species (Luiselli and Akani, 2002; Chiaraviglio, 2006; Woolrich-Piña et al., 2015). However, the direction of the thermoregulatory changes varies among species. For example, in some species, pregnant females exhibit a lower T_b than nonpregnant females, males, or both (Beuchat, 1986; Mathies and Andrews, 1997). In other species, pregnant females exhibit higher and/or less variable T_b than nonpregnant females or males (Charland and Gregory, 1990; Blouin-Demers and Weatherhead, 2001; Chiaraviglio, 2006; Gao et al., 2010). Nevertheless, the effect of pregnancy on thermoregulation is absent or undetectable in other species (Bovo et al., 2010).

Viviparous females retain their embryos in the uterus throughout development and give birth to fully formed young, enabling them to enhance offspring fitness through thermoregulation. For instance, females can accelerate embryonic development (thus decreasing the duration of pregnancy) by selecting higher temperatures through behavioral thermoregulation (Shine, 1985, 2012; Dorcas and Peterson, 1998). Thus, females can reduce the costs associated with pregnancy and adjust the rate of embryonic development so that the offspring are born at a favorable time, thereby reducing mortality (Schwarzkopf and Shine, 1991; Andrews, 1997; Brown and Weatherhead, 2000).

Rattlesnakes have been intensively used as model organisms to investigate several general questions in ecology, physiology, and evolution (Schuett et al., 2017). Although most studies focus on North American rattlesnakes, several aspects of the natural history of the South American rattlesnake, *Crotalus durissus* (Linnaeus, 1758), have also been investigated (Salomão et al., 1995; Almeida-Santos and Salomão, 1997; Almeida-Santos and Orsi, 2002; Almeida-Santos et al., 2004; Tozetti and Martins, 2008; Hoyos and Almeida-Santos, 2016). However, studies on the thermal biology of rattlesnakes have mainly focused on temperate zone species, in which thermoregulation and reproductive status interact strongly in response

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to cold climate (Gier et al., 1989; Charland and Gregory, 1990; Wills and Beaupre, 2000; Gardner-Santana and Beaupre, 2009). In the tropics, the influence of pregnancy on T_b has been observed in some snake species (e.g., *Liasis fuscus* Peters, 1873; Shine and Madsen, 1996; *Afonatrix anoscopus* [Cope, 1861], *Grayia Smythii* [Leach, 1818], *Natriciteres fuliginoides* [Günther, 1858], *N. variegata* [Peters, 1861]; Luiselli and Akani, 2002), but not in others, including viperids (e.g., *Bothrops insularis* [Amaral, 1922]; Bovo et al., 2010).

We recorded the daily and seasonal pattern of T_b in pregnant and nonpregnant South American rattlesnakes (*Crotalus durissus*). Specifically, we test the hypothesis that pregnancy affects the T_b of snakes.

MATERIALS AND METHODS

Study species

The South American rattlesnake, *Crotalus durissus*, is a robust, large (2 m total length; Marinho et al., 2009) viperid snake widely distributed in the Neotropical region, from Mexico to northern Argentina (Campbell and Lamar, 2004). In Brazil, *C. durissus* occurs primarily in open areas of the Cerrado (Brazilian savanna) and Caatinga domains, but it also occurs in open enclaves and disturbed open areas in the Amazon and Atlantic forests (Nogueira et al., 2019). This species feeds primarily on endothermic prey (rodents and small marsupials; Sant'Anna and Abe, 2007; Hoyos and Almeida-Santos, 2016). Female *C. durissus* are viviparous and exhibit a biennial seasonal reproductive cycle (Almeida-Santos and Orsi, 2002). Females initiate vitellogenesis in late summer, mate in autumn, store sperm in the oviducts during winter, and ovulate in spring (Almeida-Santos and Salomão, 1997; Almeida-Santos and Orsi, 2002; Almeida-Santos et al., 2004). Pregnancy occurs in spring and summer and lasts 4–5 months (i.e., ≤ 150 d; Almeida-Santos and Orsi, 2002). Parturition occurs in summer (Almeida-Santos and Orsi, 2002).

