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# High thermal quality rookeries facilitate high thermoregulatory accuracy in pregnant female rattlesnakes

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## ABSTRACT

Temperature is a primary factor influencing organismal development, and the fluctuating daily and seasonal thermal regimes of temperate climates may challenge the ability of viviparous reptiles to optimize body temperatures during gestation. Testing how viviparous reptiles navigate highly variable thermal conditions (e.g., relatively cold nights and/or highly fluctuating temperatures) is a powerful way to understand how they use microhabitats for thermoregulatory benefits. We assessed the thermal ecology of pregnant and non-pregnant female Prairie Rattlesnakes (*Crotalus viridis*) inhabiting a high-elevation, montane shrubland in northwest Colorado throughout their short summer active season, addressing the thermal consequences of microhabitat selection with a focus on thermoregulation of pregnant females at communal rookery sites. We deployed operative temperature models to collect data on the thermal quality of microhabitats used by the snakes, and calculated thermoregulatory accuracy of the snakes by comparing their field-active body temperatures with preferred body temperatures of snakes placed in a thermal gradient. Pregnant females inhabited rocky, hilltop rookeries that had higher thermal quality due to higher and less variable nighttime temperatures compared to microhabitats in the surrounding prairie. Pregnant females therefore thermoregulated more accurately than non-pregnant females. The difference was most pronounced during the night, when pregnant females at rookeries maintained higher body temperatures than non-pregnant snakes in the prairie. Our results support the hypothesis that one major reason female rattlesnakes at high latitudes and/or high elevations forgo migration and gestate at communal, rocky, hilltop rookeries is that, relative to prairie microhabitats, they provide better conditions for thermoregulation during pregnancy.

## 1. Introduction

Animals inhabiting temperate environments face many obstacles to their fitness and survival and, for ectotherms, primary among these challenges are variable temperatures. In extreme environments such as those at high latitudes and high elevations, great fluctuations in solar radiation and temperature occur over both short and long timescales (Stevenson 1985). In addition, high elevation imparts reduced water vapor pressure, resulting in a drying effect (Schmidt-Nielsen 1997; Porter et al., 2002; Körner 2007). In response to such affronts, ectotherms inhabiting these environments typically spend the long, cold winters in a state of quiescence within seasonal hibernacula (Gregory 1982). Active seasons can be markedly abbreviated (Gannon and Secoy 1984; Duvall et al., 1985) and bear variable temperature extremes, forcing ectotherms to periodically reduce activity and potentially making them susceptible to predation (Gregory 1982; Graves and Duvall 1987). Ultimately, such conditions can constrain energy acquisition, growth, and development of young. For example, Black Ratsnakes from a high-latitude population with a brief active season showed lower growth rates and later age at maturity than a lower-latitude population (Blouin-Demers et al., 2006). Temperature has dramatic impacts on the development of embryonic reptiles, impacting many factors including hatching success, survival, and offspring phenotype (Refsnider and Janzen 2010; Singh et al., 2020). Therefore, regulating body temperature ($T_b$) through microhabitat selection and behavioral adjustments while minimizing risks may be critical for reproduction in these challenging environments (Stevenson 1985). Investigating the thermal ecology of ectotherms in extreme environments (e.g., high latitude, high elevation, and others) provides an excellent opportunity to observe such relationships between behavioral and physiological processes key to fitness (Beaupre 1995; Taylor et al., 2020). In general, thermoregulatory accuracy (the ability to achieve $T_b$ close to preferred body temperature

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as defined by Hertz et al., 1993) is important for successful embryogenesis, gestation (in viviparous ectotherms; Evans et al., 2021), and characteristics and survival of offspring (O'Donnell and Arnold 2005; Singh et al., 2020; Pettersen et al., 2023).

Behavioral thermoregulation by shuttling among thermally variable microhabitats may be essential for maintaining thermal homeostasis, especially in scenarios where extreme temperature fluctuations may occur over short time scales. These movements serve to control heat exchange, which helps animals meet energy requirements at hourly, daily, seasonal, and annual rates (McCafferty et al., 2018). As the range of available microhabitats in an environment can obviously impact the range of available $T_b$ for ectotherms (Beaupre 1995), these animals need refuges (e.g., shrubs, burrows, or crevices) with suitable thermal characteristics to escape extreme temperatures (Klauber, 1972; King 1987), hide from predators, or both. As such, heterogeneity of the environment plays an important role in providing animals with thermoregulatory opportunities (Gaudenti et al., 2021; Sears et al., 2016). At basking sites, ectotherms may position their bodies to be partly shaded and partly exposed to direct sunlight, taking advantage of the full range of available environmental temperatures to make fine-scale adjustments in body temperatures (*sensu* Klauber, 1972). Even in the shade or inside a refugium, reptiles can behaviorally thermoregulate by altering their contact with warm or cool surfaces to alter conductive heat transfer, by making physiological adjustments such as altering heart rate and blood flow to the skin, or through many other strategies (Black et al., 2019).

Prairie Rattlesnakes (*Crotalus viridis*) are a widespread and common snake ranging from southern Canada through the central United States into northern Mexico (Ashton and de Queiroz 2001). At high-latitude and high-elevation sites in the Canadian and American prairies, these snakes often overwinter in communal hibernacula (i.e., dens) with hundreds or even thousands of snakes (see Gregory 1984). Large hibernacula aggregations are, at least in part, the result of rare geological features like deep rocky fissures that allow the snakes to escape below the frostline during the winter and then bask in thermally favorable south-facing areas in spring and fall (Hamilton and Nowak 2009; Gienger and Beck 2011). While male and nonreproductive female *C. viridis* disperse long distances from the hibernacula in summer to search for prey and mates (Reinert 1984; Duvall et al., 1985), pregnant females remain in communal "nurseries" or rookeries at or near the hibernacula (Klauber 1956; Graves and Duvall 1995). The rookeries are typically rocky, south-facing shelters on hills, often near but distinct from hibernacula, where pregnant females aggregate, rarely move or forage, then give birth and attend their young in late summer (Duvall et al., 1985; Gannon and Secoy 1985; Graves and Duvall 1993). Because the active season is so short, opportunities for feeding after late summer parturition are few. Female rattlesnakes that reproduce must therefore spend the following year (or longer) foraging to replace energy reserves before they can gestate again, resulting in biennial or triennial reproduction (Bull and Shine 1979; Gannon and Secoy 1984; Macartney and Gregory 1988).

The major benefit of remaining in hilltop rookeries for pregnant *C. viridis* may be to better facilitate accurate thermoregulation (Graves and Duvall 1993). Viviparous snakes are particularly sensitive to temperature variation and must maintain relatively constant $T_b$ for proper embryonic development and, by extension, offspring survival and maternal fitness (Lourdais et al., 2004; O'Donnell and Arnold 2005; Webb et al., 2007; Gao et al., 2010; Pettersen et al., 2023). Accessibility of sufficient refugia is an essential component of successful thermoregulation, and rookeries often include adjacent exposed and shaded areas, presenting a variety of thermoregulatory opportunities for pregnant snakes (Gregory 1975; Reinert 1984; Duvall et al., 1985; Gannon and Secoy 1985; Graves et al., 1986; Graves and Duvall 1987, 1993; Shipley et al., 2013). Compared to non-pregnant snakes, pregnant *C. viridis* inhabiting rookeries move infrequently, bask at short distances from rock openings, retreat to cooler areas beneath rocks when it is very hot (Graves and Duvall 1993), and spend more time aboveground thermoregulating (Gannon and Secoy 1985; Goode and Duvall 1989). As a result, pregnant snakes often maintain higher and more stable daily and seasonal body temperatures compared to non-pregnant snakes (Gannon and Secoy 1985; Graves and Duvall 1993; Gardner-Santana and Beaupre 2009; but see Charland and Gregory 1990; Isaac and Gregory 2004). These factors suggest that rookeries serve an important role in facilitating thermoregulation in viviparous ectotherms while simultaneously minimizing predator exposure (Gregory 1975; Naulleau 1979; Graves and Duvall 1993; but see Herr et al., 2020). Notably, however, these behavioral observations have not yet been paired with a quantitative thermal ecology study (e.g., Hertz et al., 1993; Taylor et al., 2020; Crowell et al., 2021) that assesses available environmental temperatures, preferred $T_b$, field-active $T_b$, thermal quality of various habitats, and ultimately thermoregulatory accuracy of both pregnant and non-pregnant snakes.

We conducted a study to quantify the thermal ecology of a high-elevation population of *C. viridis* to better understand how microhabitat use by pregnant and non-pregnant female rattlesnakes is related to their thermal environments. We hypothesized that rookeries are high quality thermal refugia that facilitate high thermoregulatory accuracy for pregnant female *C. viridis*. We expected to find that pregnant snakes using the rookeries would exhibit higher and less variable body temperatures, based on the observation that gestating snakes often appear to prioritize avoidance of extreme temperatures (Gannon and Secoy 1985; Graves and Duvall 1993; Gardner-Santana and Beaupre 2009). Additionally, we hypothesized that while prairie sites may possess some advantages over rookeries (e.g., higher food availability), these sites have lower thermal quality that translates into lower thermoregulatory accuracy for non-pregnant snakes. We used radiotelemetry and temperature data loggers to quantify the snakes' $T_b$, operative temperature models to quantify sampled representative microhabitat temperatures ($T_e$), and a thermal gradient to quantify the range of preferred $T_b$ of pregnant and non-pregnant snakes ($T_{set}$). We then used these variables to calculate microhabitat thermal quality (i.e., the relationship of $T_e$ to $T_{set}$) and snake thermoregulatory accuracy (i.e., the relationship of $T_b$ to $T_{set}$). By understanding these parameters, we can reveal the relative importance of such habitat features to thermoregulation during gestation in viviparous ectotherms like rattlesnakes.

