

## RESEARCH ARTICLE

# Modeling the costs and benefits associated with the evolution of endothermy using a robotic python

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## ABSTRACT

Endothermy provides considerable benefits to an organism but requires large energy investment. To understand potential driving forces that would lead to the evolution of endothermy, it is important to understand the energy costs and potential benefits of intermediate steps between ectothermy and homeothermic endothermy as well as the influences of environmental conditions on energetic costs. However, efforts to examine intermediate conditions are greatly limited by the predominant natural dichotomy between ectothermy and endothermy. Facultative endothermy by brooding pythons provides a fortunate study system where endothermy is beneficial but not essential. As one cannot control the extent of energy investment in heat production by a female python, we created an artificial snake with controllable heating capability. This enabled us to determine the energetic costs of maintaining a clutch at a preferred temperature, and to determine the relative thermal benefit of limited energy-producing capability (i.e. 50% of the required energy to maintain the preferred developmental temperature). We manipulated the pseudoserpent's clutch size (5, 10, 15 eggs), diel ambient temperature cycle (2, 4, 6°C) and insulation (with and without) at each of these power levels: unlimited power, half required power and no power. We found no significant effect of clutch size on either power requirements or developmental temperature. Energy requirements increased with the amplitude of the diel cycle and decreased with the addition of insulation, while the quality of the thermal environment decreased with the amplitude of the diel cycle. Interestingly, the quality of the thermal environment also decreased with the addition of insulation. We discuss these results within the context of the reproductive model of the evolution of endothermy.

**KEY WORDS:** Facultative thermogenesis, Nest site selection, Parental care, Pseudoserpent

## INTRODUCTION

The maintenance of high and relatively constant temperature by metabolic means (homeothermic endothermy) constitutes a major innovation that appeared independently in two vertebrate lineages: birds and mammals (Bennett and Ruben, 1979). While daily and seasonal heterothermic endothermy are widespread among birds and mammals and mitigate energetic costs (Grigg et al., 2004), the extreme complexity and energetic demands of homeothermic

endothermy suggest that there must have been substantial selective advantages driving its evolution (Bennett and Ruben, 1979; Koteja, 2004). While one can postulate advantages to the ability to maintain elevated temperature, it is unclear which selective forces may have initiated the evolution of endothermy (Hayes and Garland, 1995; Koteja, 2000; Ruben, 1995).

The evolution of homeothermic endothermy has generated considerable scientific controversy, and several hypotheses have been advanced (Hayes and Garland, 1995; Ruben, 1995). These include thermoregulation per se (Crompton et al., 1978; Heinrich, 1977), increased aerobic capacity (Bennett and Ruben, 1979) and intense parental care (Farmer, 2000, 2003; Koteja, 2000). The reproductive model was formulated by Farmer (2000), and it divides the evolution of homeothermic endothermy into two steps. As a first step, increased thermogenesis was selected for (at low associated costs) to provide embryos with a developmental environment that had a relatively high mean temperature and low variation. During the second step, additional benefits, such as the capacity for prolonged physical activity, would have then permitted the emergence of extensive postnatal parental care including food provisioning to progeny, a common feature of birds and mammals (Koteja, 2000). This second step would thereby result in the fixation of high performance (and thus high-energy demand systems) and homeothermic endothermy as displayed by birds and mammals. The reproductive model is distinct from previous theories in that endothermy is primarily beneficial to the progeny rather than the adult (Farmer, 2000).

The reproductive model has been extensively discussed and debated (Angilletta and Sears, 2003; Farmer, 2003), with the debate centering on whether the thermal benefits accrued by the offspring during development (e.g. increased survivability, decreased incubation period) outweigh the energetic costs exacted on the parents. Opponents not only suggest that these benefits are unlikely to exceed the costs but also point to the mixed results attained by studies that have attempted to quantify these benefits (Andrews et al., 2000; Shine et al., 1997). Accurate assessment of the problem, however, requires that the energetic costs to the parent must be assessed relative to these benefits, including factors that may affect such costs (e.g. nest site insulation).

While studies on heterothermic organisms have provided valuable comparative data (Grigg et al., 2004), empirical tests of any model for the evolution of homeothermic endothermy are rare. The unique characteristics of pythons, in which females of some species are facultatively endothermic during egg brooding (Vinegar et al., 1970), have been used to support arguments for and against the reproductive model (Angilletta and Sears, 2003; Farmer, 2000). More recently, Tattersall et al. (2016) provided evidence that the relatively large tegu lizard, *Salvator merianae*, also employs facultative endothermy to support reproductive efforts. However, little work has been conducted to empirically examine the relationship between the energetic costs to the female and the

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**List of symbols and abbreviations**

GLM	generalized linear model
$T_{\text{Clutch}}$	clutch temperature
$T_{\text{Env}}$	environmental temperature
$T_{\text{Int}}$	pseudoserpent–pseudo-egg interface temperature
$T_{\text{Nest}}$	nest temperature
$T_{\text{Set}}$	target incubation temperature
$\Delta T$	temperature differential
$\Delta T_{\text{Clutch}}$	difference between set temperature and clutch temperature
$\Delta T_{\text{Int}}$	difference between set temperature and interface temperature

thermal benefits to the developmental environment, or how biotic and abiotic factors related to the reproductive event may influence these costs and benefits. In fact, while python brooding is widely mentioned in discussions of facultative endothermy, only a few species are known to have significant thermogenic capability (Brashears and DeNardo, 2015; Stahlschmidt and DeNardo, 2010).

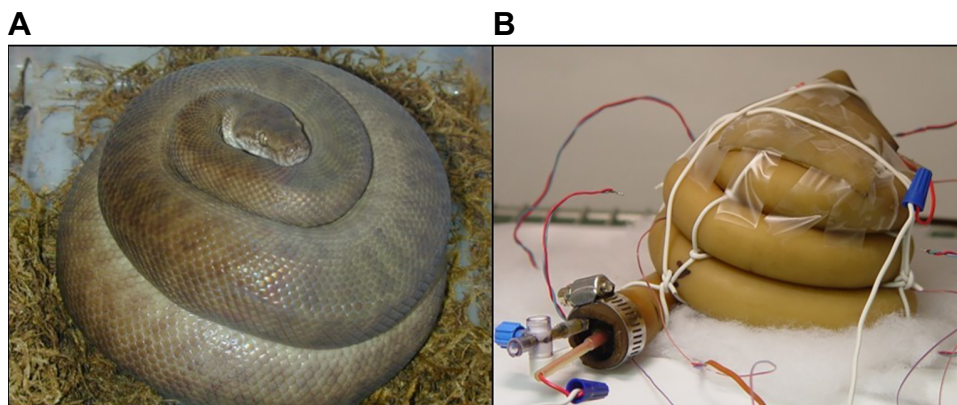
As female snakes cannot be induced to produce heat or refrain from producing heat, we designed a pseudoserpent (an artificial snake) that mimicked the insulating properties of a Children's python (*Antaresia childreni*), a species that has become an effective model for examining parental care tradeoffs (Lorion et al., 2012; Stahlschmidt and DeNardo, 2009a,b). While *A. childreni* does not use facultative thermogenesis when brooding, we were able to regulate the thermogenic capability of the pseudoserpent. We used this pseudoserpent to (1) calculate the power required to create a constant preferred developmental temperature under various conditions with respect to environment and clutch, and (2) determine the thermal consequences of the various conditions when thermogenic capability was absent or limited to 50% of the power required for developmental homeothermy. Specifically, we conducted thermometric sets of trials that included three thermogenic potentials (homeothermy, ectothermy and an intermediate condition). We imposed these three sets of condition under multiple diel cycles of ambient temperature (2, 4, 6°C amplitude with the zenith at the preferred developmental temperature) and multiple clutch sizes (5, 10 and 15 eggs). Additionally, we conducted trials in both non-insulated and insulated nest conditions. As heat production depended on the power supplied to the pseudoserpent, for each trial condition we were able to directly estimate the energetic costs and thermal consequences for the developmental environment.