Study site

This study was conducted at the serpentarium of the Instituto Butantan (São Paulo state, southeastern Brazil). The serpentarium is a semi-natural outdoor enclosure (500 m²) divided into three physically separated compartments, each designed for the maintenance and public display of a different snake species. The rattlesnake enclosure (183.22 m²) mimics the natural conditions of its habitat, including sandy soil, trees, shrubs, clusters of rocks, a small water body, and concrete shelters (for photo and schematic drawing, see Gomes and Almeida-Santos, 2012). The rattlesnake enclosure has no artificial heating system, but it receives direct sunlight throughout the year. Therefore, rattlesnakes were exposed to natural variations in temperature and photoperiod, but they had opportunities to thermoregulate by moving freely in a sun-to-shade mosaic.

Specimens and reproductive status

We studied 17 adult female *Crotalus durissus* collected in São Paulo state, southeastern Brazil (Appendix 1). The snakes were collected and brought to the Instituto Butantan by laypersons during summer and spring 2016 and summer 2017. During the study period, the snakes were fed mice (*Mus musculus* Linnaeus, 1758) once per month. Because *C. durissus* exhibits postprandial thermophily (Andrade et al., 1997; Tattersall et al., 2004), we discarded all T_b data measured during the digestion period, as they might mislead results. We assessed female reproductive status through ultrasonographic scans using a portable ultrasound system with a 7.5-MHz linear array probe, with a 60-mm field of view (LOGIQ™ 100 PRO, GE Healthcare, Milwaukee, USA). The depth and screen contrast settings on the instruments were adjusted to allow for the best visualization of the internal anatomy. Snakes were restrained with a hook and a transparent plastic tube and held throughout the examination. A layer of conductive gel was applied to the snake skin, and a series of lateral and ventral scans were performed on the posterior third of each snake body. Based on the ultrasound scans, we assigned females to nonpregnant or pregnant categories. Nonpregnant (i.e., nonreproductive) snakes had small (< 10 mm; Almeida-Santos and Orsi, 2002) and non-echogenic ovarian follicles, and pregnant snakes had large, echogenic, and linearly arranged yolk masses containing visible germinal discs or embryos (Taylor and DeNardo, 2005). After completing the scanning procedures, the gel was gently removed, and the animal was released into the enclosure. Ultrasonographic evaluations were performed in early summer and spring 2016. Nonpregnant females were 800–1,100 mm SVL ($\bar{x} \pm SD = 950.5 \pm 9.4$ mm, $n = 7$) and 510–1,394.3 g body mass ($\bar{x} \pm SD = 871.4 \pm 330.4$ g). Pregnant females were 900–930 mm snout–vent length (SVL; $\bar{x} \pm SD = 920.0 \pm 1.4$ mm; $n = 6$) and 517–1,363 g body mass ($\bar{x} \pm SD = 796.66 \pm 323.95$ g).

Snake body temperatures

A temperature-sensitive datalogger (WatchDog B100 2K, Spectrum Technologies, Aurora, IL, USA; diameter = 1.6 cm and mass = 3 g) was implanted in the body cavity of each snake during summer/spring 2016 and summer 2017. For this, snakes were restrained in transparent plastic tubes and then anesthetized with isoflurane. A small incision was made in the lateral edge of the ventral scales of anesthetized females (Reinert and Cundall, 1982). In pregnant females, a section of the uterus was everted to expose a developing embryo (adapted from Mathies and Andrews, 1997). We then determined the embryonic developmental stage following criteria by Hubert and Dufaure (1968) for the viperid *Vipera aspis* (Linnaeus, 1858). In this classification, embryonic development is divided into a series of 43 stages, with stage 1 marking the beginning of cell division and stage 43 indicating a full-term embryo (Hubert and Dufaure, 1968). We then used the

