

Revisiting Python Thermogenesis: Brooding Burmese Pythons (*Python Bivittatus*) Cue on Body, not Clutch, Temperature

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ABSTRACT.—Previous studies have shown that brooding Burmese Pythons, *Python bivittatus*, use endogenous heat production to buffer clutch temperature against suboptimal environmental temperatures and that heat production is correlated with body muscle twitch rate and metabolic rate. Improving our understanding of the patterns of thermogenesis and the mechanisms that regulate it will provide insight into the proposed link between parental care and the evolution of endothermy. We measured body, clutch, and nest temperatures and also muscle twitch rate and metabolic rate to evaluate the buffering capability of thermogenesis during brooding as well as the thermal cues regulating thermogenesis. We found that, as expected, both muscle twitch rate and metabolic rate were correlated negatively with nest temperature. Furthermore, at nest temperature 6 degrees below optimal developmental temperature, females maintained body temperature at the optimal temperature. However, while thermogenesis increased clutch temperature significantly, clutch temperature decreases with decreasing nest temperature. Our results confirm general patterns of facultative thermogenesis reported previously and, in addition, strongly suggest that females use core body temperature to regulate their thermogenic activity.

While metabolic heat production is typically considered a trait of endothermic vertebrates (i.e., birds and mammals), some ectotherms have thermogenesis that is limited spatially to specific body regions or temporally to specific periods (reviewed in Block, 1994). These convergent instances of limited endothermy can be valuable for a better understanding of the driving forces that lead to endothermy. Interestingly, multiple models for the evolution of endothermy contend that enhancement of the developmental environment of offspring was the primary initial driving force for the evolution of endothermy in birds and mammals (Farmer, 2000; Koteja, 2000). Studying ectothermic species that have significant thermogenic activity limited to parental activities should provide valuable insight into the interaction between parental investments and the evolution of endothermy.

All pythons (Squamata: Pythonidae) provide parental care to their offspring by brooding their eggs (Stahlschmidt and DeNardo, 2010). Postoviposition a female coils around her clutch, which benefits the clutch by providing protection, preventing desiccation, and buffering developmental temperature (Stahlschmidt et al., 2008). Three species are known to further regulate developmental temperature through endogenous heat production when environmental temperatures are suboptimal for development (Van Mierop and Barnard, 1978; Slip and Shine, 1988), with most studies focusing on the Burmese Python, *Python bivittatus*, and the closely related Indian Python, *Python molurus* (note: these two species have traditionally been considered subspecies within *P. molurus*, but *P. bivittatus* has recently been elevated to specific rank; Jacobs et al., 2009).

Previous studies agree on the general attributes of facultative thermogenesis in brooding *P. bivittatus*. Decreasing environmental temperature (T_{env}) below optimal developmental temperature leads to increases in both muscular twitch rate (R_{tw}) and metabolic rate, which results in substantial endogenous heat production (Van Mierop and Barnard, 1978). This heat production is sufficient enough to provide a relatively homeothermic developmental environment unless T_{env} falls below a critical threshold of approximately 24°C. While *P. bivittatus* is unable to fully buffer against T_{env} below 24°C, the thermogenic

activity of the female can provide a temperature differential between the clutch and the environment ($T_{\Delta cl}$) as high as 8.3°C (Van Mierop and Barnard, 1978); however, the range of reported values is wide for both $T_{\Delta cl}$ and R_{tw} (Dowling, 1960; Hutchison et al., 1966).

Early studies concluded that all heat production was generated by muscular twitching based upon correlations between $T_{\Delta cl}$ and R_{tw} (Vinegar et al., 1970), but later studies suggest this relationship is curvilinear and report females with elevated metabolic rate and body temperature (T_b) even when no twitches were visible (Van Mierop and Barnard, 1978). Studies have also disagreed on whether or not twitches and metabolic rate decrease at night. A diel cycle, if present, can affect offspring phenotype and therefore fitness (Shine et al., 1997). Thus, despite previous work on facultative thermogenesis by Pythons during egg brooding, considerable work remains to be done to understand fully this interesting and evolutionarily insightful form of parental care.

We thus initiated a study of *P. bivittatus* to clarify some of the inconsistencies in the literature as part of a long-term goal of investigating proximate mechanisms of brooding in Pythons. We wanted to determine T_b , $T_{\Delta cl}$, R_{tw} , and carbon dioxide production (VCO_2) during brooding at three temperatures within the reported thermogenic range. Additionally, we wanted to measure internal T_b directly, something not previously done, and assess the potential for the presence of a diel cycle for each of the variables.