**MATERIALS AND METHODS****Pseudoserpent construction**

The pseudoserpent was designed to mimic the size, shape and insulation properties of a brooding Children's python (*A. childreni*). The body of the pseudoserpent was constructed by inserting a 130 cm spring into a 135 cm length of latex tubing (outside diameter 24 mm, inside diameter 18 mm; McMaster-Carr, Santa Fe Springs, CA, USA). To provide the pseudoserpent with the ability to generate heat, we soldered 18 resistors (0.33  $\Omega$ , All Electronics Corporation, Van Nuys, CA, USA) in series with sufficient distance between them that the total length approximated the length of the pseudoserpent's body. A piece of 18 G wire was soldered to each end of the resistor string, and then the resistors were enclosed in a piece of waterproof heat shrinkable tubing. The sealed resistor array was then inserted into the body of the pseudoserpent. A no. 3 rubber stopper was inserted into each end of the tubing, with the circuit wire exiting through a small (~3 mm) hole in each of the stoppers. Additionally, a syringe needle (18 G, 2.5 cm) fitted with a stopcock penetrated one of the stoppers. The stopcock and needle were used for filling the pseudoserpent with water prior to each set of trials.

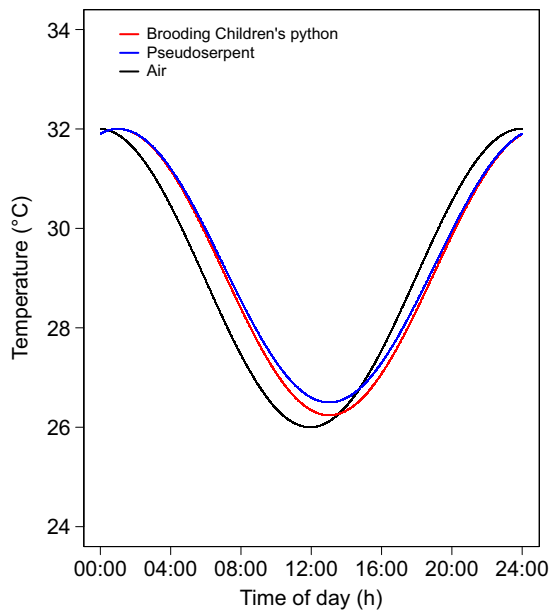
The complete pseudoserpent was then coiled into a beehive posture typical of a brooding female and secured in that position using fine wire (Fig. 1). To mimic the seal typically provided by the snake's head, we secured a piece of latex over the opening at the top of the coil. *Antaresia childreni* females coil so effectively around their clutch that the eggs are completely confined when the female is in a tightly coiled position (Stahlschmidt and DeNardo, 2008). Therefore, we placed the brooding female and her clutch on a thin bed of synthetic insulation and filled any small gaps between coils with small pieces of the same insulation. Pseudo-eggs were made by removing the fingers of large-sized latex gloves and filling them with 15±0.5 ml of water before knotting the end. The desired number of eggs (depending on trial set, see below) was then placed within the female's coils. The insulating properties of the resulting brooding pseudoserpent closely mimicked those of a live *A. childreni* female brooding her eggs (Fig. 2).

To regulate the thermogenic activity of the pseudoserpent, we supplied power to the pseudoserpent through an amplifying circuit that was controlled by a datalogger (21X micrologger, Campbell Scientific Instruments, Logan, UT, USA). The program increased power to the pseudoserpent, and thus current and heat production, in proportion to the difference between the pseudoserpent–pseudo-egg interface temperature ( $T_{\text{Int}}$ ) and the target incubation temperature ( $T_{\text{Set}}$ , 30.5°C, which approximates the preferred incubation temperature of *A. childreni*; Lourdais et al., 2007).



**Fig. 1. Pseudoserpent construction.**

A Children's python (*Antaresia childreni*) brooding her clutch in a beehive posture (A) and the pseudoserpent brooding a clutch of pseudo-eggs in a similar posture (B).



**Fig. 2. Comparison of clutch temperatures.** Temperature is shown for a clutch being brooded by a female Children's python and an artificial clutch surrounded by a similarly sized pseudoserpent simultaneously maintained in a 6°C diel cycle. As *A. childreni* is ectothermic, the model was set to zero thermogenic potential to compare the insulating properties of the female and the pseudoserpent. Air temperature is also shown.

## Trials

Trials were conducted in a darkened, temperature-controlled test chamber that had a sinusoidal diel thermal cycle tightly controlled by the same datalogger used to power the pseudoserpent. Chamber temperature was controlled using feedback from a 3-thermocouple array that surrounded the pseudoserpent at a distance of 10 cm, thus averaging its immediate environmental temperature ( $T_{Env}$ ). The environmental chamber was equipped with a small fan to maximize thermal homogeneity within the chamber.

In addition to chamber temperature, the datalogger also recorded input from a thermocouple at the center of the clutch ( $T_{Clutch}$ ) and from a thermocouple array averaging  $T_{Int}$  over three points of contact. During sets of trials with nest insulation,  $T_{Env}$  was measured just outside the insulating box, and we additionally measured nest temperature using a thermocouple placed within the insulating box but not in contact with the pseudoserpent ( $T_{Nest}$ ).

Trial sets were designed to test the relationship between power consumption,  $T_{Clutch}$  and  $T_{Int}$  at a specified set point ( $T_{Set}$ ) in the presence of a diel cycle. Each trial set lasted 80 h and consisted of constant environmental conditions and clutch characteristics (see below), but variable thermogenic capability as the trial set progressed. On day 1, the pseudoserpent was supplied with unlimited power (up to 4 W, which was ~300 mW above the power needed to maintain  $T_{Clutch}$  even in the most demanding conditions). On day 2, the power limit was set to half of the maximum power consumed on the previous day. At the end of day 2, power to the pseudoserpent was discontinued (i.e. an ectothermic condition was imposed). The first 8 h of day 3 were used as an equilibration period to assure that starting temperatures were the same for all three power conditions. After the 8 h equilibration period, we recorded all temperatures for a continuous 24 h period.