stages of development to divide gestation into three stages: early pregnancy (stages 1–27, from cleavage to mid-organogenesis), mid-pregnancy (stages 28–32, from mid- to late organogenesis), and late pregnancy (stages 33–43, from early to late growth). These three phases allowed us to assess seasonal differences in T_b throughout gestation. Next, the uterine section was reinserted into the body cavity, the dataloggers were implanted intraperitoneally, and the incision was sutured. We returned snakes to their enclosure after a post-surgery recovery period of approximately one week. The data loggers were programmed to record T_b every 2 h, totaling 12 records per day. At the end of each summer, the dataloggers were removed from each female using the same procedures described above. T_b records were extracted from the dataloggers using SpecWare 9 Basic software (Spectrum Technologies, Inc., 2016).

Operative environmental temperatures (T_e , thermal models)

We compared the potential T_b available to the snakes with the actual observed temperatures (Charland and Gregory, 1990). For this, we used physical models (T_e) made of water-filled copper pipes 60 cm long and 16 cm in diameter (Bovo et al., 2012). To check the accuracy of our physical models, we compared their thermal profiles with those of a freshly dead *Crotalus durissus* (Bovo et al., 2012). For this, we inserted dataloggers inside the snake carcass and the models. We sampled snake carcass and copper model temperatures under similar conditions for heat exchange/equilibration expected to occur in nature (Bovo et al., 2012). Temperatures were recorded every 15 minutes for 24 h.

To sample the T_e available for rattlesnakes, we distributed six physical models along the microhabitats (thermal sites) most used by *Crotalus durissus* in the enclosure (Gomes and Almeida-Santos, 2012). We placed these physical models exposed to solar radiation (leaning against the walls), at shaded areas on the ground, and inside the shelters. The temperature of these models was recorded at 2-h intervals.

Statistical analysis

All statistical tests were performed using Statistica 7.0 (StatSoft, 2004) and GraphPad Prism 5.0 (GraphPad, 2007). Values are presented as $\bar{x} \pm SD$, and the significance level for all statistical tests was set to $\alpha = 0.05$. We used correlation analysis to examine the relationship between T_b and T_e and thus provide information on T_b modulation. We calculated the daily mean T_b for each snake based on the temperatures recorded every 2 h, representative of the gestation period. We tested for differences in mean T_b between pregnant and nonpregnant females within seasons using repeated-measures analysis of variance (ANOVA) and among seasons using generalized linear

mixed models (GLMM), with individual females as a random factor nested within reproductive state and season. We included in the model the minimum number of parameters estimated by the Akaike Information Criterion (AIC) to explain the variation in T_b .

RESULTS

Reproductive status

In summer 2016, we detected four nonpregnant females and one pregnant female through ultrasound scans. In spring 2016, we identified three nonpregnant females and one pregnant female. Lastly, in summer 2017, we detected three nonpregnant females and five pregnant females.

Thermal models

The temperatures of the snake carcass and copper model were significantly correlated ($n = 100$; $r = 0.935$; $P = 0.004$). We considered that the operational temperatures obtained from the thermal models were adequate and provided a better resolution of the landscape temperature, compatible with individual body size.

Daily variation in body temperature and model temperature

We recorded the daily pattern of T_b of pregnant and nonpregnant females, as well as the daily pattern of T_e , in the three study periods (summer 2016, spring 2016, and summer 2017). Female T_b varied throughout the day, oscillating between higher than or lower than the temperatures of the models. Mean T_b was higher than the mean temperature of the models; however, in both summers, the mean temperature and standard variation of the models surpassed T_b between 10:00 and 16:00. Snake T_b slowly declined after 16:00. The lowest values of T_b were recorded during spring at dawn, while the highest values were recorded during both summers at midday (Fig. 1).