## 2. Methods

### 2.1. Study site

This study was conducted on an approximately 1000 ha privately owned ranch in Routt County, Colorado. A large rattlesnake hibernaculum is located on an igneous rock butte (2247 m elevation) surrounded by Rocky Mountain Gambel Oak-mixed montane shrubland with some livestock pastures. The region's climate is cold and dry, with a mean annual temperature of 4.17 °C and 625 mm of precipitation, the vast majority of which occurs as winter snow. Ambient air temperatures during the study period ranged from -5–34.4 °C (Applied Climate Information System: National Oceanic and Atmospheric Administration Station USC00057936; https://www.weather.gov/wrh/climate).

### 2.2. Surgical implants

In late May to early June 2020, we used snake tongs to capture 20 female *C. viridis* (ten pregnant, ten non-pregnant) weighing over 340 g (Table S1) from the hibernaculum. Within two days of capture, we surgically implanted snakes with radio-transmitters (following Lind et al., 2010) and thermal data loggers (Thermochron iButton, models DSL1922L-F5# or DSL1921G-F5#) that were coated in Plasti-dip (Plasti Dip International, Minnesota, USA). iButtons were programmed to record hourly body temperatures ($T_b \pm 0.5$ °C). Prior to implantation (and use in operative temperature models, below), iButtons were submerged in a water bath and their temperatures compared to a reference thermometer, which matched in all cases and thus did not require further calibration. Smaller females ($N = 5$; 343–427 g) received one of two Holohil radio-transmitters (5 g SB-2 or 11 g SI-2 models, Holohil Systems Ltd., Carp, Ontario), and larger females ($N = 15$; 353–858 g) received 13 g ATS R1535 radio-transmitters (Advanced Telemetry Systems, Minnesota, USA). Implant net mass (transmitter + data logger) did not exceed 5% of body mass. Pregnant and non-pregnant snakes did not differ in mean snout-vent-length (SVL) (pregnant: 871.25 mm, non-pregnant: 844.70 mm; $t_{(21.039)} = 0.921$, $p = 0.367$) but differed significantly in mean mass due to additional embryo mass in pregnant females (pregnant: 568.93 g, non-pregnant: 393.13 g; $t_{(17.161)} = 4.869$, $p < 0.001$). Post-surgery, we monitored recovery of snakes in glass terraria with heating strips (resulting in a thermal gradient with maximum temperatures of 33–35 °C) and water *ad libitum* until release at their capture sites 12–24 h later. Rattlesnakes were radio-tracked throughout their active season (daily: 28 May through 14 June; biweekly: 14 June until 22 August, then once on 02 October); we used this information to determine when non-pregnant snakes left hibernacula to establish summer ranges in the prairies, and to confirm that pregnant snakes remained at rookeries.

### 2.3. Preferred body temperature ($T_{set}$)

In late May and early June 2021, we collected additional pregnant ($N = 13$) and non-pregnant ($N = 12$; Table S1) female rattlesnakes to establish preferred body temperature range ($T_{set}$; i.e., fundamental thermal niche, Giacometti et al., 2024) using a thermal gradient. In a room with ambient temperatures between 25 and 30 °C and constant lighting, we followed the design of Bovo et al. (2012) and Crowell et al. (2021) to construct a thermal gradient with three lanes (250 × 20 × 25 cm) separated by solid wooden dividers and filled with a thin layer of sand. We affixed copper pipes underneath the floor, covered them with fiberglass and insulating foam, and connected closed water circuits at each end. The water circuits were attached to a heater (Stiebel Eltron model SHC4, Holzminden, Germany) on one side and a cooler (Active-AQUA Water Chiller model AACH10, Petaluma, California, USA) on the other, creating a surface gradient that ranged from 10 to 48 °C. Thermocouples (model 5SRTC-TT-K-40-72) connected to data loggers (model RDXL4SD; both: Omega Engineering, Surrey, United Kingdom) were inserted into a rattlesnake's cloaca and secured with medical and duct tape. Snakes were in the gradient for ca. 24 h, with $T_b$ recorded every 5 min. Between trials, we mixed the sand to minimize the impact of scent on behavior of subsequent snakes.

We described $T_{set}$ using the interquartile range of the data (Blouin-Demers and Weatherhead 2001; Fitzgerald et al., 2003; Crowell et al., 2021) to encompass the range of preferred body temperatures as described by the dual set-point mode (Barber and Crawford 1977, 1979). We compared mean of the median $T_{set}$, the first (IQ1) and the third interquartile (IQ3) between reproductive groups and calculated thermal quality and thermoregulatory accuracy using the appropriate reproductive group's $T_{set}$.

### 2.4. Operative temperatures ($T_e$)

From 01 June (approximate date that non-pregnant snakes dispersed into their summer foraging ranges in prairie and mixed-forest habitats) to August 21, 2020 (date of the first known birth of neonates at the rookeries), we deployed 16 operative temperature models (OTMs) in areas used by rattlesnakes to collect data on a sample of representative microhabitat operative temperatures ($T_e$). OTMs were 40 cm × 2.5 cm hollow copper pipes spray-painted with a tan base and brown diamonds (Rust-Oleum Corporation, Illinois, USA) to resemble rattlesnake color patterns (Taylor et al., 2020; Crowell et al., 2021) and mimic solar spectral absorptance (Bakken 1992; Dzialowski 2005; Herr et al., 2020) for a closely related species, *C. oreganus* (Crowell et al., 2021). Pipes had soldered copper caps at one end and screw plastic caps at the other end (Bakken 1992; Lutterschmidt and Reinert 2012; Crowell et al., 2021), with an iButton set to record temperature hourly (DSL1921G-F5# model) suspended inside using 3D-printed plastic inserts to record $T_e$ without touching metal walls (Bakken 1992; Herr et al., 2020). In a previous study using the same OTMs (Crowell et al., 2021), OTMs were validated using two recently-deceased adult rattlesnakes (570 g, 740 g) with implanted iButtons. Snake carcasses and models were cooled for 1 h in a fridge, then placed in direct sunlight for 2.5 h. Models heated slightly faster than carcasses but snake and OTM temperatures were highly correlated (Pearson linear regression, $r^2 = 0.97$, $p < 0.001$). All OTMs were anchored in place at microhabitats chosen to represent a range of representative environmental temperatures (Dzialowski 2005), including rookeries ($N = 8$), grass and forb-dominated meadows ($N = 3$), rock outcrops ($N = 2$), Gambel oak groves ($N = 2$), and mixed conifer and deciduous forest ($N = 1$). All locations were frequented by *C. viridis* in previous years (Boback, Unpublished data). For simplicity, in analyses we refer to OTMs at rookeries as "rookery" and non-rookery sites as "prairie." Rookery sites are within a mean of 18 ± 9 m of the den, while prairie sites extend to a mean of 968 ± 692 m away. We paired OTMs at each location (except for one in the meadow and one in the forest), with one exposed to direct sunlight and one in full shade. At rookery sites, exposed OTMs were placed where snakes basked and shaded OTMs were under rocks where they retreated.

### 2.5. Habitat thermal quality ($d_e$)

We calculated thermal quality ($d_e$) using methods developed by Hertz et al. (1993), adjusted to provide directional information, using both observed and model-estimated $T_e$. That is, rather than using the absolute value of the differences between $T_{set}$ and either habitat-observed or model-estimated $T_e$, we used the actual (mathematical) difference, to capture not only how far $T_e$ is offset from $T_{set}$, but whether the microhabitat is warmer or cooler. We used the bounds of mean IQ1 and IQ3 of $T_{set}$ for non-pregnant snakes to calculate prairie $d_e$, and bounds of $T_{set}$ for pregnant snakes to calculate rookery $d_e$. When either observed $T_e$ or model-estimated $T_e$ was higher than mean IQ3, then $d_e = T_e$ - mean IQ3 ; when either $T_e$ was lower than mean IQ1, then $d_e = T_e$ - mean IQ1 ; and when either $T_e$ was between mean IQ1 and mean IQ3 we defined $d_e$ as zero. Here, the closer $d_e$ is to 0, the better the thermal quality. When $d_e$ is positive, it is warmer than the upper bound of $T_{set}$, and when $d_e$ is negative, it is cooler than the lower bound of $T_{set}$. We use "poor" and "good" to discuss thermal quality, rather than "low" or "high," for clarity.

### 2.6. Field active body temperatures ($T_b$)

We recovered iButtons from 14 of our 20 telemetered rattlesnakes ($N = 7$ pregnant, $N = 7$ non-pregnant), capturing temperature data between 28 May to 30 September, 2020. Data from remaining snakes was not recovered due to radio failure, predation, or overwinter mortality. We downloaded iButton data using OneWireViewer (Version 3.19.47). For descriptive analyses, we excluded data after August, and earlier for two non-pregnant snakes who died on 26 July and 24 August. We removed $T_b$ from individuals when in "alternate habitats," rare instances of pregnant snakes briefly visiting the prairie and non-pregnant snakes before dispersing. Statistical analyses use $T_b$ from 30 June (earliest date that all individuals were settled in final locations) through 24 August (date that second non-pregnant snake died).