## Diel cycle amplitude trials

Trial sets were conducted with  $T_{Env}$  following a sinusoidal diel cycle with a zenith temperature of 30.5°C and a nadir temperature of 28.5, 26.5 or 24.5°C (a 2, 4 or 6°C diel cycle, respectively). This range of temperature cycles reflects the range reported for free-ranging nesting females of a python species that is sympatric to *A. childreni* (Shine et al., 1997). Three sets of trials were conducted at each of the three diel cycles, and clutch size for these trials was 10 eggs, which approximates a typical clutch size for *A. childreni* (Stahlschmidt and DeNardo, 2008).

## Clutch size trials

Trial sets were conducted in triplicate for clutch sizes of 5, 10 and 15 eggs, which approximates the clutch size range of captive *A. childreni* (D.F.D., unpublished data).

## Insulation trials

While the sets of trials using clutch size as a treatment mimicked the situation of brooding a clutch in open space on the surface, this set of trials provided insulation similar to what might be provided if a female broods her eggs in a more restricted environment (e.g. within a burrow chamber). We examined both conditions, as pythons, in general, are known to brood either on the surface or within burrows, and the location of brooding in *A. childreni* is unknown. Trial sets were similarly conducted in triplicate for clutch sizes of 5, 10 and 15 eggs, but, for these trial sets, we placed the pseudoserpent within a rectangular box (24×17×16 cm) constructed by pinning a single layer of shade cloth (Synthesis Commercial 95, Arizona Sun Supply Inc., Phoenix, AZ, USA) to the six faces of a wooden frame. We determined the thermal conductivity ( $k$ ,  $W\ ^\circ C^{-1}\ m^{-2}$ ) of the shade cloth by inserting a high wattage ceramic resistor within the center of the box suspended from the top layer. We placed the box within the test chamber and set the chamber to maintain 25.5°C to provide a constant heat sink. Thermocouples were placed at three points on the inner surface of the shade cloth and three points on the outer surface of the shade cloth. We then provided constant power (1.6 W) across the resistor and allowed the system to reach steady-state conditions (~1.5 h). To calculate the thermal conductivity of the system, we used the equation:

$$k = \frac{Q \cdot \Delta d}{A \cdot \Delta T}, \quad (1)$$

where  $Q$  is heat flow in W,  $\Delta d$  is the thickness of the shade cloth,  $A$  is the surface area of the box and  $\Delta T$  is the temperature differential across the shade cloth. We did this under two conditions: first with no air circulation within the environmental chamber, then with the small fan turned on, which we used during trials to ensure homogeneity of the thermal environment. For our material,  $k=0.6\pm 0.2\ W\ ^\circ C^{-1}\ m^{-2}$  with no air circulation and  $2.0\pm 0.2\ W\ ^\circ C^{-1}\ m^{-2}$  with air circulation.

## Data analysis

Data were analyzed by fitting multiple models to the data and comparing Akaike information criterion (AIC) values. Independent factors were the type of diel cycle (2, 4, 6°C), the clutch size (5, 10, 15 eggs) and insulation (no insulation, one layer). Supplied power (zero, half, full) fitted as an independent factor produced the lowest AIC values, but, as these power levels were programmed into the trials, and we were interested in the relationships within the levels, the final model was a general linear model (GLM) with supplied power as a covariate. All data were tested for parametric assumptions. The percentage of time the clutch ( $T_{Clutch}$ ) and



interface ( $T_{\text{Int}}$ ) spent at suboptimal temperatures ( $\Delta T \geq 1.0^\circ\text{C}$ ) was calculated for each trial, then arcsine transformed and tested across each treatment (diel, clutch size, insulation) with supplied power as a covariate. All data are presented as means  $\pm$  s.e.m. Analyses were performed using R 2.14.0 (R Development Core Team, 2011) and models were run using the lme4 package (Bates et al., 2011).

## RESULTS

The power required by the pseudoserpent during the trials reflected the energetic costs of maintaining  $T_{\text{Clutch}}$  above  $T_{\text{Env}}$  (Table 1). Power metrics modeled a female's metabolic rate during brooding. The maximum recorded power during a 3 day trial increased with the amplitude of the diel cycle ( $F_{2,71}=31.431$ ,  $P<0.001$ ) but was not significantly decreased with the addition of insulation ( $F_{1,71}=3.190$ ,  $P=0.078$ ). The total power supplied during a single 24 h period reflected the average power over that period. Total power was increased by the diel cycle amplitude ( $F_{2,71}=33.734$ ,  $P<0.001$ ) as well as clutch size ( $F_{2,71}=5.928$ ,  $P=0.004$ ). The addition of insulation strongly decreased total power ( $F_{1,71}=18.866$ ,  $P<0.001$ ).

The mean interface temperature difference ( $\Delta T_{\text{Int}}=T_{\text{Set}}-T_{\text{Int}}$ ) increased with diel cycle amplitude ( $F_{2,71}=34.417$ ,  $P<0.001$ ) and was weakly decreased by the addition of insulation ( $F_{2,71}=3.806$ ,  $P=0.055$ ; Table 2). Both the maximum recorded  $\Delta T_{\text{Int}}$  and the percentage of time during which  $\Delta T_{\text{Int}}$  was at a suboptimal temperature ( $\Delta T \geq 1.0^\circ\text{C}$ ) increased with diel cycle amplitude (maximum  $\Delta T_{\text{Int}}$ :  $F_{2,71}=57.623$ ,  $P<0.001$ ; percentage time:  $F_{2,22}=25.672$ ,  $P<0.001$ ; Table 2) but decreased with the addition of insulation (maximum  $\Delta T_{\text{Int}}$ :  $F_{1,71}=6.790$ ,  $P=0.011$ ; percentage time:  $F_{1,65}=14.045$ ,  $P<0.001$ ; Table 2). The variance of  $T_{\text{Int}}$  increased only in response to the amplitude of the diel cycle ( $F_{2,71}=20.107$ ,  $P<0.001$ ; Table 3). Clutch size had no effect on any interface metrics.