We also tested for daily variation in the T_b of pregnant and nonpregnant females and T_e per season. For this, we divided the day into three periods: 1) sunrise (02:00–08:00 h), 2) daytime (10:00–16:00 h), and 3) evening (18:00–24:00 h). T_b s were highest during the daytime in all seasons (summer 2016: $F_{2,31} = 22.44$, $P < 0.001$; spring 2016: $F_{2,31} = 6.16$, $P < 0.01$; summer 2017: $F_{2,31} = 28.30$, $P < 0.001$). However, T_b s were higher in pregnant snakes than nonpregnant snakes and T_e only in the spring 2016 ($F_{2,31} = 10.66$, $P < 0.01$). Female T_b and T_e were similar in the summers of 2016 ($F_{2,31} = 0.63$, $P = 0.552$) and 2017 ($F_{2,31} = 1.190$, $P = 0.204$).

We analyzed temperature data for each season separately and found significant differences among the model and body temperatures (per reproductive stage).

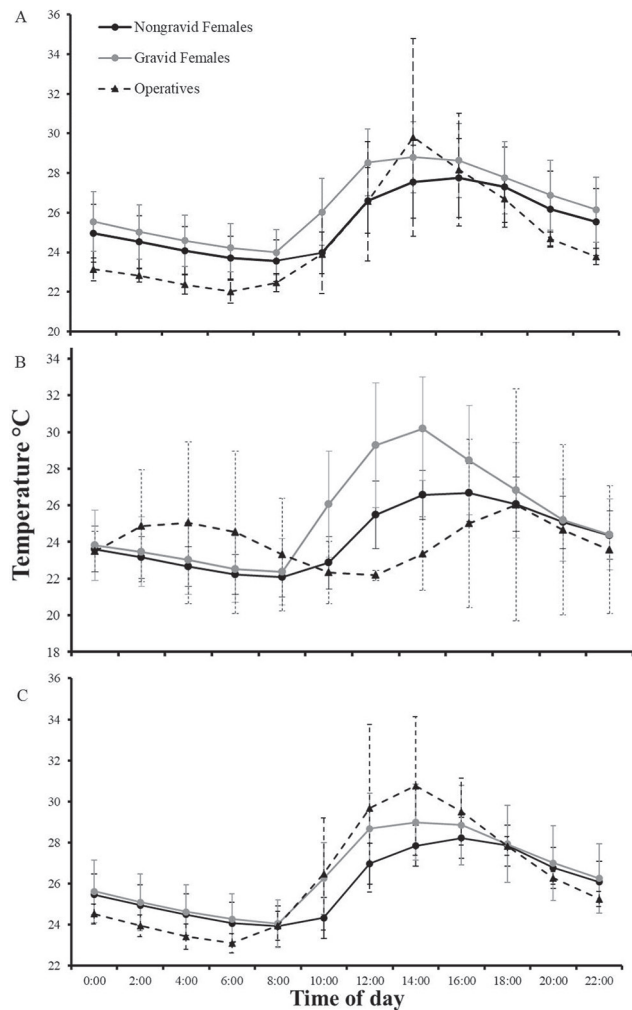


Figure 1. Daily variation in body and operative temperature (T_e) for *Crotalus durissus* rattlesnakes. (A) Summer 2016, (B) spring 2016, and (C) summer 2017.

In summer 2016, the model had the highest temperatures ($F_{5,155} = 14.637$, $P < 0.001$), followed by nonpregnant and pregnant females (Fig. 2A). Pregnant females showed the highest temperatures in spring 2016 ($F_{4,2536} = 86.828$, $P < 0.001$; Fig. 2B) and summer 2017 ($F_{8,8805} = 15.481$, $P < 0.001$; Fig. 2C).

Seasonal variation in body temperatures

Body temperature was higher in pregnant than nonpregnant snakes in spring 2016 and summer 2017 (Table 1; Fig. 3). The most parsimonious linear mixed model was the Full-3 (AIC = 31214, $wAIC = 1$; Table 2), which included: body temperature $\sim T_e +$ reproductive status + (1|individuals female). We observed in this model that the variation in body temperature in the three seasons depended primarily on reproductive status and a thermal factor.