### 2.7. Snake thermoregulatory accuracy ($d_b$)

We calculated thermoregulatory accuracy ($d_b$), the difference between either observed or model-estimated $T_b$ and $T_{set}$, with methods developed by Hertz et al. (1993) adjusted to give directional information. When either observed $T_b$ or model-estimated $T_b$ was higher than mean IQ3, then $d_b = T_b$ - mean IQ3. When $T_b$ was lower than mean IQ1, then $d_b = T_b$ - mean IQ1, again using each reproductive group's respective $T_{set}$ to calculate their $d_b$. When $T_b$ was between mean IQ1 and mean IQ3, we defined $d_b$ as zero. Values close to 0 suggest a snake is thermoregulating close to its $T_{set}$.

### 2.8. Statistical analyses

We fit two models, one to $T_b$ and a second to $T_e$; estimates for $d_b$ and $d_e$ were calculated by substituting model estimates for $T_b$ and $T_e$ in the calculations described above in sections 2.5 and 2.7. Models for $T_b$ and $T_e$ employed an extended linear mixed model framework where mean temperatures are represented as continuous functions of time (Crowell et al., 2021). Fixed model terms account for both daily mean temperature fluctuations and longer-term shifts associated with seasonal changes during the study period. Each model specifies that these fluctuations depend on location type and, in the case of the $T_e$ model, on sun exposure. Random coefficients in each model account for individual-level variability (individual snake for $T_b$) or site-level variability (individual OTM for $T_e$); these terms allow for estimation of individual- and site-specific curves of mean temperatures over time. Finally, an autoregressive error term is included in each model to account for serial correlation in the data. Models were formulated based on graphical assessments of temporal patterns and refined using standard residual diagnostic techniques. Full specifications and additional details are provided in Supplemental Methods.

All analyses were run in R version 4.2.2 (R Core Team, 2022): model parameters were estimated via restricted maximum likelihood (REML) using the *nlme* package (Pinheiro and Bates 2000; Pinheiro et al., 2023); figures were created using the *ggplot2* package (Wickham 2016); additional analyses and data summaries utilized *tidyverse* packages (Wickham et al., 2019). All results are presented as the mean ± standard deviation (SD).

## 3. Results

### 3.1. Preferred body temperatures ($T_{set}$)

For pregnant snakes ($N = 13$), the mean of median $T_{set}$ was 30.36 ± 1.24 °C with an IQR of 29.07 ± 1.33 °C–31.85 ± 0.85 °C. The mean of median $T_{set}$ for non-pregnant snakes ($N = 12$) was higher at 32.10 ± 1.88 °C, with an IQR of 30.63 ± 2.43 °C–33.45 ± 1.74 °C. Non-pregnant snakes had greater variance around the mean of body temperatures within the gradient ($F_{(2411,\ 3609)} = 1.54, p < 0.001$). Means of the median $T_{set}$ significantly differed between reproductive groups, which appeared to be driven by the upper bound (IQ3) of $T_{set}$ between groups (median $T_{set}$: $t_{(18.84)} = -2.71$, $p = 0.014$; IQ1: $t_{(16.73)} = -1.97$, $p = 0.065$; IQ3: $t_{(15.72)} = -2.89$, $p = 0.011$). For all cases, non-pregnant females had higher $T_{set}$.

### 3.2. Operative temperatures ($T_e$)

We analyzed hourly $T_e$ from 16 OTMs for 84 days of the active season. For raw $T_e$ at each microhabitat, see Fig. S1, summarized by time of day in Fig. 1 and across season in Fig. 2. Hour of the day, exposure, and location were all associated with significant differences in $T_e$ (respectively: $F_{(4,\ 29824)} = 152.04, p < 0.001$; $F_{(1,\ 11)} = 1270.96, p < 0.001$; $F_{(1,\ 11)} = 145.06, p < 0.001$), with a significant interaction between location and exposure ($F_{(1,\ 11)} = 20.91, p < 0.001$; Table S2). Estimated marginal means (EMMs) indicate that temperatures are greater in exposed sites when averaged across location and time: mean $T_e$ in exposed sites were estimated to exceed those in shaded sites by 5.37 °C averaging over location, hour of the day, and day of the season (Table 1). Comparing rookery and prairie locations indicated that temperatures were lower in rookery locations averaged across exposure and time: mean operative temperatures in rookery locations were estimated to be 2.39 °C lower than those in prairie locations, averaging over exposure, hour of the day, and day of the season (Table 1). However, these estimated differences are driven largely by daytime temperatures, which reach greater

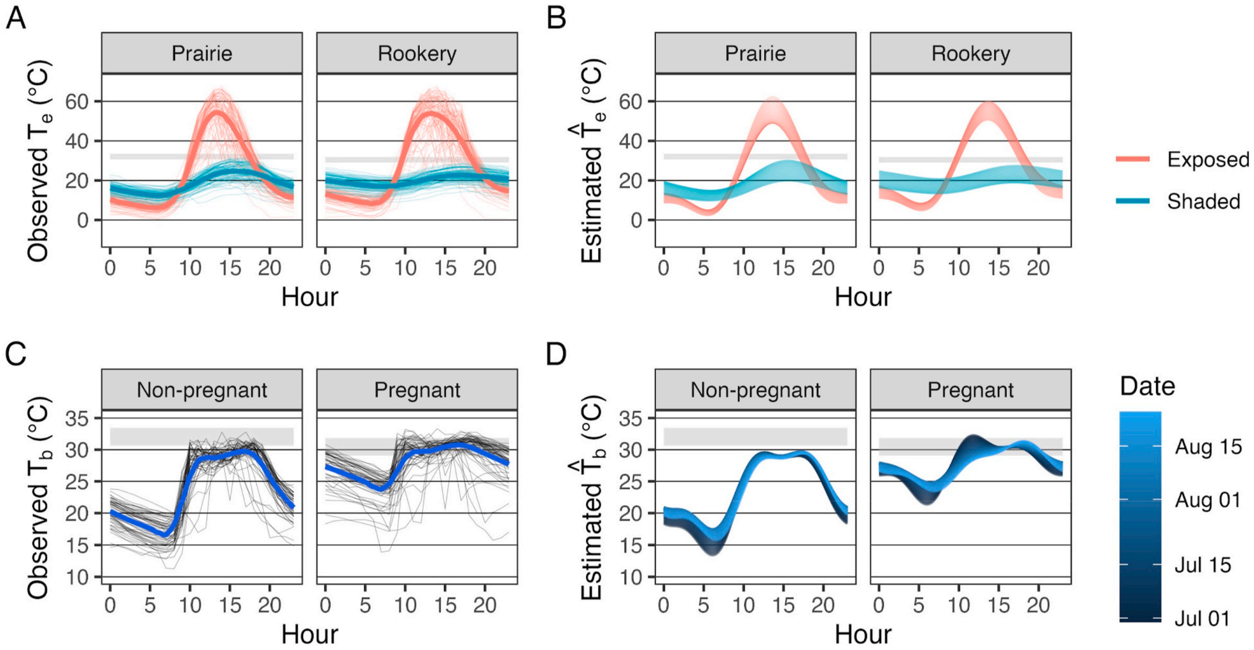

**Fig. 1. Hourly trends in environmental temperature ($T_e$) and Prairie Rattlesnake (*Crotalus viridis*) body temperature ($T_b$).** Observed (left panel sets A, C) and estimated mean (right panel sets B, D) $T_e$ (top panel sets A, B) and $T_b$ (bottom panel sets C, D) by hour of day. For $T_e$, location and exposure are differentiated by panel and color, respectively; for $T_b$, pregnancy status is differentiated by panel. Individual semi-transparent curves correspond to each day in the study period; for estimated mean $T_b$ (D) the color gradient, indicating day of the active season, shows shifts in the mean hourly temperature-curve as the season progresses. Gray polygons show the bounds of interquartile ranges around $T_{set}$ for all snakes in the corresponding group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