MATERIALS AND METHODS

Animals and Maintenance.—We used three gravid female *P. bivittatus* (10.5–17.7 kg) that were part of a captive Python colony maintained at Arizona State University (ASU). Animal rooms were maintained at 27°C under a 12:12 h photoperiod with supplemental heat provided by a subsurface heating element (Flexwatt, Flexwatt Corp., Wareham, MA) below one end of each cage.

Experimental Procedure.—Females ($N = 3$) were implanted surgically with a small (~3 g) temperature logger (iButton, Maxim, USA) during early gravidity. The logger was programmed to record temperature every 45 min. Approximately 1 week before oviposition, the mass of a gravid female was

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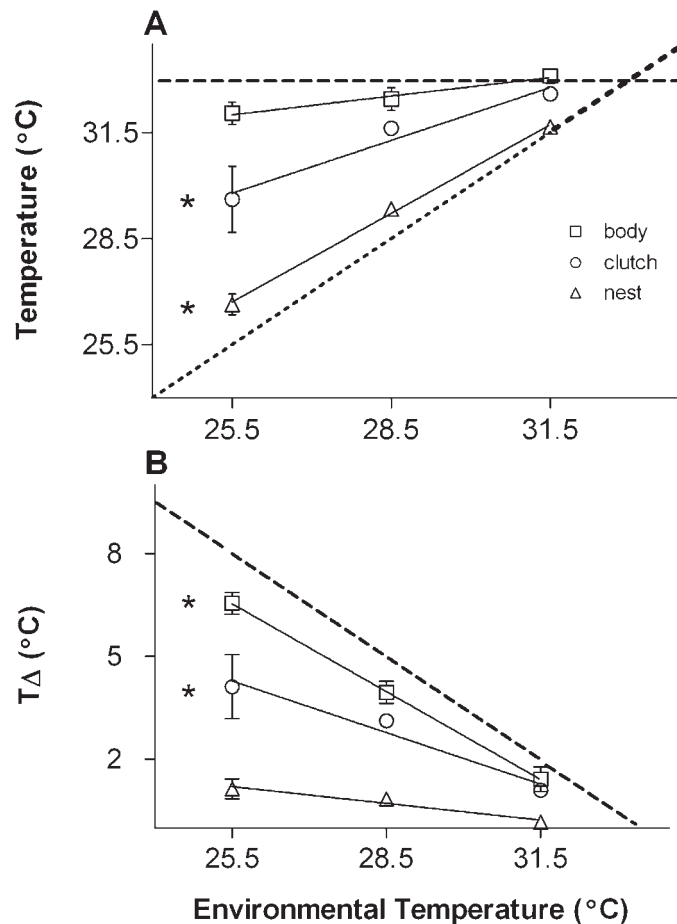


FIG. 1. The effect of environmental temperature on (A) the temperature of the female's body, clutch, and nest, and (B) temperature differentials between these three temperatures and the environmental temperature in female Burmese Pythons (*Python bivittatus*, $N = 3$). Dashed lines represent the predicted result if optimal temperature of 33°C had been maintained while the dotted line represents temperature without thermogenesis. Asterisks indicate statistically significant effects of environmental temperature using rmANOVA. Values are presented as mean \pm SE.

determined and she was moved to a respirometry unit housed in a dark environmental chamber maintained at $31.5 \pm 0.3^\circ\text{C}$ between trials. From this point the female was not provided food or drinking water until the end of the study.

The respirometry unit was a 126-L square container fabricated from wood, lined with sheet metal, and caulked to make it airtight. The lid on the unit was supplied by one influx and one efflux port placed diagonally across from each other. Saturated air was supplied through the influx port by bubbling building supply air through a 1.6 m water-filled hydrator. During trials, one thermocouple was inserted through the influx port ~15 cm into the container and another into the clutch through a resealable port on the bottom of the container.