The amplitude of the diel cycle significantly increased the amount of time  $T_{\text{Clutch}}$  experienced a suboptimal environmental temperature by all metrics of  $T_{\text{Clutch}}$ : the calculated mean  $\Delta T_{\text{Clutch}}$  ( $\Delta T_{\text{Clutch}}=T_{\text{Set}}-T_{\text{Clutch}}$ ;  $F_{2,71}=72.722$ ,  $P<0.001$ ), the maximum recorded  $\Delta T_{\text{Clutch}}$  ( $F_{2,71}=87.118$ ,  $P<0.001$ ), the percentage of time

the clutch spent at a suboptimal temperature ( $F_{2,22}=15.411$ ,  $P<0.001$ ) and the variance in temperature the clutch experienced ( $F_{2,71}=23.053$ ,  $P<0.001$ ). Interestingly, the addition of insulation also increased the mean  $\Delta T_{\text{Clutch}}$  ( $F_{1,71}=6.921$ ,  $P=0.010$ ), the maximum recorded  $\Delta T_{\text{Clutch}}$  ( $F_{1,71}=8.406$ ,  $P=0.005$ ), the thermal variance that the clutch experienced ( $F_{1,71}=3.610$ ,  $P=0.062$ ) and the percentage of time the clutch spent at a suboptimal temperature ( $F_{1,65}=26.398$ ,  $P<0.001$ ). Clutch size did not affect most metrics, but larger clutches increased the percentage of time a clutch experienced suboptimal temperatures ( $F_{2,43}=8.357$ ,  $P<0.001$ ).

## DISCUSSION

### Costs and benefits of homeothermic endothermy

We designed an artificial snake model (pseudoserpent) to estimate the cost and benefits associated with reproductive endothermy in pythons. The pseudoserpent mimicked a brooding Children's python (*A. childreni*), which, although not facultatively endothermic, has become a useful model for examining brooding tradeoffs (Lorion et al., 2012; Stahlschmidt and DeNardo, 2009a,b).

The unlimited thermogenic pseudoserpent was able to maintain the temperature of the clutch within an optimal range (Table 2; Fig. 3), but doing so required considerable amounts of energy (Table 1; Fig. 3). At the  $6^\circ\text{C}$  amplitude with a 10-egg clutch, the pseudoserpent used  $148 \pm 4$  kJ over the 24 h trial (Table 1). In comparison, a much larger (16 kg) brooding and facultatively endothermic Burmese python (*Python molurus*) maintaining a  $6^\circ\text{C}$  gradient averages approximately  $772$  kJ day $^{-1}$  (21 g fat day $^{-1}$ ; Brashears and DeNardo, 2013). Despite heating a much larger clutch mass (50-fold), energy requirements to maintain a similar developmental homeothermic state are only 5-fold higher for the Burmese python. Such large differences in energy requirements per gram of clutch are a result of the high thermal conductivity of smaller females. Using the maximum recorded power and the temperature differential the pseudoserpent maintained at that power, the thermal conductivity of our pseudoserpent is  $0.63 \pm 0.1$  W kg $^{-1}$  °C $^{-1}$ , closely matching the thermal conductivity of  $0.65$  W kg $^{-1}$  °C $^{-1}$  reported for the slightly larger ball pythons

**Table 1. Maximum power, mean energy and total energy used by an artificial snake during trials**

	Diel cycle/ clutch size	Power supplied	Max power (mW)	Mean energy (J)	Total energy (kJ)	Relative cost (no. of eggs)
No insulation, 10-egg clutch	2°C	Full	1213 $\pm$ 16	37 $\pm$ 1	53 $\pm$ 2	0.7
		Half	618 $\pm$ 10	27 $\pm$ 1	39 $\pm$ 2	0.5
	4°C	Full	2323 $\pm$ 18	70 $\pm$ 1	100 $\pm$ 1	1.3
		Half	1172 $\pm$ 7	49 $\pm$ 1	71 $\pm$ 2	0.9
	6°C	Full	3410 $\pm$ 51	103 $\pm$ 2	148 $\pm$ 4	1.9
		Half	1721 $\pm$ 32	72 $\pm$ 2	104 $\pm$ 3	1.3
No insulation, 6°C diel cycle	5 eggs	Full	3802 $\pm$ 29	76 $\pm$ 1	109 $\pm$ 1	1.4
		Half	1369 $\pm$ 33	56 $\pm$ 5	81 $\pm$ 8	1.0
	10 eggs	Full	3903 $\pm$ 72	84 $\pm$ 1	121 $\pm$ 1	1.6
		Half	1414 $\pm$ 16	58 $\pm$ 1	84 $\pm$ 1	1.1
	15 eggs	Full	3960 $\pm$ 98	86 $\pm$ 5	123 $\pm$ 8	1.6
		Half	1479 $\pm$ 63	61 $\pm$ 3	87 $\pm$ 4	1.1
Insulation, 6°C diel cycle	5 eggs	Full	3554 $\pm$ 18	64 $\pm$ 1	93 $\pm$ 1	1.2
		Half	1112 $\pm$ 14	45 $\pm$ 1	65 $\pm$ 2	0.8
	10 eggs	Full	3555 $\pm$ 34	72 $\pm$ 1	104 $\pm$ 2	1.3
		Half	1162 $\pm$ 36	51 $\pm$ 0	73 $\pm$ 0	0.9
	15 eggs	Full	3605 $\pm$ 1	73 $\pm$ 6	105 $\pm$ 8	1.4
		Half	1166 $\pm$ 51	51 $\pm$ 4	73 $\pm$ 6	0.9

Power supplied was the thermogenic limit put on the pseudoserpent (see Materials and methods for details). We manipulated the amplitude of the diel cycle (2, 4 or  $6^\circ\text{C}$ ) using a 10-egg clutch size, as well as clutch size (5, 10 or 15 eggs) using a  $6^\circ\text{C}$  diel cycle under both insulated and non-insulated conditions (see Materials and methods). Total energy is expressed as the cost relative to egg production using a 15 g egg with a composition of 14% lipid. All trials were conducted in triplicate and values are presented as means  $\pm$  s.e.m.

**Table 2. Differences between set temperature ( $T_{\text{Set}}$ ) of the pseudoserpent and both clutch ( $T_{\text{Clutch}}$ ) and interface temperature ( $T_{\text{Int}}$ ) during trials**