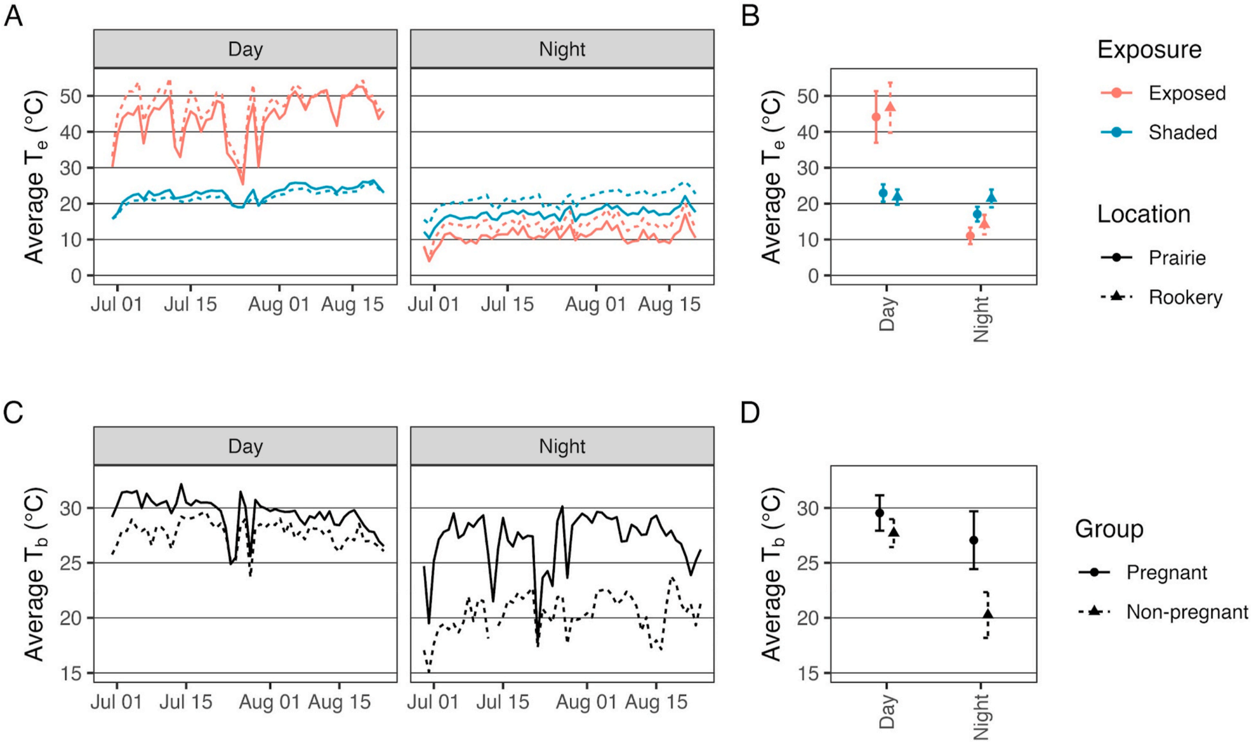

**Fig. 2. Daytime and nighttime trends in environmental temperatures ($T_e$) and Prairie Rattlesnake (*Crotalus viridis*) body temperature ($T_b$).** Summary comparisons between daytime and nighttime average operative temperatures (top panel sets A, B) and average body temperatures (bottom panel sets C, D) by date (left panel sets A, C) and averaged across the active season (right panels B, D). Operative temperatures are differentiated by location (linetype and point shape) and exposure (color); body temperatures are differentiated by pregnancy status (linetype and point shape). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**
Estimated marginal mean operative temperatures (°C) and standard error (SE) by location and exposure type with 95% pointwise confidence intervals (CIs). Each estimate is time-averaged across the entire study period; estimated differences between location types are averaged across exposure types, and estimated differences between exposure types are averaged across location types.

| | | Estimate (°C) | SE | 95% CI |
|---|---|---|---|---|
| **Means** | Prairie, Exposed | 22.95 | 0.168 | (22.58, 23.32) |
| | Rookery, Exposed | 26.05 | 0.168 | (25.68, 26.42) |
| | Prairie, Shaded | 18.29 | 0.194 | (17.86, 18.72) |
| | Rookery, Shaded | 19.96 | 0.168 | (19.59, 20.33) |
| **Contrasts** | Prairie - Rookery | −2.39 | 0.175 | (−2.77, −2.00) |
| | Exposed - Shaded | 5.37 | 0.175 | (4.99, 5.76) |

extremes; opposite trends exist during nighttime hours (Figs. 1 and 2).

### *3.3. Habitat thermal quality ($d_e$)*

Thermal quality ($d_e$) of prairie and rookery sites was similar during daytime hours (Fig. 3). Overall, $d_e$ was poorer in exposed sites than in shaded sites because solar radiation caused ambient temperatures to rise well above $T_{set}$. During daytime hours (0900–1700), average $d_e$ was markedly poorer at exposed rookery sites (15.35 °C) compared with exposed prairie sites (11.44 °C) and slightly better at shaded rookery sites (−7.35 °C) compared with shaded prairie sites (−7.99 °C). Signs indicate that shaded sites were below $T_{set}$, and exposed sites were above. However, during nighttime hours (2100–0500), average $d_e$ was below $T_{set}$ at all sites but was uniformly better among rookery sites: exposed rookery sites (−14.91 °C) were markedly better than exposed prairie sites (−19.62 °C) and shaded rookery sites (−7.74 °C) were markedly better than shaded prairie sites (−13.58 °C). Notably, at rookery sites, average $d_e$ was substantially similar regardless of time of day; at shaded sites average $d_e$ remained near constant, while nighttime exposed sites had slightly poorer average $d_e$ than during the day.

In both prairie and rookery sites, exposed daytime $d_e$ got worse throughout the active season because temperatures rose far above $T_{set}$ (Fig. 4). However, $d_e$ in other sites (daytime shaded, nighttime exposed, and nighttime shaded) improved because temperatures rose closer to $T_{set}$. In both prairie and rookery, nighttime exposed $d_e$ was poorer than shaded $d_e$ because exposed sites got colder, while daytime exposed $d_e$ was poorer than shaded $d_e$ because exposed sites got much hotter.

### *3.4. Field active body temperatures ($T_b$)*

We gathered hourly $T_b$ of 14 snakes for 79 days of the active season and analyzed data for 13 snakes (7 pregnant, 6 non-pregnant) who were alive during the 56 days when all individuals were settled in either prairie or rookery locations. For raw body temperatures of all individuals, see Fig. S2, summarized by time of day in Fig. 1 and across the season in Fig. 2. Reproductive group (synonymous with location for $T_b$) and hour of the day significantly impacted $T_b$ (respectively: $F_{(1,\ 11)} = 621.83$, $p < 0.001$; $F_{(6,\ 17419)} = 65.39$, $p < 0.001$), with a significant interaction between reproductive group and hour ($F_{(6,\ 17419)} = 8.95$, $p < 0.001$; Table S3). Pregnant females maintained higher estimated mean $T_b$ of 4.38 °C averaged across time (Table 2), a difference driven largely by higher nighttime $T_b$ (Figs. 1 and 2).

### *3.5. Thermoregulatory accuracy ($d_b$)*

Thermoregulatory accuracy ($d_b$) of pregnant snakes was closer to 0 than $d_b$ of non-pregnant snakes, both during daytime and nighttime hours (Fig. 4). Overall, average $d_b$ was higher for all snakes during the day (0900–1700 h) than at night, but average $d_b$ dropped more at night

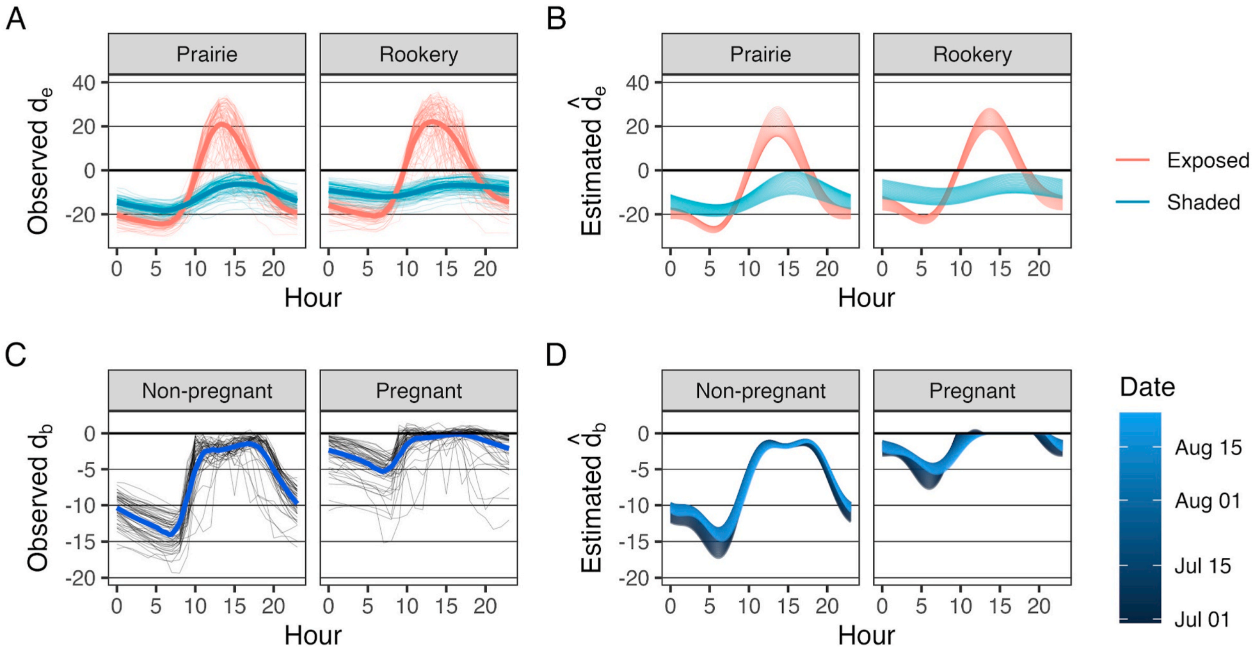

**Fig. 3. Hourly trends in habitat thermal quality ($d_e$) and Prairie Rattlesnake (*Crotalus viridis*) thermoregulatory accuracy ($d_b$).** Observed (left panel sets A, C) and estimated (right panel sets B, D) $d_e$ (top panel sets A, B) and $d_b$ (bottom panel sets C, D) by hour of day. For $d_e$, panel and color differentiate location and exposure, respectively; for $d_b$, panel differentiates pregnancy status. Individual curves correspond to each day in the study period; for estimated mean $d_b$ (D) the color gradient shows shifts in the mean daily temperature-curve over the season. A bold zero reference line indicates ideal quality or accuracy in all panels. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