We found no effect of reproductive status on the precision of thermoregulation during the 2016 and 2017 summers. However, pregnant females thermoregulated more precisely (i.e., lower variance) in both summers than in spring (Levene’s test, $F_{2,2592} = 26.103$, $P < 0.001$; summer 2016: $S^2 = 5.15$, spring 2016: $S^2 = 12.32$, summer

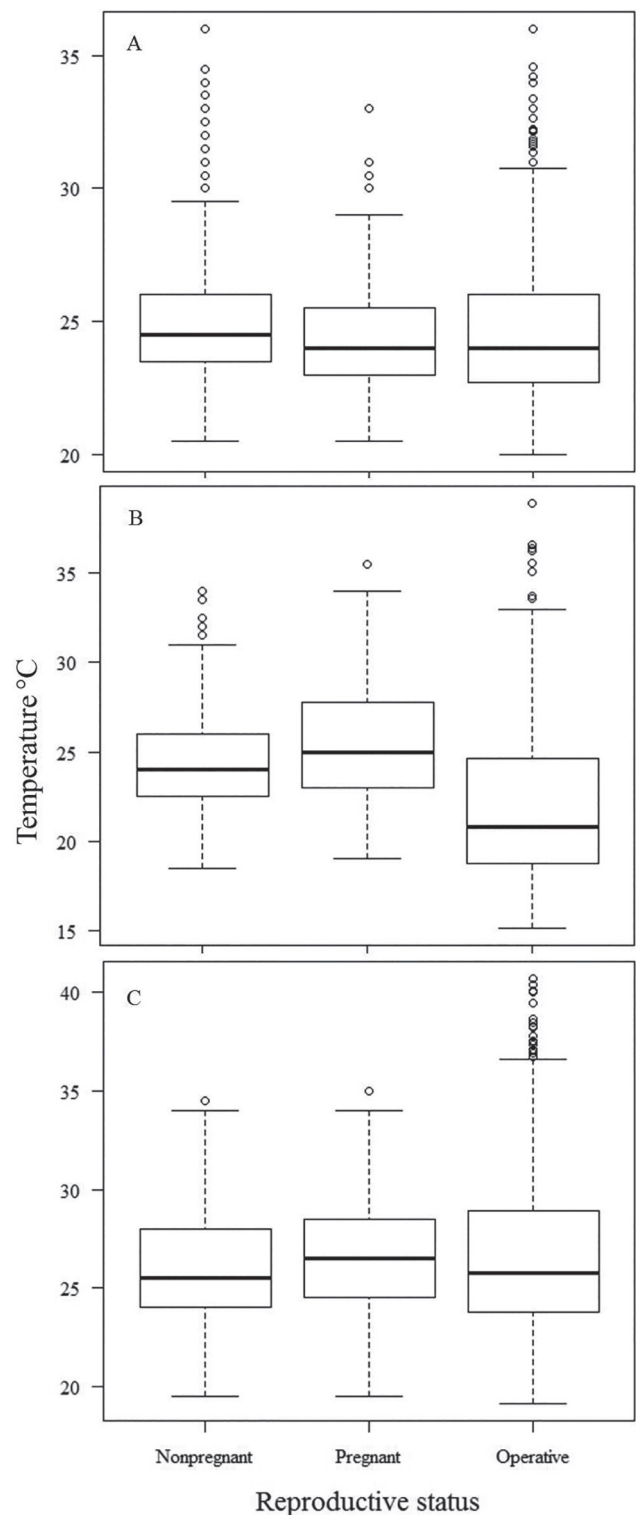


Figure 2. Variation in body temperature (pregnant and nonpregnant females) during the study period for *Crotalus durissus* rattlesnakes. (A) Summer 2016, (B) spring 2016, and (C) summer 2017.

2017: $S^2 = 5.42$). Nonpregnant females showed no significant seasonal changes in the precision of thermoregulation (Table 2). We measured T_b throughout pregnancy in a single female. In this individual, T_b was higher during mid- and late pregnancy than early pregnancy ($F_{2,1410} = 83.84$, $P < 0.001$; Fig. 4). In each season, mean T_b (nonpregnant and pregnant females) was positively correlated with mean T_e ($P < 0.001$ for all cases; Table 1).