	Diel cycle/ clutch size	Power	$T_{\text{Clutch}}$ (°C)			$T_{\text{Int}}$ (°C)		
			Mean $\Delta T_{\text{Clutch}}$	Max. $\Delta T_{\text{Clutch}}$	% Time $\Delta T \geq 1^\circ\text{C}$	Mean $\Delta T_{\text{Int}}$	Max. $\Delta T_{\text{Int}}$	% Time $\Delta T \geq 1^\circ\text{C}$
No insulation, 10-egg clutch	2°C	Full	0.17±0.01	0.24±0.02	0.0±0.0	0.01±0.00	0.05±0.00	0.0±0.0
		Half	0.48±0.01	1.06±0.02	10.8±1.4	0.31±0.01	0.92±0.02	0.0±0.0
		None	1.32±0.07	2.23±0.06	61.7±1.6	1.12±0.06	2.06±0.05	54.2±1.3
	4°C	Full	0.18±0.06	0.26±0.06	0.0±0.0	0.02±0.00	0.06±0.01	0.0±0.0
		Half	0.75±0.05	1.91±0.09	34.5±1.2	0.59±0.02	1.80±0.05	34.2±2.3
		None	2.22±0.02	4.01±0.02	74.0±0.3	2.06±0.05	3.92±0.04	69.4±0.5
	6°C	Full	0.16±0.06	0.24±0.06	0.0±0.0	0.03±0.01	0.07±0.01	0.0±0.0
		Half	1.03±0.08	2.78±0.11	43.3±2.2	0.90±0.01	2.73±0.01	38.8±0.1
		None	3.18±0.01	5.87±0.03	79.7±0.2	3.04±0.05	5.82±0.02	76.1±0.5
No insulation, 6°C diel cycle	5 eggs	Full	0.73±0.09	1.28±0.61	0.0±0.0	0.08±0.01	0.24±0.01	0.0±0.0
		Half	1.52±0.15	3.08±0.05	42.5±1.4	0.91±0.15	2.57±0.08	38.4±0.2
		None	3.10±0.16	5.75±0.11	79.9±0.7	2.73±0.05	5.47±0.09	75.5±0.5
	10 eggs	Full	0.69±0.19	1.19±0.17	0.0±0.0	0.08±0.01	0.44±0.31	0.0±0.0
		Half	1.48±0.22	3.19±0.29	42.7±0.3	0.86±0.03	2.56±0.09	37.1±0.3
		None	3.37±0.14	5.88±0.12	79.1±0.5	2.95±0.07	5.59±0.07	79.5±0.5
	15 eggs	Full	0.73±0.19	1.15±0.30	0.0±0.0	0.08±0.01	0.25±0.04	0.0±0.0
		Half	1.48±0.14	3.22±0.19	47.7±0.9	0.84±0.03	2.56±0.04	38.7±0.1
		None	3.39±0.07	5.94±0.08	82.9±0.3	3.01±0.01	5.68±0.03	90.7±0.8
	5 eggs	Full	1.0±0.4	2.0±1.0	17.7±11	0.3±0.4	1.0±1.3	0.0±0.0
		Half	1.8±0.7	3.9±1.4	52.4±2.3	1.4±0.9	3.5±1.6	35.8±0.1
		None	9.1±10.3	14.0±14.0	89.2±2.6	8.9±10.6	13.8±14.4	80.2±1.5
Insulation, 6°C diel cycle	10 eggs	Full	0.7±0.2	1.2±0.2	0.0±0.0	0.08±0.01	0.4±0.3	0.0±0.0
		Half	1.5±0.2	3.2±0.3	50.9±1.1	0.86±0.03	2.6±0.09	39.6±0.8
		None	3.4±0.1	5.9±0.1	89.4±1.3	2.95±0.07	5.6±0.07	87.6±2.1
	15 eggs	Full	0.7±0.2	1.2±0.3	0.0±0.0	0.08±0.01	0.25±0.03	0.0±0.0
		Half	1.5±0.1	3.2±0.2	49.8±1.2	0.84±0.03	2.56±0.04	40.3±0.8
		None	3.4±0.1	5.9±0.1	90.6±1.1	3.01±0.01	5.68±0.03	88.5±1.9

Power refers to the thermogenic limit put on the pseudoserpent (see Materials and methods for details). We manipulated the amplitude of the diel cycle (2, 4 or 6°C) using a 10-egg clutch size, as well as clutch size (5, 10 or 15 eggs) using a 6°C diel cycle under both insulated and non-insulated conditions (see Materials and methods). All trials were conducted in triplicate, and values are presented as means±s.e.m.

(*Python regius*) (Ellis and Chappell, 1987). In contrast, the two facultatively endothermic species, the Burmese python (*P. molurus*) and the diamond python (*Morelia spilota*), are considerably larger and have reduced thermal conductivity (0.27 and 0.114 W kg<sup>-1</sup> °C<sup>-1</sup>, respectively) (Ellis and Chappell, 1987).

In addition to energy savings resulting from size-based differences in conductivity, larger snakes also have greater energy reserves. At 148 kJ day<sup>-1</sup>, a female *A. childreni* would use 188 g of fat over a species-typical 48 day brooding period (Lorion, et al., 2012), or 63% of body mass assuming a 300 g post-parturient body mass (Stahlschmidt and DeNardo, 2008). If she were to maintain the maximum power we recorded during the entirety of the same trial, she would need to use, over the 48 day brooding period, 375 g fat, which is more than her entire body mass. However, over a species-typical 60 day incubation period (Ramesh and Bhupathy, 2010), the female Burmese python, at 772 kJ day<sup>-1</sup>, would require a total of 1.2 kg of fat, which represents only 7.5% of her body mass, a very feasible investment.