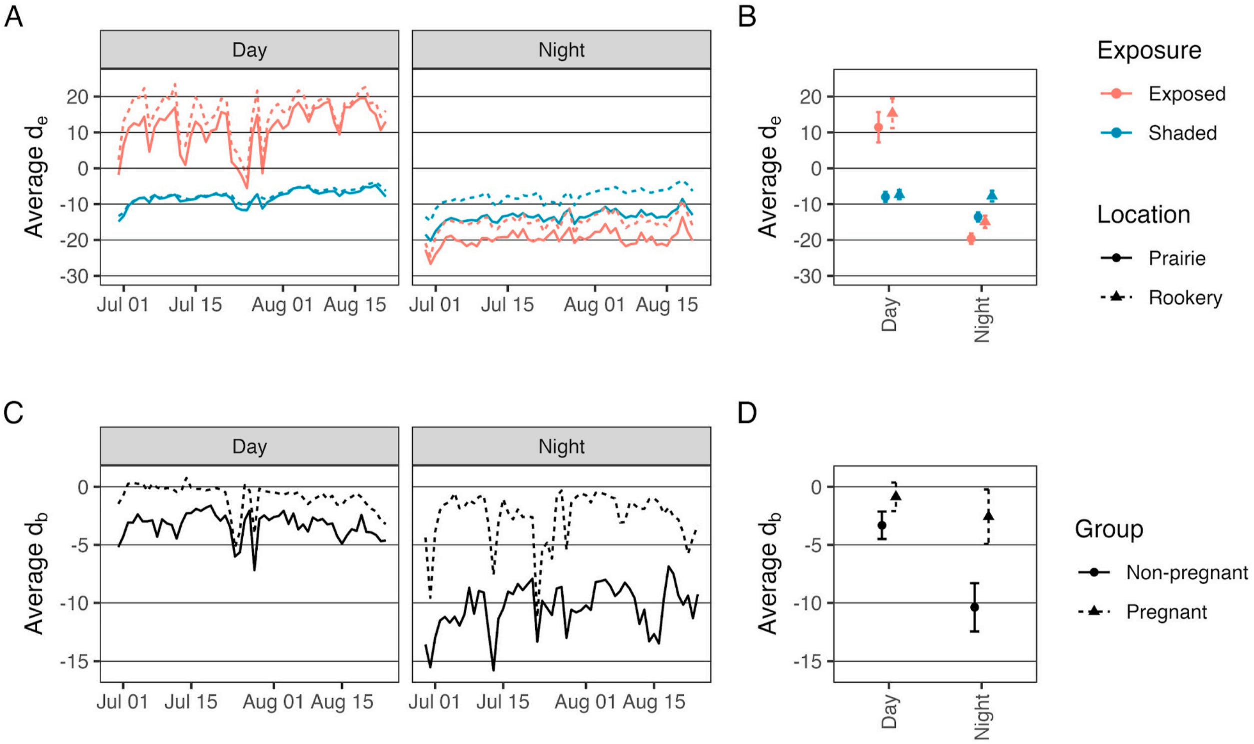

**Fig. 4. Daytime and nighttime trends in habitat thermal quality ($d_e$) and Prairie Rattlesnake (*Crotalus viridis*) thermoregulatory accuracy ($d_b$).** Summary comparisons between daytime and nighttime average $d_e$ (top panel sets A, B) and average $d_b$ (bottom panel sets C, D) by date (left panel sets A, C) and across the active season (right panels B, D). For $d_e$, linetype and point shape differentiate location and and color differentiates exposure; for $d_b$, linetype and pointshape differentiate pregnancy status. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

for non-pregnant snakes (decrease of 7.01 °C) than for pregnant snakes (decrease of 1.71 °C). Notably, average $d_b$ was low because $T_b$ was below preferred ranges for both groups regardless of time of day, but quite close for pregnant snakes during the daytime (−0.86 °C). Seasonally, snakes are able to thermoregulate accurately during daytime hours from June through August, then $d_b$ drops off in late August as environmental

**Table 2**
Estimated marginal means and contrasts for body temperatures (°C) and standard error (SE) of pregnant and non-pregnant Prairie Rattlesnakes (*Crotalus viridis*) with 95% pointwise confidence intervals (CIs). Estimates are time-averaged across the entire study period.

| Group | Estimate (°C) | SE | 95% CI |
|---|---|---|---|
| Pregnant | 27.927 | 0.117 | (27.67, 28.19) |
| Non-pregnant | 23.545 | 0.127 | (23.27, 23.82) |
| Pregnant - Non-pregnant | 4.382 | 0.173 | (4.00, 4.76) |

temperatures begin to drop.

## 4. Discussion

Our hypothesis that rookeries act as high quality thermal refugia for pregnant rattlesnakes was supported by our data. Rookeries facilitated maintenance of higher body temperatures, which could allow pregnant females to thermoregulate more accurately than non-pregnant females in the prairie, especially during the night when snakes cannot use solar radiation to actively thermoregulate. Daytime solar radiation allowed both pregnant snakes at rookeries and non-pregnant snakes at prairie sites to thermoregulate more accurately since they could move between exposed and shaded microhabitats. At night, reduced thermal quality of all microhabitats precluded snakes from thermoregulating as accurately, but rookeries had higher and less variable operative temperatures, allowing pregnant snakes to maintain higher nighttime body temperatures than non-pregnant snakes. Habitat temperatures changed over the active season, with rising temperatures causing increasingly poorer quality of exposed microhabitats but better quality of shaded microhabitats. Given the short active season for rattlesnakes at this high-elevation site, the ability to maximize time close to preferred temperature is especially important. Whereas non-pregnant snakes likely choose sites based on multiple factors (e.g., hunting, digesting, mating), thermoregulating to maintain optimal body temperature for developing embryos must be a central factor impacting gestation site choice. Although other benefits of communal gestation are not mutually exclusive from thermoregulation, our study shows the thermal benefits of hilltop rookeries are important for high-elevation rattlesnakes.

Rigorous investigation of the thermal ecology of a population as it relates to reproduction requires measurement of all variables ($T_{set}$, field-active $T_b$, $T_e$; Taylor et al., 2020) to compare thermal quality of habitats and thermoregulatory accuracy between groups. In this population of *C. viridis*, pregnant and non-pregnant female snakes share the same habitat during the spring (e.g., basking at dens), but for the duration of their active season, pregnant females stayed at rookeries near their winter den (mean distance from den: 18 ± 9 m), while non-pregnant snakes dispersed into the prairie to forage (mean distance from den: 968 ± 692 m; Boback et al., Unpublished data). Though daytime temperatures of prairie sites were, on average, higher, this trend reversed during nighttime hours. Pregnant snakes at rookeries consistently maintained higher body temperatures, even during the day, however, suggesting that their thermoregulatory behavior (e.g., basking) is also tightly modulated. Pregnant snakes also maintained more stable daily body temperatures than non-pregnant snakes, possibly because their added mass conferred higher thermal inertia (Tattersall et al., 2016), but also probably because they thermoregulated more tightly around a set-point. Marinho et al. (2022) showed that pregnant South American Rattlesnakes (*C. durissus*) in Brazil selected higher mean $T_b$ than non-pregnant snakes in a semi-natural enclosure, suggesting that rattlesnakes may thermoregulate at higher temperatures when pregnant even when choosing from the same range of microhabitats. In our study, non-pregnant females occupied areas with long durations of lower temperatures (and therefore poorer thermal quality) and maintained lower body temperatures even though their preferred temperature range was higher than that of pregnant snakes. This caused non-pregnant snakes to have far lower thermoregulatory accuracy than pregnant snakes, which we hypothesize to represent a trade-off driven by a need to forage during the active season. Ambush foraging requires snakes to choose a spot and remain immobile for extended periods of time (Clark 2016), and although snakes may use temperature to choose ambush sites (Signore et al., 2022), the optimal temperature for hunting may be different from the preferred body temperature measured in a gradient (i.e., optimal $T_b$ may not track $T_{set}$; Giacometti et al., 2024). The body temperature of pregnant females, which rarely eat during pregnancy, are therefore less constrained by activity trade-offs and are closer to their preferred temperatures.