Table 1. Body temperature (T_b) and operative temperature (T_e) in *Crotalus durissus* rattlesnakes. Precision was calculated as the variance (S^2) of individual mean T_b . Differences were considered significant at $P < 0.05$.

Season	Reproductive status	T_b		T_e	T_b VS T_e	P-value
		$\bar{x} \pm SD$	S^2			
Summer 2016	Nonpregnant	24.77 ± 1.78	3.16	24.83 ± 3.08	0.70	0.0109
	Pregnant	24.35 ± 2.27	5.15			
Spring 2016	Nonpregnant	24.24 ± 2.09	4.36	23.16 ± 3.93	0.40	0.0001
	Pregnant	25.46 ± 3.51	12.32			
Summer 2017	Nonpregnant	25.92 ± 2.36	5.56	26.86 ± 4.48	0.75	0.0001
	Pregnant	26.45 ± 2.33	5.42			

Table 2. Generalized linear mixed model (GLMM) based on different combinations and interactions of parameters such as body temperature, operative temperature, reproductive status, and season. The most parsimonious linear mixed model was Full-3. Combinations and interactions of the parameters in GLMM models:

1. Full-3: lmer (formula = Body temperature ~ Operative temperature + Season + Reproductive status + (1|Number individual), data = Snake)
2. Full-2: lmer (formula = Body temperature ~ Operative temperature + Season + Reproductive status + (1|Number individual)* (1|Season), data = Snake)
3. Full-1: lmer (formula = Body temperature ~ Reproductive status + (1|Number individual)* (1|Season), data = Snake)
4. Null model: lmer (formula = Body temperature ~ 1 + (1|Number individual), data = Snake)

Models	K	AIC	Delta AIC	wAIC
1. Full-3	5	31,214	0	1
2. Full-2	4	34,716	3,502	0
3. Full-1	5	34,715	3,501	0
4. Null model	3	35,108	3,894	0

showed higher T_b than solitary conspecifics under similar conditions due to the reduction in surface–volume ratio (Graves and Duvall, 1987). By making postural adjustments (such as body coiling), snakes might reduce heat loss and maintain thermal inertia (Ayers and Shine, 1997). Both the thermal environment and the behavior of the snake determine the daily patterns of T_b variation (Peterson, 1987). Another possibility is to increase basking. Several authors have suggested that, in viviparous reptiles, pregnant females bask more than nonpregnant females or males, which allows the female or her offspring to benefit from accelerated embryogenesis and early birth (reviewed by Shine, 1980). Apparently, pregnancy in *C. durissus* induces a female to bask earlier in the day during spring. The importance of behavior in determining T_b patterns generally increases with increasing T_e .

DISCUSSION

Daily pattern of T_b variation

Daily variation in the T_b of *Crotalus durissus* was similar to the “oscillating” pattern reported in the free-ranging garter snake, *Thamnophis elegans vagrans* (Baird and Girard, 1853) (Peterson, 1987). In the morning, female rattlesnakes maintain their T_b higher than T_e . This thermal profile suggests that snakes keep T_b at a higher level than the T_e available at 00:00–06:00 (Fig. 1). In the early afternoon, T_e begins to rise and peak at 14:00–16:00. By that time, the T_b s of rattlesnakes are cooler than T_e . The divergence between the daily variation in T_b and that of the physical model indicates that female rattlesnakes can modify their T_b by selecting cooler microenvironments (Charland and Gregory, 1990). This strategy might be used to avoid potentially lethal temperatures. In nature, *C. durissus* apparently reduces the risks of overheating by selecting cooler microhabitats during the day and avoids lower temperatures at night by selecting warmer microhabitats (Tozetti and Martins, 2008). After 16:00, environmental temperatures decline, and female rattlesnakes maintain a higher T_b at night.