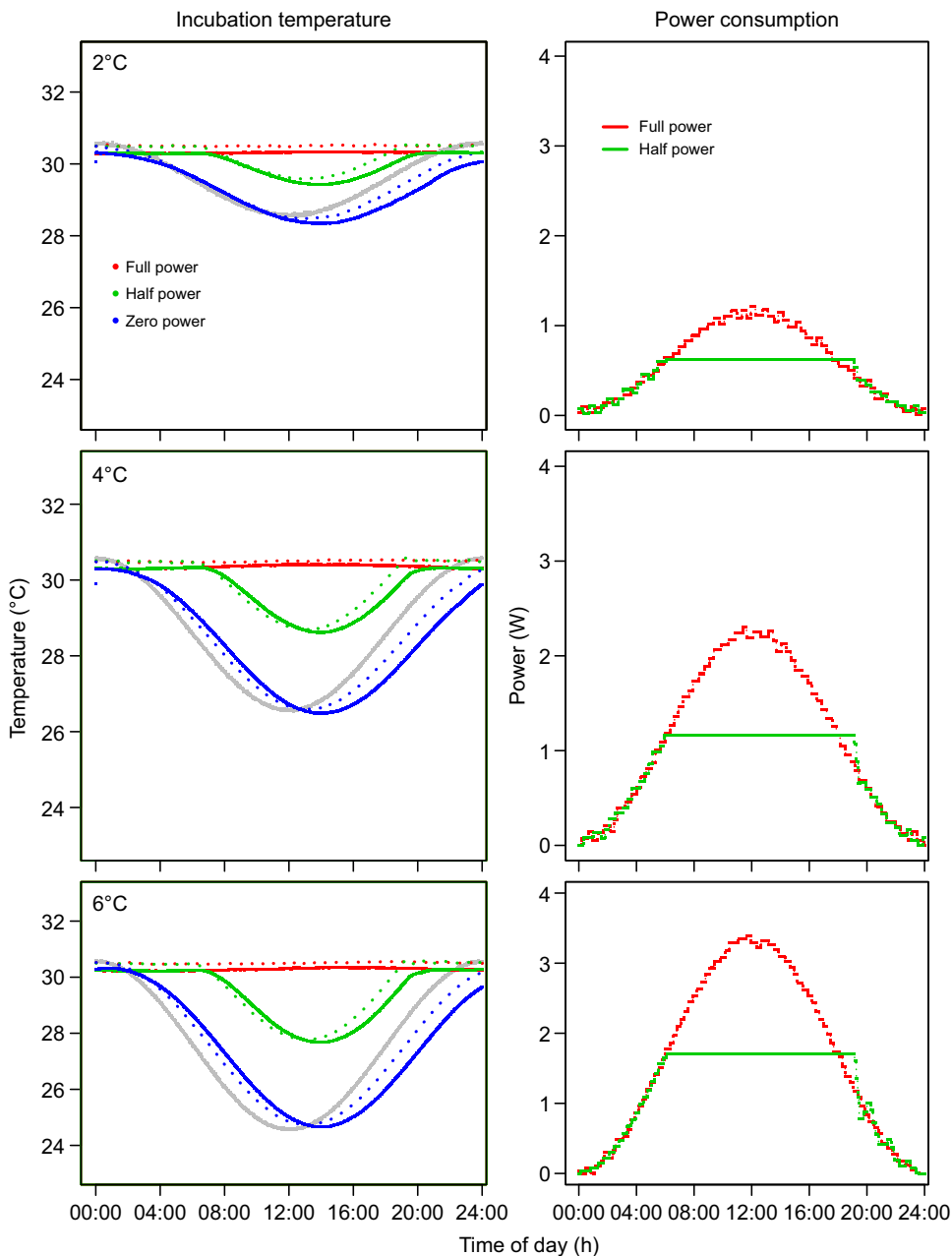
While endothermy buffers the developmental thermal environment from ambient conditions, the costs of endothermy are still highly sensitive to ambient conditions. Thus, even for facultatively endothermic brooders, nest site selection can be critically important for balancing the costs and benefits of endothermy. For the pseudoserpent, the cost of heating a 10-egg clutch for 48 days went from 188 g of fat (63% of body mass) for the 6°C diel cycle to 67 g of fat (22% of body mass) for the 2°C cycle.

In addition to the thermal variation of a nest, heat loss through conductivity also greatly influences the cost of endothermy (Tattersall, 2016). While reptiles do not possess an insulating coat (e.g. fur or feathers), it is plausible that an endothermic python could

reduce the energy costs of regulating the egg developmental environment by altering blood perfusion and directing greater peripheral flow to her body wall that faces her eggs, while reducing flow to the side away from the eggs. Alteration in blood flow is a well-documented component of thermoregulation in squamates (Porter and Witmer, 2015; Tattersall et al., 2016; Weathers, 1971), and peripheral flow adjustments associated with brooding remain unexplored and deserve attention.

While we could not evaluate changes in conductivity associated with altered perfusion, we could evaluate the effect of nest insulation on conductive heat loss, which assuredly would influence the cost of regulating the developmental environment (Farmer, 2001; Tattersall, 2016). The addition of a single layer of insulation during the 6°C diel cycle required 132 g of fat (44% of body mass), which is 30% less than the 188 g of fat calculated for our non-insulated trials. The thermal conductivity of the insulated nest environment we created for our experimental trials was 2.0±0.3 W °C<sup>-1</sup> m<sup>-1</sup>. In comparison, the thermal conductivity of soil can range widely, from 0.3 to 4 W °C<sup>-1</sup> m<sup>-1</sup> depending on the type of soil and the amount of moisture. For dry soil, the thermal conductivity is approximately 1 W °C<sup>-1</sup> m<sup>-1</sup> (Somerton, 1992), and a burrow with this conductivity would reduce the energetic costs of our model even further, requiring 98 g of fat (31% body mass) during a 48 day brooding period.

It is pertinent to note that convection within our test chamber, created by a small fan used to avoid thermal stratification, may have led to an overestimation of the energetic costs associated with brooding if the female nested in a location with minimal air flow. However, the natural nesting environments of *A. childreni* are currently unknown, and both facultatively endothermic species



**Fig. 3. Pseudoserpent and artificial clutch metrics over a 3 day trial.** Example of power consumed and temperature data collected during a single trial using a pseudoserpent model with variable power potential (4 W, half the maximum power required for clutch homeothermy, and no power) and variable thermal diel cycles (2, 4 and 6°C) set to maintain interface temperature (the temperature at the contact zone between the pseudoserpent and artificial clutch) at 30.5°C. Trials were conducted in triplicate at different thermal diel cycles (amplitude of 2, 4, or 6°C), and the pseudoserpent was programmed to have a heating potential of either full or half power. Plots on the left show the incubation temperatures of the artificial clutch ( $T_{\text{Clutch}}$ ; solid lines) and the interface between the pseudoserpent and clutch ( $T_{\text{Int}}$ ; dotted lines). Gray lines are air temperature. Plots on the right show the power consumed by the snake when it was provided full power potential and half power potential.

(*P. molurus* and *M. spilota*) have been reported brooding on the surface where convection could easily equal or exceed that present in our chamber (Ramesh and Bhupathy, 2010; Slip and Shine, 1988).

The tight brooding posture of *A. childreni* (Fig. 1) reduces clutch evaporative water loss 15-fold (Lourdais et al., 2007), but water loss still occurs from the female and her clutch, and the entropy of vaporization associated with this water loss contributes to heat loss from the brooding female. In a previous study, water loss from brooding females and their eggs was calculated to be, on average, 90.47 mg h<sup>-1</sup> (Lourdais et al., 2007), which would result in 7.24 g of water lost over an 80 h period. Over the 80 h experimental trials, the pseudoserpent and her artificial eggs lost an average of 8.1 g, all attributable to water loss. These similar values suggest an insignificant difference in heat loss due to vaporization between the natural and artificial systems.

Clearly, microhabitat selection, altered blood perfusion and behaviors such as intermittent basking (Slip and Shine, 1988) can reduce the cost of homeothermic endothermy, and these influences are especially important to small snakes, which have limited energy reserves to support endothermy. Even using such mechanisms to reduce costs, homeothermic endothermy might still be beyond the capability of some female pythons as they invest enormous amounts of fat and muscle reserves into the production of the clutch (Lourdais et al., 2013), and brooding occurs immediately following this investment.