Our expectation that pregnant females would choose higher temperatures than non-pregnant snakes was based on other observations that free-ranging pregnant rattlesnakes have higher body temperatures compared to non-pregnant snakes (Graves and Duvall 1993; Gardner-Santana and Beaupre 2009; Marinho et al., 2022; but see Charland and Gregory 1990). Higher and more stable body temperatures are often suggested to be beneficial to embryonic development, but we do not have any data on how temperature or its fluctuations impact development of rattlesnake embryos. In the absence of such data, the accurate thermoregulation of pregnant female rattlesnakes at higher temperatures suggest that these conditions benefit the offspring, but further study is needed to confirm this. While we did observe that pregnant snakes had higher field-active body temperatures than non-pregnant females, we found that, on average, pregnant snakes chose lower temperatures in the lab. This is consistent with other viviparous squamates and could indicate a conflict between the mother's thermal optima (slightly higher) and that of her developing embryos (slightly lower; Beauchat 1986). An animal's preferred body temperature is not a static variable but rather can vary, for example seasonally (e.g., Giacometti et al., 2024), and here we show that it may also be impacted by reproductive condition. In addition, preferred body temperature measurements rely on certain assumptions. Measuring preferred temperatures using laboratory gradients has the benefit of removing external confounding variables, but simultaneously introduces others (Figueroa-Huitrón et al., 2019) and snakes may alter behavioral thermoregulation in response to stress from capture and transport. However, the fact that snakes in this study thermoregulated extremely accurately in the field during the periods when availability of both solar radiation and shade allows them to achieve their preferred temperature, suggests we attained good estimates of $T_{set}$ in the lab. Indeed, the $T_{set}$ values near 30 °C align with those from other rattlesnake thermal ecology studies (Lutterschmidt 1991; Figueroa-Huitrón et al., 2019; Jaramillo-Alba et al., 2020; Crowell et al., 2021), suggesting preferred body temperature may be somewhat conserved among rattlesnakes; however, this hypothesis remains to be tested formally.

Theoretically, snakes should thermoregulate within their range of preferred body temperatures if only temperature impacts microhabitat selection, and if such temperatures were always available. However, in field settings, many biotic and abiotic factors must be balanced including humidity, solar and wind exposure, predation risk, and finding prey. When the benefits of thermoregulating outweigh potential costs (Huey and Slatkin 1976), thermoregulatory behavior should be influenced by thermal quality where positive $d_e$ values cause animals to move into cooler microclimates and under negative $d_e$ values, animals move into warmer microclimates such that snakes stay within the range of their preferred temperatures (Barber and Crawford 1977, 1979; Giacometti et al., 2021). We found that snakes typically had field-active body temperatures below preferred, suggesting they do face thermoregulatory constraints of available thermal quality. Further, shaded operative temperatures were typically below $T_{set}$'s lower bound, meaning snakes in shaded microhabitats cannot achieve their preferred body temperature without exposing themselves to bask in direct sun. Multiple studies have shown that gestating snakes may prioritize maintaining less variable temperatures (Gannon and Secoy 1985; Graves and Duvall 1993; Gardner-Santana and Beaupre 2009; but see Charland and Gregory

1990), instead thermoregulating to avoid high and low temperatures, which we found in both in the laboratory and field. Avoiding extreme temperatures and maintaining more constant body temperatures may promote embryonic development (e.g., Shine 1995; Pettersen et al., 2023), and the reduced variability of rookeries seem to provide this benefit.

Interestingly, during midday, both exposed and shaded microhabitats at this high-elevation site dropped in thermal quality, for opposite reasons: solar radiation raised exposed operative temperatures above $T_{set}$, while operative temperatures were usually below $T_{set}$ at shaded sites. At night, when solar basking is unavailable, all microhabitats had poor thermal quality due to temperatures below $T_{set}$. We recommend other thermal ecology studies to use mathematical values (rather than absolute) when calculating $d_e$ and $d_b$ to better differentiate thermal constraints from high and low temperatures, which likely impact animals differently. However, thermal quality of rookeries for pregnant snakes was better than that of prairie habitat for non-pregnant snakes, likely because the large igneous rocks that make up rookery sites absorb solar radiation during the day which is re-emitted at night (Waples and Waples 2004), giving snakes at rookeries greater access to conductive heat gain at night. Shaded $T_e$ under rocks at rookeries, where pregnant snakes retreated overnight, were higher than those inside prairie retreats used by non-pregnant snakes. These large differences in thermal quality, combined with a slightly lower $T_{set}$ of pregnant snakes, led to higher thermoregulatory accuracy of pregnant snakes. Though other studies, including one on *C. viridis* (Graves and Duvall 1993), have demonstrated that pregnant snakes have higher, less variable body temperatures than non-pregnant conspecifics, ours is the first to demonstrate this difference in thermoregulatory accuracy between female rattlesnakes in different reproductive states. Future studies could begin to disentangle the mechanisms behind such differences, for example by studying how pregnant and non-pregnant snakes differ in thermoregulatory behaviors including shuttling and postures, the role of inertia in heating and cooling rates, and other variables.

This thermal benefit of rookeries does not exclude other benefits of gestating at rookeries (reviewed in Graves and Duvall 1995). The presence of other snakes could benefit individuals thermally and/or hydrically, such as through heat transfer among individuals by conduction; which may beneficially impact energy usage, ecdysis, and other processes (Reiserer et al., 2007). Rattlesnakes are adapted to arid areas, and pregnant and neonate rattlesnakes alike may be hydrically stressed (Bedard and Taylor et al., Unpublished results); huddling in large groups could reduce surface area of exposed skin and thereby reduce surface area exposed to evaporative water loss and heat loss (Graves et al., 1986; Heinen 1993). Pregnant snakes gestating at or near their winter dens do not incur the locomotor costs that non-pregnant females and males expend to disperse long distances (occasionally >10 km) to summer home ranges (Duvall et al., 1985; Didiuk 1999; Gardiner et al., 2013). Gestating in large groups may be an example of the selfish herd effect, where greater numbers of snakes dilute the chance of predation of any given individual (De Vos and O'Riain 2009), and increases fitness through higher likelihood of offspring survival. Finally, presence of conspecifics may provide a social buffering effect, reducing the effects of stressors on individuals (Martin et al., 2023).

We demonstrated that rookeries have high thermal quality, which may be especially important to snakes at high-elevation sites or other extreme environments. Snakes in this population have very brief active seasons, with a short window of time where conditions are suitable for thermoregulating accurately (Gannon and Secoy 1984). This is especially important for pregnant poikilothermic vertebrates, whose embryos appear particularly susceptible to low incubation temperatures during development (Yntema 1960; Fox et al., 1961; Vinegar 1973; Burger et al., 1987). Thus, rookeries represent an important thermal buffer for pregnant snakes by reducing variation in thermal fluctuation and offering higher nighttime temperatures. However, the benefit of occupying communal areas of good thermal quality can come at the expense of greater predation risk (Herr et al., 2020). We have game camera evidence that the snakes at our site are regularly preyed upon by avian predators which likely revisit the butte as a known food source. The costs and benefits of communal denning and gestation of rattlesnakes presents a complex puzzle, and future studies will continue to tease apart the reasons why snakes gather in large numbers in specific areas. Our study provides a piece of this puzzle by confirming, using quantitative thermal ecology techniques, the thermal benefits of rookeries to pregnant female *C. viridis*.

## Data accessibility

The raw and processed data required to reproduce the above findings are publicly archived on Zenodo (https://doi.org/10.5281/zenodo.13177060), with code hosted on GitHub (https://github.com/ruizt/viridis).

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## CRediT authorship contribution statement

**Haley A. Moniz:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Jack H. Buck:** Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Hayley L. Crowell:** Writing – review & editing, Methodology, Investigation. **Scott M. Goetz:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Conceptualization. **Trevor D. Ruiz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Emily N. Taylor:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Scott M. Boback:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The raw and processed data required to reproduce the above findings are publicly archived on Zenodo (https://doi.org/10.5281/zenodo.13177060), with code hosted on GitHub (https://github.com/ruizt/viridis).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2024.103948.

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**Specifications for $T_b$ and $T_e$ models**

The $T_b$ model is specified in terms of location type *i*, hour of day *h*, day of year *d*, and individual *k*, as follows:

$$Y_{idhk} \;=\; \mu + \tau_i + f_{ik}(h, d) \;+ \epsilon_{ik}(h, d)$$

The $T_b$ response for snake *k* on day *d* at hour *h* in location type *i* is denoted by $Y_{idhk}$; μ denotes the population mean $T_b$; $\tau_i$ denotes deviation in mean $T_b$ for location type *i* (prairie or rookery) and is subject to a sum constraint so that $\tau_1 + \tau_2 = 0$; the function $f_{ik}(h, d)$ represents fluctuations in mean $T_b$ over time for location type *i* and snake *k* and is represented using two sets of Fourier bases $\left\{\psi_j^{(h)}\right\}$ and $\left\{\psi_l^{(d)}\right\}$. The first set of basis functions is used to obtain an approximation of periodic daily fluctuations and comprises three sine-cosine pairs with a period of 24; the second set is used to obtain an approximation of longer-term seasonal fluctuations and comprises a single sine-cosine pair with a period of 365. The model specifies both fixed and random coefficients for each set of basis functions and includes interaction terms. The full specification of $f_{ik}(h, d)$ in terms of model parameters is given by:

$$f_{ik} = \sum_j \left(\alpha_j + \overline{\alpha}_{ij} + \delta_{jik}\right)\psi_j^{(h)} + \sum_l \left(\beta_l + \overline{\beta}_{il}\right)\psi_l^{(d)} + \sum_j\sum_l\left(\gamma_{jl} + \overline{\gamma}_{ijl}\right)\psi_j^{(h)}\psi_l^{(d)}$$