Within 10 days after oviposition the brooding female was put through a random sequence of three trials, one each at environmental temperatures (T_{env}) of 25.5, 28.5, and 31.5°C. Each treatment lasted 24 h and was monitored in real time using an infrared camera (Model OC960, Wisecomm, USA) and time-lapse video recorder (Ganz CTR-03-ONC-2, CBC Corporation, USA). Clutch (T_{cl}), nest (container, T_{n}), and environmental chamber (T_{env}) temperatures were recorded minutely using copper-constantin thermocouples (TT-T-245-SLE, Omega, USA)

connected to a datalogger (21X, Campbell Scientific Instruments, USA). Temperature differentials were calculated with reference to environmental temperature ($T_{\Delta x} = T_x - T_{\text{env}}$).

Gas data were collected minutely using a portable carbon dioxide analyzer (FoxBox, Sable Systems International, USA). The 99% equilibration period was ~33 min for the largest female and ~60 min for the smallest female (Lasiewski et al., 1966). Air was dried using CaSO_4 before entering the analyzer, and a baseline recording was taken for 1 h before and after brooding by diverting supply air directly to the CaSO_4 . During her first trial, female no. 3 cracked the respirometry chamber, making collection of \dot{V}_{CO_2} data impossible for all of her trials (removing the female to repair the chamber would have led to clutch abandonment). Females and clutches were weighed at the end of the experiment. \dot{V}_{CO_2} was calculated using the equations in Lighton (2008). The mass used for these equations was determined by subtracting clutch mass from gravid female mass and then averaging this with the postexperiment female mass. Using the same respirometry system we collected non-brooding respirometric data from each female 3 months after the brooding experiment.

Video was analyzed after the experiments to determine R_{tw} . A twitch was defined as a spasmodic, muscular movement that involved greater than 50% of the length of the body. Sporadic, localized twitches were present but were not included in the count. Hourly R_{tw} (twitches/min) were calculated by randomly selecting three nonoverlapping, 15-min segments from each hour, counting the total number of twitches during each segment, and dividing the mean of each hour's three counts by 15.

Statistical Analysis.—Results were analyzed using R software (R Development Core Team, 2011). Treatment results were analyzed using rmANOVA. To test for the presence of a diel cycle, 24-h sets of data were averaged over 1-h periods (the maximum equilibration time) beginning at the top of each hour and analyzed using rmANOVA. All values are displayed as mean \pm SE and statistical significance was set at $\alpha = 0.05$.

RESULTS

Immediately following oviposition, each female achieved a tight coil around her clutch and began periodic twitching. All females were able to maintain T_{b} significantly above T_{env} during all treatments (Fig. 1A). Although the temperature differential was small ($1.6 \pm 0.1^\circ\text{C}$; Fig. 1B) at the highest T_{env} (31.5°C), the low variance in T_{b} and T_{env} data resulted in a statistically significant difference ($t(94) = 7.93$, $P < 0.0001$). The T_{b} was remarkably stable and did not significantly vary across treatments ($F(2, 4) = 1.21$, $P = 0.39$). While females were able to maintain their T_{b} at a relative constant, T_{cl} decreased proportionally to T_{env} ($F(2, 4) = 10.34$, $P = 0.026$; Fig. 1A); however, T_{c} at 31.5°C was $32.7 \pm 0.1^\circ\text{C}$, closely approximating T_{b} . Although female mass and clutch size varied considerably (Table 1), this did not affect a female's ability to thermoregulate. The nest environment was most susceptible to changes in T_{env} causing T_{n} to significantly decrease proportional to T_{env} ($F(2, 4) = 193.5$, $P < 0.0001$; Fig. 1A).

At 31.5°C, the highest T_{env} females loosened their coils enough to expose their clutches. However, twitches were still present and mean R_{tw} increased by 52% as T_{env} was lowered to 25.5°C (treatment effect: $F(2, 4) = 25.26$, $P = 0.0054$; Fig. 2). Linear regression analysis showed that R_{tw} had a significant negative slope ($\beta = -0.22$, $F(1, 7) = 12.46$, $P = 0.0095$) and

TABLE 1. Body mass, snout-vent length (SVL), and clutch data for female Burmese Pythons (*Python bivittatus*) used in the experiment.

Python (ID no.)	SVL (m)	Prelay mass (kg)	Postlay mass (kg)	Clutch mass (g)	Fertile eggs (N)	Total eggs (N)
1	2.6	11.8	7.3	2,078	16	24
2	3.2	25.5	16.3	6,108	39	42
3	3.0	20.9	14.5	5,110	37	37

moderate linearity ($r^2 = 0.64$). Correlations between R_{tw} and treatment means showed that no relationship between R_{tw} and T_b , R_{tw} and T_{cl} had a significant negative slope ($\beta = -2.03$, $F(1, 7) = 10.44$, $P = 0.014$, $r^2 = 0.60$), and R_{tw} and \dot{V}_{CO_2} were strongly correlated ($r^2 = 0.91$) and had a significant positive relationship ($\beta = 52.35$, $F(1, 4) = 40.71$, $P = 0.0031$).