The effect of the thermal developmental environment on offspring fitness is still being explored in pythons. Early studies showed that *P. molurus* eggs are sensitive to increases in  $\Delta T_{\text{Clutch}}$ , with survival rates quickly approaching zero when  $\Delta T_{\text{Clutch}} > 3^\circ\text{C}$  (Vinegar, 1973). In the ectothermic water python (*Liasis fuscus*), however, egg survival seems robust to daily temperature fluctuations (maximum

**Table 3. Variance of clutch ( $T_{\text{Clutch}}$ ) and interface temperature ( $T_{\text{Int}}$ )**

	Diel cycle/ clutch size	Power supplied	$T_{\text{Clutch}}$ variance (°C)	$T_{\text{Int}}$ variance (°C)
No insulation, 10-egg clutch	2°C	Full	0.001±0.001	0.000±0.000
		Half	0.105±0.008	0.123±0.005
		None	0.405±0.010	0.432±0.013
	4°C	Full	0.002±0.001	0.001±0.000
		Half	0.419±0.047	0.470±0.024
		None	1.601±0.048	1.722±0.028
	6°C	Full	0.005±0.005	0.001±0.000
		Half	0.983±0.067	1.091±0.013
		None	3.608±0.059	3.840±0.037
No insulation, 6°C diel cycle	5 eggs	Full	0.064±0.053	0.003±0.001
		Half	0.921±0.047	0.921±0.047
		None	3.276±0.241	3.276±0.241
	10 eggs	Full	0.040±0.011	0.004±0.001
		Half	0.989±0.072	0.922±0.069
		None	3.063±0.043	3.397±0.152
	15 eggs	Full	0.074±0.046	0.003±0.001
		Half	1.022±0.060	0.886±0.048
		None	3.136±0.055	3.424±0.038
Insulation, 6°C diel cycle	5 eggs	Full	0.104±0.079	0.023±0.012
		Half	0.920±0.081	0.896±0.075
		None	2.987±0.112	3.433±0.027
	10 eggs	Full	0.091±0.092	0.022±0.013
		Half	0.901±0.052	0.911±0.089
		None	2.866±0.141	3.223±0.176
	15 eggs	Full	0.064±0.013	0.019±0.009
		Half	0.691±0.544	0.639±0.540
		None	1.838±1.537	1.956±1.678

Power supplied was the thermogenic limit put on the pseudoserpent (see Materials and methods for details). We manipulated the amplitude of the diel cycle (2, 4 or 6°C) using a 10-egg clutch size, as well as clutch size (5, 10 or 15 eggs) using a 6°C diel cycle under both insulated and non-insulated conditions (see Materials and methods). All trials were conducted in triplicate and values are presented as means±s.e.m.

$\Delta T_{\text{Clutch}}=8^{\circ}\text{C}$ ), although higher developmental temperatures decrease the length of incubation (Shine et al., 1997), which can result in an increase in hatchling survival rate (Madsen and Shine, 1999).

The pseudoserpent was able to maintain an optimal environment for the artificial clutch with unlimited power, mimicking a thermogenic python capable of homeothermic endothermy. The fraction of time the clutch spent at suboptimal temperatures was greatly reduced, as were the mean  $\Delta T_{\text{Clutch}}$  (Table 2) and the variance of  $T_{\text{Clutch}}$  (Table 3). When the pseudoserpent was supplied with zero power, mimicking the ectothermic condition, the mean  $\Delta T_{\text{Clutch}}$  increased by approximately  $3^{\circ}\text{C}$ , and the maximum  $\Delta T_{\text{Clutch}}$  increased by approximately  $5^{\circ}\text{C}$  during the  $6^{\circ}\text{C}$  diel cycle (10-egg clutch; Table 2). Additionally, the clutch was below optimal developmental temperature 61–83% of the time (Table 2) and the variance of  $T_{\text{Clutch}}$  increased by approximately  $3.5^{\circ}\text{C}$  (Table 3).

As there is considerable overlap in the ranges of *A. childreni* and *L. fuscus*, it is likely that the egg development in *A. childreni* is similarly robust to the effects of temperature. Although pre-oviposition body temperature in a female has been shown to affect offspring fitness (Lorion et al., 2012), no data currently exist on the relationship between clutch temperature and offspring fitness in *A. childreni*. Even if the *A. childreni* eggs are as thermally sensitive as those of *P. molurus*, it seems highly improbable that the benefits to offspring fitness provided by a fully thermogenic female would outweigh the energetic costs to the female, even if these energetic costs were reduced environmentally. Thus, we conclude that the high thermal conductance of small females, combined with

their inability to store the large fat reserves that thermogenesis would require, prohibits them from being facultatively endothermic.

### Costs and benefits of limited thermogenesis

The reproductive model for the evolution of endothermy posits that thermogenesis was initially selected for the benefits it provided to the developing offspring (Farmer, 2000). In pythons, this would have entailed small increases in thermogenesis that provided sufficient offspring benefits to drive full facultative endothermy. As much of the debate regarding the reproductive model centers on how the costs of such limited thermogenesis would be offset by increases in offspring benefits (Angilletta and Sears, 2003; Farmer, 2003), we examined these costs when the maximum amount of power supplied to the pseudoserpent was reduced by half, mimicking a limited thermogenic state.

In the limited thermogenic state, the pseudoserpent spent more time drawing its maximum power. Thus, mean and total energy did not decrease by 50%, but decreased by around 30% across treatments. This reduced the total energy cost that an unlimited thermogenic, 600 g female would have to expend during 60 days under a  $6^{\circ}\text{C}$  diel cycle from 235 g of fat (39% body mass) to 165 g of fat (28% body mass). These costs were offset by substantial benefits to the clutch over the fully ectothermic state. Under a  $6^{\circ}\text{C}$  diel cycle, the maximum  $\Delta T_{\text{Clutch}}$  and the percentage of time the clutch spent below optimal developmental temperature decreased by 52% and 46%, respectively. The mean  $\Delta T_{\text{Clutch}}$  decreased by 58%, and the variance of  $T_{\text{Clutch}}$  decreased by 73%. This trend was repeated under the 2 and  $4^{\circ}\text{C}$  diel cycles. Thus, a limited thermogenic female under a  $2^{\circ}\text{C}$  diel cycle would lose only 62 g of fat (10% initial body mass) but still gain a 50–75% reduction in thermal costs.

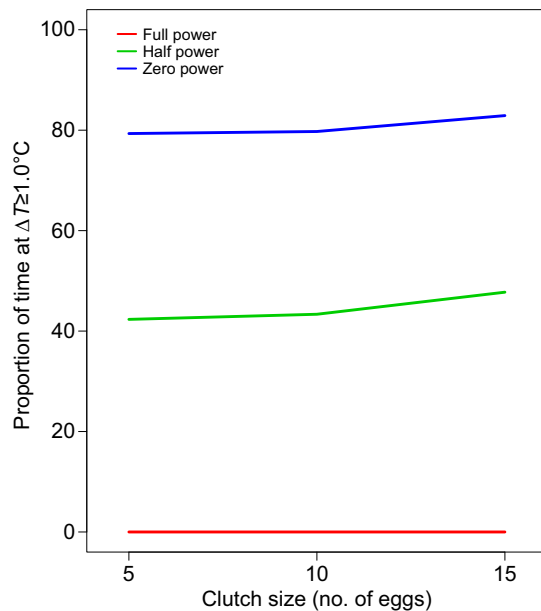
These results suggest that the evolution of limited thermogenesis might confer disproportionate benefits to the offspring, especially in the reduction of thermal variance, which can affect fitness (Bozinovic et al., 2011; DuRant et al., 2013; Shine et al., 1997). Although the high thermal conductivity of a small species would likely still make the energetic cost prohibitive, a full explanatory model must include the reduction in costs conferred by environmental variables (e.g. tropical environment, nest site selection). Our results also suggest that a female's maximum metabolic rate might be a constraint in the evolution of limited thermogenesis.