The coefficients α, β, γ are all treated as fixed model parameters and are thus subject to sum constraints:

$$\sum_j \alpha_j = \sum_j \overline{\alpha}_{ij} = \sum_l \beta_l = \sum_l \overline{\beta}_{il} = \sum_j\sum_l \gamma_{jl} = \sum_l \overline{\gamma}_{jl} = \sum_j \overline{\gamma}_{jl} = \sum_j\sum_l \overline{\gamma}_{jl} = 0$$

The coefficients δ are random – $\delta_{jik} \sim N\left(0,\ \sigma_j^2\right)$ – and capture individual variability in daily fluctuations relative to population means from snake to snake. Lastly, $\epsilon_{ik}(h,\ d)$ represents the unexplained error for snake *k* in location type *i* and is specified as an autoregressive process with parameter ϕ. Expressed as a function of consecutive time-points *t* corresponding to each unique combination of hour and day and arranged in sequence, the error model is:

$$\epsilon_{ik}(t) = \phi \cdot \epsilon_{ik}(t - 1) + w_t \text{ where } w_t \sim N\left(0, \sigma^2\right).$$

Parameters of $T_b$ model and their explanations.

| **Model parameter** | **Count** | **Df** | **Interpretation** |
|---|---|---|---|
| $\mu$ | 1 | 1 | Overall mean temperature |
| $\tau_i$ | 2 | 1 | Temperature shift due to location type |
| $\alpha_j, \alpha_{ij}$ | 18 | 12 | Coefficients determining daily fluctuation curves by location type, exposure type, and combination |
| $\beta_l, \beta_{il}$ | 6 | 4 | Coefficients determining long-term fluctuation curves by location type, exposure type, and combination |
| $\gamma_{jl}, \gamma_{ijl}$ | 36 | 24 | Coefficients adjusting daily fluctuation curves for day of year |
| $\sigma_j^2$ | 6 | | Variance across snakes, relative to group means given location type, hour of day, and day of year |
| $\sigma^2$ | 1 | | Error variance |
| $\phi$ | 1 | | Error autocorrelation |

The model for $T_e$ is specified similarly, but random model terms account for variability by site, rather than OTM, since variation between temperatures from each OTM is largely attributable to site rather than differences in the physical devices. As such, the fixed portion of the model specifies that temporal fluctuations in $T_e$ depend on location type, exposure type and their interaction; the random portion of the model captures variation by site in the overall temperature fluctuations as well as site-level variation in the differences between shaded and exposed areas. In detail, the model specified in terms of location type *i*, exposure type *m,* hour of day *h*, day of year *d*, and site *k* is:

$$Y_{imhdk} = \mu + \tau_i + \omega_m + (\tau\omega)_{im} + f_{imk}(h, d) + \epsilon_{imk}(h, d)$$

The response $Y_{imhdk}$ represents $T_e$ for location type *i*, exposure type *m*, hour of day *h*, day of year *d*, and site *k*. Unique combinations of *imk* specify exactly each OTM. Parameters μ, τ, ω, and (τω) represent grand mean $T_e$, deviations by location, deviations by exposure, and the interaction of location type and exposure type, respectively.

The function $f_{imk}(h, d)$ is parametrized in an analogous manner as in the $T_b$ model save for three details: (a) one less Fourier basis pair was used for the hour of day component in light of the raw data reflecting simpler periodicity in daily fluctuations; (b) sets of four (rather than two) α, β, γ coefficients were specified in the basis expansion of $f_{imk}(h, d)$ so that the function depends on location type, exposure type, and the combination of exposure type with location type (*e.g.*, specified as $\left(\alpha_j + \alpha_{ij} + \alpha_{mj} + \alpha_{imj}\right) \cdot \psi_j^{(h)}$); (c) one set of random coefficients is associated with each exposure type.

Parameters of $T_e$ model and explanations.

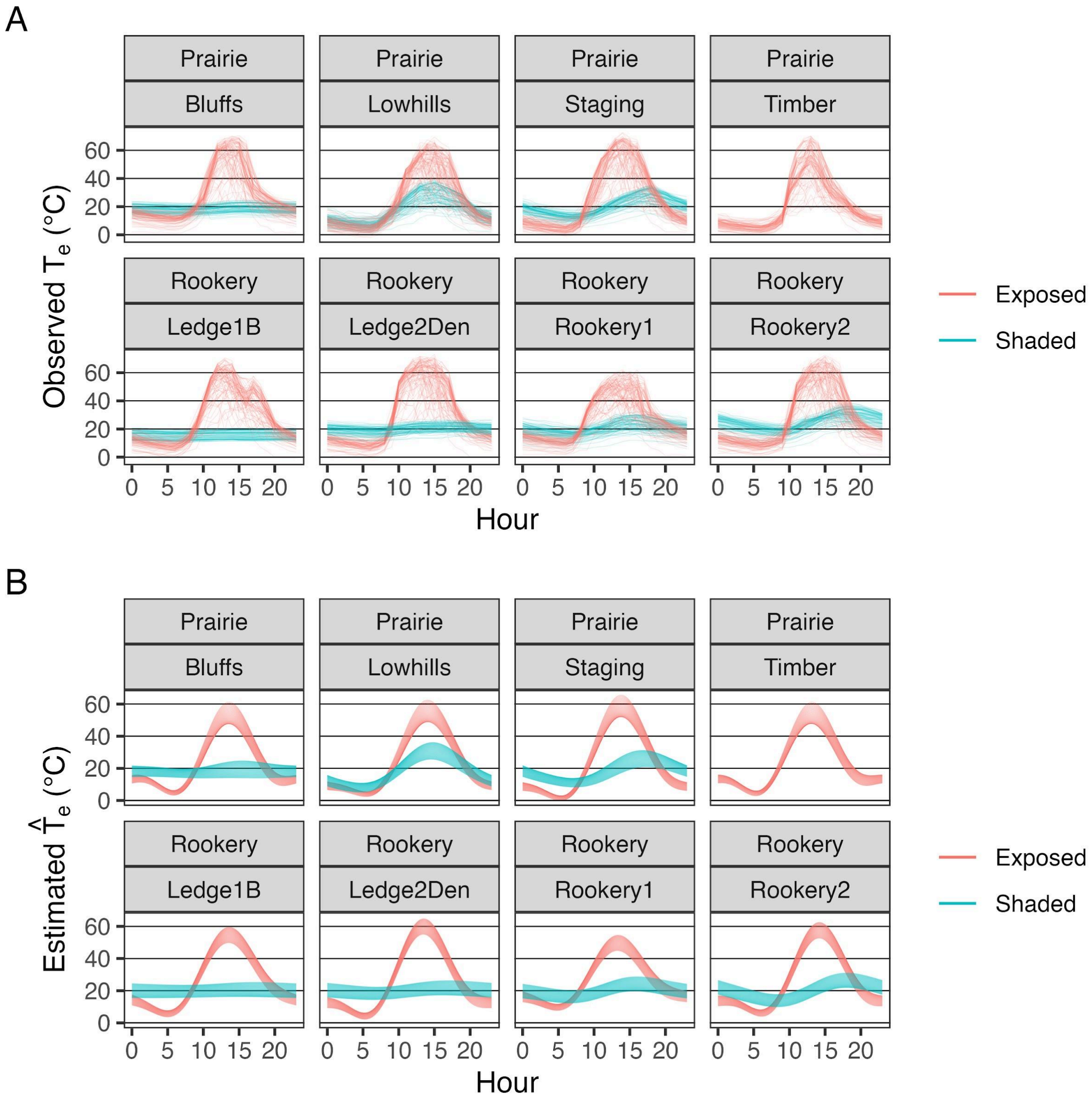
| **Model parameter** | **Count** | **Df** | **Interpretation** |
|---|---|---|---|
| $\mu$ | 1 | 1 | Overall mean temperature |
| $\tau_i$ | 2 | 1 | Temperature shift due to location type |
| $\omega_m$ | 2 | 1 | Temperature shift due to exposure type |
| $(\tau\omega)_{im}$ | 4 | 1 | Adjustment to temperature shifts by location type and exposure type combination |
| $\alpha_j, \alpha_{ij}, \alpha_{mj}, \alpha_{imj}$ | 36 | 16 | Coefficients determining daily fluctuation curves by location type, exposure type, and combination |
| $\beta_l, \beta_{il}, \beta_{ml}, \beta_{iml}$ | 18 | 8 | Coefficients determining long-term fluctuation curves by location type, exposure type, and combination |
| $\gamma_{jl}, \gamma_{ijl}, \gamma_{mjl}, \gamma_{imjl}$ | 72 | 32 | Coefficients adjusting daily fluctuation curves for day of year |

| Model parameter | Count | Df | Interpretation |
|---|---|---|---|
| $\sigma_j^2$ | 8 | | Variance across sites, relative to group means given location type, exposure type, hour of day, and day of year |
| $\sigma^2$ | 1 | | Error variance |
| $\phi$ | 1 | | Error autocorrelation |