Respirometric data were obtained from two brooding females (Table 1), both of whom had similar, mass-specific metabolic rates that followed an endothermic pattern (Table 1, Fig. 3), temperature coefficients (Q_{10}) being 0.10 and 0.16. \dot{V}_{CO_2} showed the greatest variation during the 24-h treatment period (Fig. 4) but statistical analyses revealed no significant hourly differences in mean \dot{V}_{CO_2} , mean R_{tw} , T_b , or T_{cl} . When measured 3 months postbrooding, females were not able to maintain a thermal differential with their environment. Nonreproductive females did not coil when placed into their previous brooding containers, and twitches were not present during any of the postreproductive trials. Mean Q_{10} for nonreproductive females was 4.09 ± 0.06 .

DISCUSSION

Our results confirm the general pattern of thermoregulation in brooding *P. bivittatus* documented previously. Our calculated temperature differentials were within the reported range and, at our highest T_{env} , females maintained a T_{Ab} of approximately 1°C, supporting the most-commonly reported brooding female preferred temperature of 3°C (Hutchison et al., 1966). For brooding females our calculated maximum metabolic rates at 25.5°C and our calculated Q_{10} values were within the range of the two previously reported values ($\dot{V}_{O_2} = 100$ ml/kg/hr, $Q_{10} =$

0.26: Hutchison et al., 1966; $\dot{V}_{O_2} = 154$ ml/kg/hr, $Q_{10} = 0.11$: Van Mierop and Barnard, 1978).

The largest discrepancy with existing literature was in our R_{tw} values, which were an order of magnitude lower than values reported previously (42 twitches/min: Hutchison et al., 1966; 35 twitches/min: Van Mierop and Barnard, 1978). These studies did not report how twitch was defined, and we presume that differences in the definition of twitch caused the discrepancy rather than a true difference in contraction rates. Two types of twitches can be observed when females are brooding: large, rhythmic contractions that run the length of the body and highly localized, sporadic contractions. We only counted the former type, but when we factored the localized twitches into our calculations, R_{tw} quickly approached maximal values of 45 twitches/min.

We are the first to measure internal T_b , and the results of our study strongly suggest that females primarily regulate T_b with the maintenance of T_{cl} being indirect. That is, females consistently maintained T_b near preferred developmental temperature regardless of experimental temperature but T_{cl} decreased significantly with decreasing experimental temperature, likely because of enhanced conductive heat loss (Fig. 1). This may have important consequences for the fitness of the offspring, as incubation temperature affects both incubation duration and offspring phenotype in reptiles (Booth, 2006). In Water Pythons (*Liasis fuscus*) an incubation regimen with a minimum of 24.3°C affected offspring fitness proxies, delayed hatching up to 20 days, and resulted in a significant decrease in recapture success (Shine et al., 1997), most likely because of decreased prey acquisition by the hatchlings (Madsen and Shine, 1998). Few studies have examined these effects in *P. bivittatus*, but *P. bivittatus* eggs incubated at 27.5°C have hatching success rates approaching zero (Vinegar, 1973). Although females in our study were able to maintain T_{cl} above 29°C (Fig. 1), both T_{cl} and T_b approximate 33°C when provided a 31.5°C environment, suggesting that this may be their optimal preferred incubation temperature and that variation within an incubation range of 29–33°C likely has fitness consequences. However, future studies of thermal influences on offspring phenotype should examine temperatures that more closely approximate optimal developmental temperature.

Our results do not eliminate the possibility of nonshivering thermogenesis. Similar to all previous studies, we found a high correlation between R_{tw} and \dot{V}_{CO_2} ($r^2 = 0.91$), despite our differences in R_{tw} as compared to previous studies. This correlation has been used to argue against the presence of nonshivering thermogenesis (Hutchison et al., 1966), but Van Mierop and Barnard (1978) found this correlation to have a significant lack of fit and concluded that the correlation was the result of both metabolic rate and R_{tw} being correlated with temperature. Our correlation did not show a significant lack of fit ($F(1, 4) = 3.0$, $P = 0.18$), possibly due to our definition of twitch (i.e., large muscular contractions may correlate better with oxygen consumption). However, R_{tw} was weakly linear ($r^2 = 0.64$), suggesting that the fit may be due to low sample size.