### Relationship of the model to the evolution of endothermy

Our results highlight several novel aspects of python endothermy. Unexpectedly, clutch size had no significant effect on any energetic or thermal metric except the percentage of time the clutch spent at optimal temperature ( $\Delta T \geq 1.0^{\circ}\text{C}$ ; Tables 1–3), with a large clutch size slightly depreciating the thermal environment (Fig. 4). This result suggests there might not be an increased energetic cost associated with the thermal inertia of larger clutches, a result supported by data from *P. regius* showing that energy expenditure from coiling does not increase even with a 50% increase in clutch size (Aubret et al., 2005). The depreciation of the thermal environment, however, might act as a selective force in determining species clutch size.

Previous work has indicated that brooding *P. molurus* regulate their own body temperature during facultative endothermy and only indirectly regulate clutch temperature (Brashears and DeNardo, 2013). Similarly, the pseudoserpent increased heat production in proportion to the magnitude of  $\Delta T_{\text{Int}}$ . As the thermocouples were in direct contact with the inner surface of the pseudoserpent,  $\Delta T_{\text{Int}}$  was presumably largely driven by the pseudoserpent's temperature. This



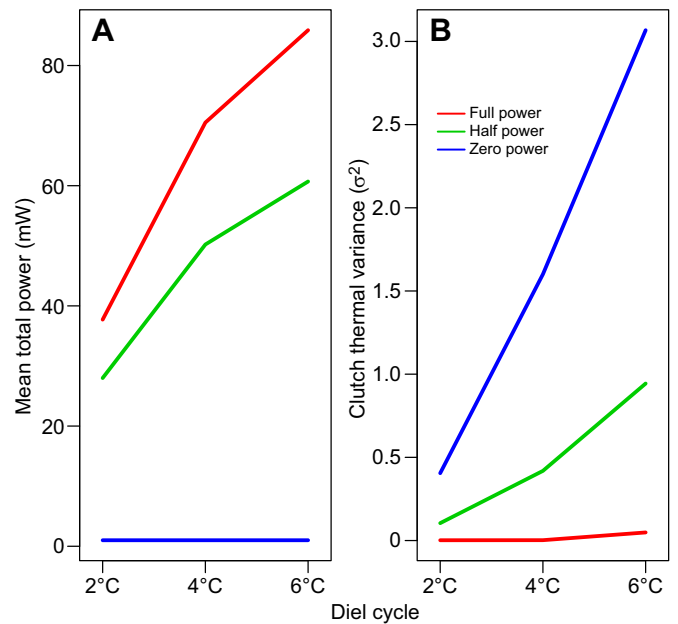


**Fig. 4. Proportion of time clutches spent at suboptimal temperature as a product of clutch size.** Suboptimal temperature was  $\Delta T \geq 1.0^\circ\text{C}$  from the preferred developmental temperature of  $30.5^\circ\text{C}$ . Supplied power was a covariate. A clutch size of 10 eggs reduces the amount of time a clutch experiences suboptimal temperatures when the pseudoserpent is supplied with power. Data are averages of three replicate trials.

had the counter-intuitive effect of reducing the benefits to the clutch when insulation was added (Table 2). Under the insulated condition, the pseudoserpent consumed less power (Table 1) to regulate its own temperature (Table 2), which ultimately decreased heat transference to the clutch. Thus, the presence of a nest environment during the evolution of endothermy would only have been adaptive if the lowered energetic costs were not offset by a depreciated nest environment.

As pythons are capital breeders, energy allocated to thermogenesis would likely come at a cost in the form of reduced clutch size. Although the exact composition of *A. childreni* eggs is unknown, lipids are responsible for 14% of the wet mass of eggs in *L. fuscus* (Speake et al., 2003). For a 15 g pseudoserpent egg, this would translate into a minimal cost of 77.7 kJ per egg. At the maximum diel cycle and without insulation, providing developmental homeothermy would cost almost two eggs (Table 1). In addition to lipids, eggs contain protein and other nutrients, and their production requires metabolic energy (Thompson and Speake, 2003). Therefore, this approximation may overestimate the relative costs. It would not be surprising if facultatively endothermic pythons had reduced clutch sizes relative to female body mass, a line of inquiry that is becoming more tractable as comparative reproductive data emerge.

Our results provide some insight into the costs and benefits that may have driven the evolution of endothermy. The mean power consumed by the pseudoserpent increased dramatically in response to increased diel cycle amplitude in both the limited and full thermogenic states (Fig. 5A). However, increasing diel cycles increased only the thermal variance of the clutch for pseudoserpents mimicking the ectothermic and limited thermogenic states, but not the full thermogenic state (Fig. 5B). An intuitive interpretation is that environmental temperatures could have driven the evolution of facultative endothermy by initially increasing offspring fitness through a reduction of clutch variance, but afterwards



**Fig. 5. Energetic costs to pseudoserpent and thermal benefits to clutch.** The mean total power used by the pseudoserpent (A) and the clutch thermal variance (B) during three 24 h diel cycles with different maximum amplitudes: 2, 4, 6°C. Each diel cycle was run in triplicate and averaged.

environmental temperatures would mainly select for a reduction of energetic costs (e.g. increased insulation). The achievement of lower energetic costs could then allow new selective pressures to dominate. As heterothermy is likely the ancestral condition for birds and mammals (Grigg et al., 2004), a scenario in which new selection pressures extended endothermy to other life stages is plausible.

In summary, our results demonstrate that pseudoserpent thermometry is a useful tool for investigating the evolution of python endothermy and it provides insight into the evolution of endothermy in general. Our model was able to quantify many of the energetic costs and thermal benefits associated with python brooding. Future research will benefit from the expansion of the model to facultatively endothermic python species to gain greater understanding of the role played by body size in the evolution of endothermy.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: D.F.D.; Methodology: T.C.M.H., D.F.D.; Software: T.C.M.H.; Validation: T.C.M.H., D.F.D.; Formal analysis: J.A.B.; Investigation: J.A.B.; Writing - original draft: J.A.B.; Writing - review & editing: J.A.B., T.C.M.H., D.F.D.; Funding acquisition: D.F.D.

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