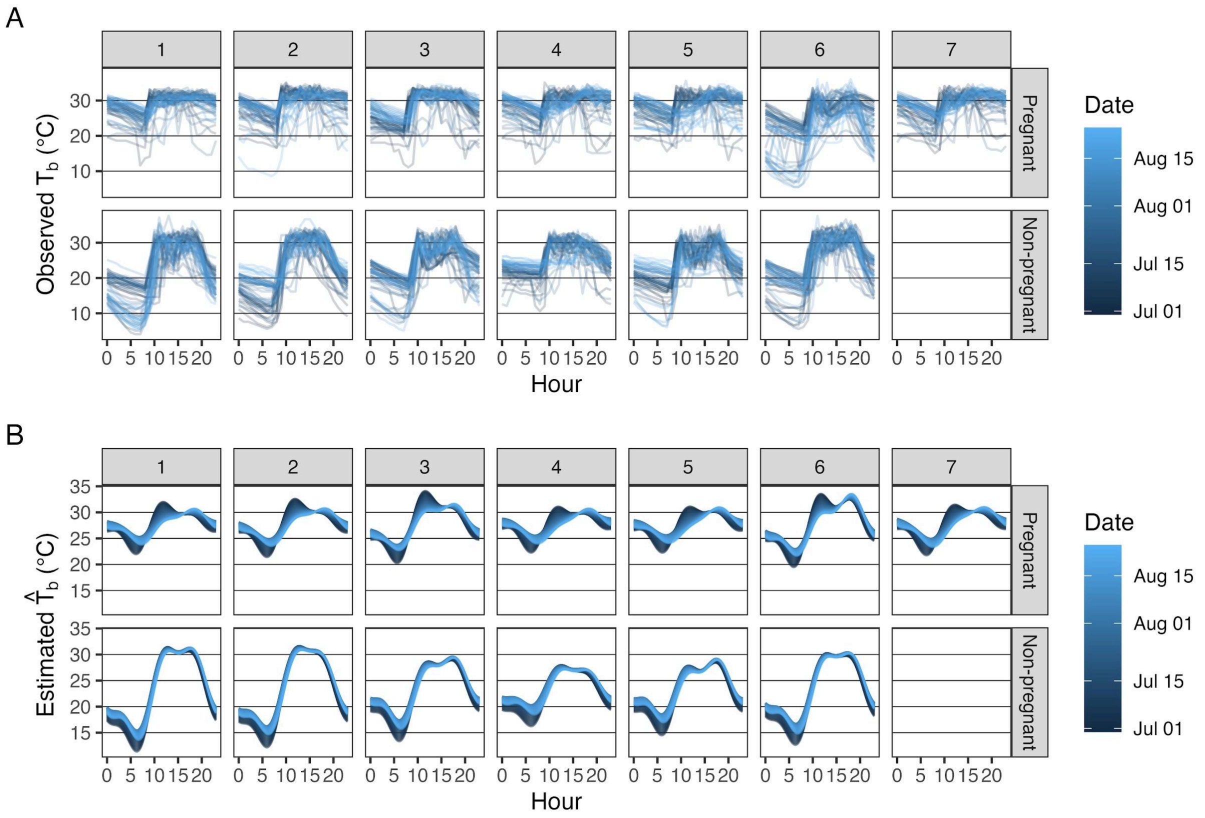
Specifications for fixed time components $f_{ik}(h, d)$ and $f_{imk}(h, d)$ in each model, respectively, were developed based on visual assessments of temporal patterns by hour of day for each dataset. Residual diagnostics were used to specify the order of autoregression for the error term in the model.

# Supplemental Figures

**Supplemental Figure S1. Individual operative temperature model curves.** Raw operative temperatures (top panel set) and fitted values from the $T_e$ model (bottom panel set) for each individual OTM, for each of the 84 days analyzed.



**Supplemental Figure S2. Individual Prairie Rattlesnake (*Crotalus viridis*) body temperature-curves.** Raw body temperatures (top panel set) and fitted values from the $T_b$ model (bottom panel set) for each individual snake by hour of day, for each of the 56 days analyzed. The color gradient indicates day of the active season, showing shifts in the hourly temperature-curve as the season progresses.



## Supplemental Tables

**Supplemental Table S1.** Morphometrics and reproductive status for rattlesnakes included in thermal gradient analysis and with implanted thermal loggers for field-active body temperatures. Snout-vent-length (SVL in mm) and mass (g) were measured upon first capture.

| Snake ID | Data | Reproductive Status | SVL (mm) | Mass (g) |
|---|---|---|---|---|
| PBW | Thermal Gradient | Pregnant | 990 | 714.7 |
| O1 | Thermal Gradient | Pregnant | 811 | 393.9 |
| PPuY | Thermal Gradient | Pregnant | 921 | 629.3 |
| PPuW | Thermal Gradient | Pregnant | 841 | 508.1 |
| WPuW | Thermal Gradient | Pregnant | 812 | 434.2 |
| PWW | Thermal Gradient | Pregnant | 850 | 471.8 |
| PPuR | Thermal Gradient | Pregnant | 884 | 494.4 |
| PYPu | Thermal Gradient | Pregnant | 820 | 417.5 |
| PPuA | Thermal Gradient | Pregnant | 773 | 444.8 |
| O2 | Thermal Gradient | Pregnant | 803 | 346.6 |
| PGPu | Thermal Gradient | Pregnant | 767 | 344.3 |
| WWPu | Thermal Gradient | Pregnant | 810 | 472.8 |
| R2 | Thermal Gradient | Pregnant | 908 | 570.7 |
| PPB | Thermal Gradient | Non-pregnant | NA | NA |
| PYY | Thermal Gradient | Non-pregnant | 779 | 310.2 |
| WYG | Thermal Gradient | Non-pregnant | 805 | 301.4 |
| PRP | Thermal Gradient | Non-pregnant | 801 | 330.7 |
| RBP | Thermal Gradient | Non-pregnant | 868 | 325.6 |
| WWG | Thermal Gradient | Non-pregnant | 1011 | 524.1 |
| RYP_B | Thermal Gradient | Non-pregnant | 791 | 223.5 |
| RRA | Thermal Gradient | Non-pregnant | 779 | 335.4 |
| WPuR | Thermal Gradient | Non-pregnant | 829 | 281 |
| R1 | Thermal Gradient | Non-pregnant | 679 | 340.6 |
| WRA | Thermal Gradient | Non-pregnant | 679 | 278.1 |
| RRB | Thermal Gradient | Non-pregnant | 835 | 358.1 |
| G1 | Field | Pregnant | 1005 | 857.5 |

| Snake ID | Data | Reproductive Status | SVL (mm) | Mass (g) |
|---|---|---|---|---|
| G2 | Field | Pregnant | 857 | 579.4 |
| G3 | Field | Pregnant | 785 | 528 |
| G4 | Field | Pregnant | 815 | 498.4 |
| G5 | Field | Pregnant | 750 | 343 |
| G9 | Field | Pregnant | 847 | 606.5 |
| G10 | Field | Pregnant | 805 | 479.9 |
| NG1 | Field | Non-pregnant | 780 | 426.7 |
| NG2 | Field | Non-pregnant | 875 | 423.8 |
| NG4 | Field | Non-pregnant | 842 | 376 |
| NG5 | Field | Non-pregnant | 960 | 366 |
| NG8 | Field | Non-pregnant | 750 | 370.2 |
| NG9 | Field | Non-pregnant | 910 | 353 |
| NG10 | Field | Non-pregnant | 809 | 378.1 |

**Supplemental Table S2.** Conditional F tests for fixed terms in $T_e$ model. The significance of additional interactions largely indicates that daily and monthly temperature cycles vary depending on location and exposure.

| **Term** | **Numerator DF** | **Denominator DF** | ***F* statistic** | ***p* value** |
|---|---|---|---|---|
| Location | 1 | 11 | 145.06 | <0.0001 |
| Exposure | 1 | 11 | 1270.96 | <0.0001 |
| Hour | 4 | 29824 | 152.04 | <0.0001 |
| Day | 2 | 29824 | 396.20 | <0.0001 |
| Location:Exposure | 1 | 11 | 20.91 | 0.0008 |
| Location:Hour | 4 | 29824 | 1.84 | 0.1175 |
| Exposure:Hour | 4 | 29824 | 87.41 | <0.0001 |
| Location:Day | 2 | 29824 | 0.23 | 0.7908 |
| Exposure:Day | 2 | 29824 | 1.09 | 0.3362 |
| Hour:Day | 8 | 29824 | 12.39 | <0.0001 |
| Location:Exposure:Hour | 4 | 29824 | 0.60 | 0.6647 |
| Location:Exposure:Day | 2 | 29824 | 1.97 | 0.1391 |
| Location:Hour:Day | 8 | 29824 | 3.68 | 0.0003 |
| Exposure:Hour:Day | 8 | 29824 | 4.11 | <0.0001 |
| Location:Exposure:Hour:Day | 8 | 29824 | 0.76 | 0.6418 |

**Supplemental Table S3.** Conditional F tests for fixed terms in $T_b$ model.

| **Term** | **Numerator DF** | **Denominator DF** | ***F* statistic** | ***p* value** |
|---|---|---|---|---|
| Group | 1 | 11 | 621.83 | <0.0001 |
| Hour | 6 | 17419 | 65.39 | <0.0001 |
| Day | 2 | 17419 | 9.10 | 0.0001 |
| Group:Hour | 6 | 17419 | 8.95 | <0.0001 |
| Group:Day | 2 | 17419 | 1.71 | 0.1803 |
| Hour:Day | 12 | 17419 | 17.26 | <0.0001 |
| Group:Hour:Day | 12 | 17419 | 1.91 | 0.0286 |