Rattlesnake T_b often exceeded model temperatures by several degrees, presumably because the snakes selected warmer microhabitats and performed behavioral adjustments such as coiling (Peterson, 1987) or aggregation (P.S. Marinho, pers. obs.). For instance, aggregated *Crotalus viridis viridis* (Rafinesque, 1818) individuals

Effects of reproductive status on T_b

One of the most prominent benefits of the evolution of viviparity in reptiles is that pregnant females can optimize the rate of embryonic development through thermoregulation (Shine, 1985, 2014). The increase and stability of T_b during pregnancy have been considered essential to ensure embryonic development and enhance offspring fitness (Schwarzkopf and Shine, 1991; Lourdais et al., 2004; Gardner-Santana and Beaupre, 2009; Uller et al., 2011). In this scenario, we expect pregnant females to have higher or less variable values of T_b than nonpregnant females (Charland and Gregory, 1990). Our results corroborate this hypothesis. During the summer (when the second half of pregnancy and parturition occur; Almeida-Santos and Orsi, 2002), pregnant *Crotalus durissus* maintain a higher T_b than nonpregnant conspecifics. This finding suggests that the seasonal reproductive cycle of female rattlesnakes (Almeida-Santos and Orsi, 2002) follows the premise of the benefits of selecting higher temperatures during pregnancy.

Our results agree with previous studies involving other squamate species exhibiting gestational thermophily (Charland and Gregory, 1990; Tu and Hutchison, 1994; Lourdais et al., 2004; Crane and Greene, 2008; Woolrich-Piña et al., 2015). For instance, pregnant garter snakes (*Thamnophis sirtalis* [Linnaeus, 1758] and *T. elegans* [Baird and Girard, 1853]) show higher mean T_b and thermoregulate more precisely (i.e., less variable T_b) than nonpregnant conspecific females. Moreover, these differ-

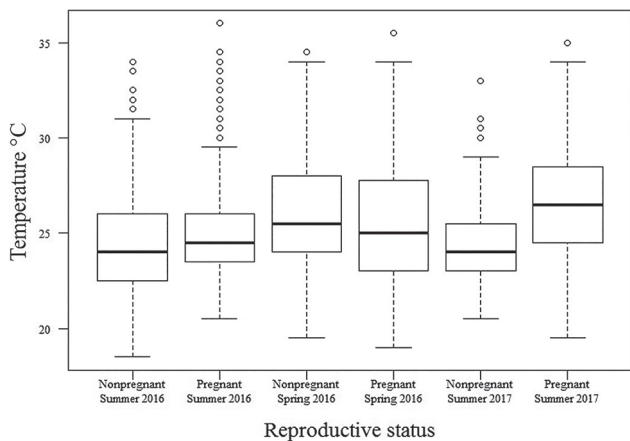


Figure 3. Seasonal variation in body temperature (T_b) of pregnant and non-pregnant *Crotalus durissus* rattlesnakes among seasons.

ences in thermoregulation persist throughout pregnancy but disappear shortly after parturition (Charland, 1995). Pregnant *Crotalus viridis* also show higher mean T_b and higher precision of thermoregulation than nonpregnant conspecifics (Charland and Gregory, 1990). Pregnant and nonpregnant *C. durissus* showed similar variance in T_b . However, the variance in T_b of pregnant females decreased significantly between spring and summer (Table 1). According to Charland (1995), the accuracy of the regulated T_b could be as important as maintaining an optimal mean T_b .