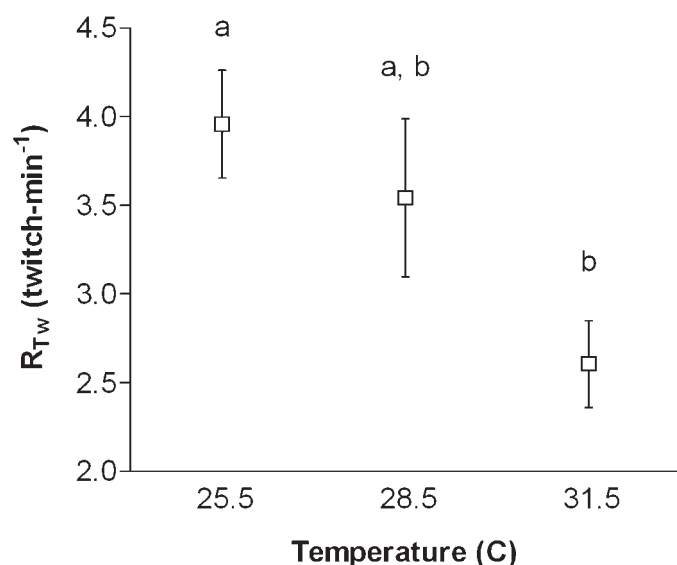


FIG. 2. The effect of environmental temperature on twitch rate (R_{tw}) in brooding female Burmese Pythons ($N = 3$). Letters indicate statistical differences among temperatures using rmANOVA. Values are presented as mean \pm SE.

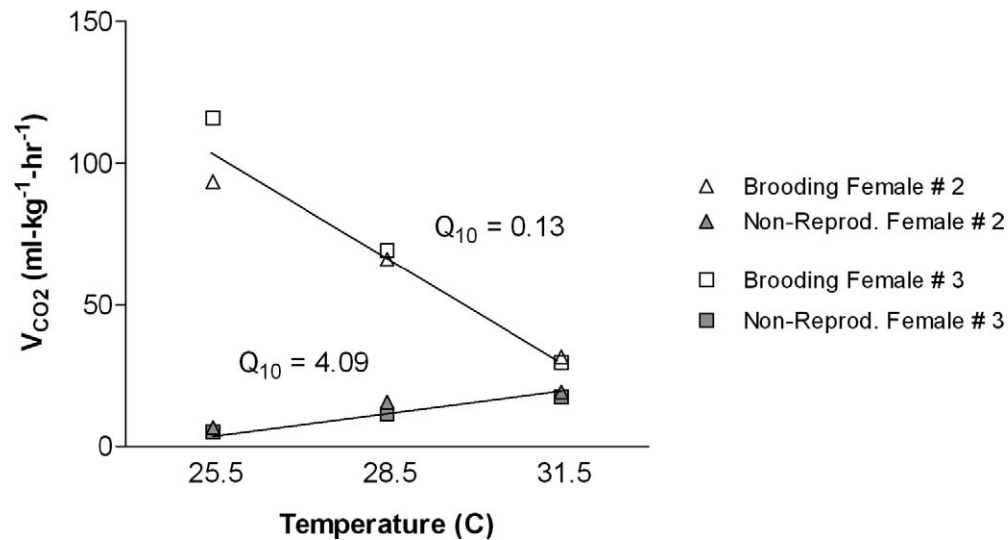


FIG. 3. The effect of environmental temperature on mean metabolic rate (\dot{V}_{CO_2}) of two female Burmese Pythons (*Python bivittatus*) during brooding and after reproduction.

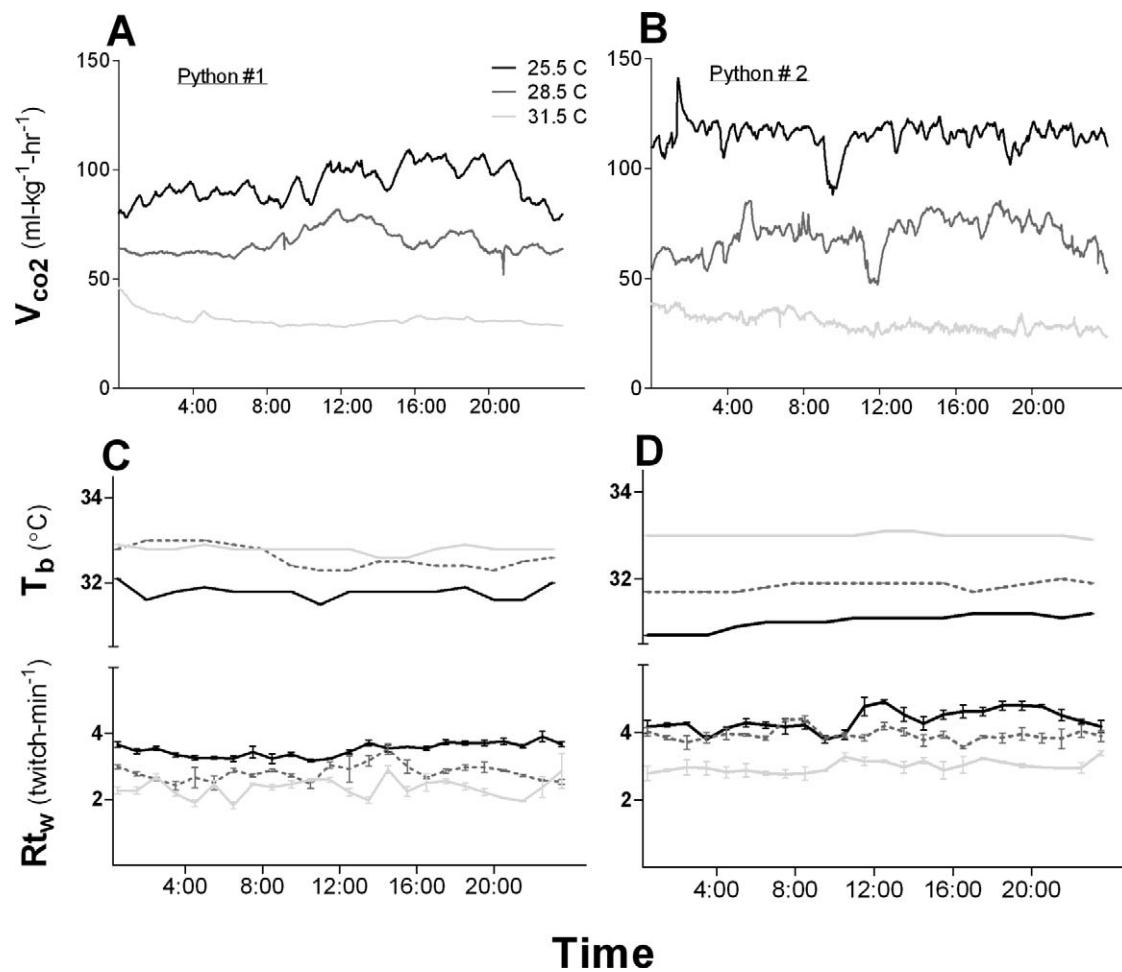


FIG. 4. Metabolic rate (\dot{V}_{CO_2}), female body temperature (T_b), and twitch rate (R_{tw}) over a 24-h period for two brooding female Burmese Pythons (*Python bivittatus*) at three environmental temperatures. Upper graphs (A, B) show metabolic rate and lower graphs (C, D) show body temperature and twitch rate on a split x-axis. Data were collected simultaneously for each female. ANOVA analysis revealed no statistical differences among hours. Values for R_{tw} were calculated for each hour and are presented as mean \pm SE.

Shivering in birds and mammals is due to the misalignment of muscle fibers during tetani (Hohtola, 2004) and this is likely true in shivering *P. bivittatus*. If so, R_{tw} may be a weak proxy for the number of muscles in tetani during brooding, resulting in weak correlations between R_{tw} and metabolic rate.

Finally, we found no evidence of a diel cycle in any measured variable (Fig. 4), although V_{CO_2} and R_{tw} fluctuated throughout the 24 h for each female. However, there were no trends when these fluctuations were aligned temporally. These data and the stability of T_b and T_{cl} (Fig. 4) across the 24-h period suggest that a female continuously modifies her heat production to some extent while brooding, resting after a warming period. Future research should also focus on direct measurements of muscle contraction in brooding females.

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