Most species in which thermoregulation has been studied inhabit temperate regions. However, differences in T_b between pregnant and nonpregnant females also occur in a range of tropical snakes (e.g., Shine and Madsen, 1996; Luiselli and Akani, 2002) and lizards (e.g., Dayananda et al., 2017). But why do pregnant females maintain higher T_b even in the tropics? Temperature during development influences numerous offspring phenotypic traits that could affect survival and fitness (reviewed by Birchard, 2004). Accordingly, females are thought to change their T_b during pregnancy to increase offspring fitness (Shine, 1995, 2012). More broadly, changes in female T_b are hypothesized to maximize their lifetime reproductive success (Schwarzkopf and Andrews, 2012a, b). Thermoregulation in tropical snakes is relevant to certain activities and life-history attributes, even in thermally favorable environments (Luiselli and Akani, 2002). Previous studies have found that metabolic rates of reproducing females increase steadily as pregnancy proceeds, which might be related to the increased metabolic demands by embryos as development proceeds (Birchard et al., 1984; DeMarco and Guillette, 1992). This could also explain the increase in T_b in the pregnant female we monitored throughout the gestational period. Increased oxygen consumption and weight-specific oxygen consumption at late pregnancy could indicate that fetal oxygen consumption is scaled to fetal and not to maternal weight (Birchard et al., 1984). On the other hand, oxygen consumption depends strongly on body temperature and mass in *Vipera aspis*, even though oxygen consumption does not differ between pregnant and nonpregnant females (Ladyman et al., 2003). Thus,

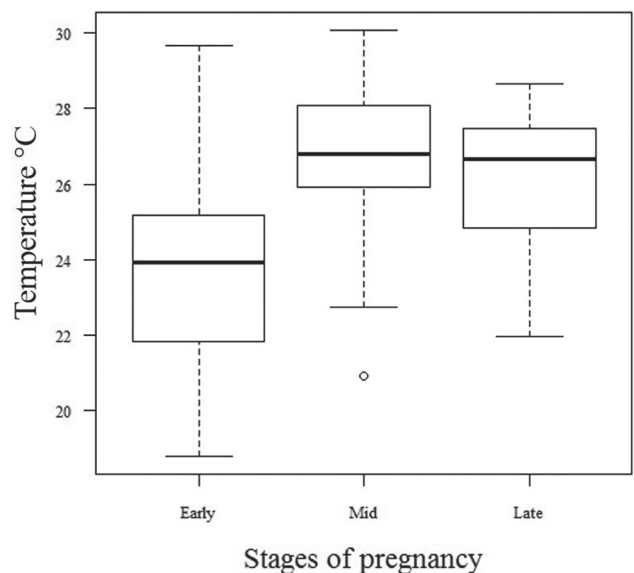


Figure 4. Variation in body temperature (T_b) throughout pregnancy in a single pregnant *Crotalus durissus* rattlesnake.

maternal metabolism is not influenced by litter size during early gestation, but it is weakly influenced during late gestation (Ladyman et al., 2003).

We emphasize that the reproductive cycle of female *Crotalus durissus* is markedly seasonal. Vitellogenesis occurs from late summer to winter, ovulation/fertilization occurs in spring, and pregnancy occurs in spring and summer (Salomão and Almeida-Santos, 2002). As pregnant females maintain high T_b , we assume that higher temperatures or more refined thermoregulatory behaviors are less important for nonreproductive than reproductive females. The daily pattern of T_b of pregnant *C. durissus* is consistent with the timing of thermoregulatory activities previously observed in reproductive females (Salomão et al., 1995). Pregnant *C. durissus* probably increase basking during the daytime to maintain high T_b overnight. Nighttime retention of part of the heat gained during the daytime could be related to circadian adjustments in regional blood flow (Amiel et al., 2011) or postural changes, such as coiling (Ayers and Shine, 1997).

In conclusion, our results suggest that reproductive status influences the selection of higher T_b in female *Crotalus durissus*. Our study is the first to demonstrate the influence of reproductive status on the thermoregulation of the South American rattlesnake. Moreover, our results agree with the hypothesis that females change their thermoregulatory behavior during pregnancy (Shine, 1995). Further studies should evaluate the costs and benefits associated with the change in T_b during pregnancy and determine whether and how higher temperatures influence maternal and offspring fitness.

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APPENDIX 1

Location of collection of the *Crotalus durissus* individuals used in this study.

State of São Paulo. Araraquara ($n = 1$), Botucatu ($n = 2$), Itu ($n = 2$), Piracaia ($n = 1$), Santana do Parnaíba ($n = 1$), São José dos Campos ($n = 7$), São Pedro ($n = 1$), Tapiratiba ($n = 1$), Votorantim ($n = 1$).