Forces driving thermogenesis and parental care in pythons

by

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ABSTRACT

Parental care provides many benefits to offspring. One widely realized

benefit is enhanced regulation of offspring's thermal environment. The

developmental thermal environment during development can be optimized

behaviorally through nest site selection and brooding, and it can be further

enhanced by physiological heat production. In fact, enhancement of the

developmental thermal environment has been proposed as the initial driving force

for the evolution of endothermy in bird and mammals. I used pythons (Squamata:

Pythonidae) to expand existing knowledge of behavioral and physiological

parental tactics used to regulate offspring thermal environment. I first

demonstrated that brooding behavior in the Children's python (*Antaresia*

childreni) is largely driven by internal mechanisms, similar to solitary birds,

suggesting that the early evolution of the parent-offspring association was

probably hormonally driven. Two species of python are known to be facultatively

thermogenic (i.e., are endothermic during reproduction). I expand current

knowledge of thermogenesis in Burmese pythons (*Python molurus*) by

demonstrating that females use their own body temperature to modulate

thermogenesis. Although pythons are commonly cited as thermogenic, the actual

extent of thermogenesis within the family Pythonidae is unknown. Thus, I

assessed the thermogenic capability of five previously unstudied species of

python to aid in understanding phylogenetic, morphological, and distributional

influences on thermogenesis in pythons. Results suggest that facultative

thermogenesis is likely rare among pythons. To understand why it is rare, I used

an artificial model to demonstrate that energetic costs to the female likely outweigh thermal benefits to the clutch in species that do not inhabit cooler latitudes or lack large energy reserves. In combination with other studies, these results show that facultative thermogenesis during brooding in pythons likely requires particular ecological and physiological factors for its evolution.

ACKNOWLEDGMENTS

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Chapter 1: Summary

INTRODUCTION

Parental care is used by a wide variety of taxa to increase offspring fitness

(Clutton-Brock, 1991). The specifics of the parental care provided vary widely,

from female earwigs removing harmful fungal spores from their eggs (Lamb,

1976) to the laborious and protracted care exhibited by anthropoid primates

(Altmann, 1987). Parental care is important for understanding the evolution of life

history trade-offs as it often reduces the energy parents can invest in their own

growth, future reproduction, and survival (Trivers, 1972; Stearns, 1976). Parental

care is often most important during early development because this period can

cause the greatest increases in offspring fitness (Lumma and Clutton-Brock,

2002).

Temperature regulation during early development is a common feature of

parental care in terrestrial animals. Vertebrate embryos require a stable, relatively

high temperature for optimal development, with suboptimal temperatures

decreasing multiple metrics of fitness: offspring growth (Braña and Ji, 2000),

performance (Ashmore and Janzen, 2003), survival (DuRant et al., 2010),

morphology (Shine and Harlow, 1996), and behavior (Sakata and Crews, 2003).

Oviparous species have thus evolved multiple mechanisms for regulating

incubation temperature (Deeming and Ferguson, 1991).

The nest is a critical aspect of the oviparous developmental environment

(Walsberg, 1981; Shine, 2004), and its thermal properties factor into many

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species' choice of nest site (insects: Jones and Oldroyd, 2007; birds: Zerba, 1983; turtles: Wilson, 1998; lizards: Warner and Andrews, 2002). Nest site selection can affect both the mean and variance of incubation temperature (Walsberg and King, 1978a; De Souza and Vogt, 1994), and is thus able to have a substantial effect on offspring quality: size (Packard and Packard, 1988), performance (Van Damme et al., 1999), and survival (Resetaris, 1996). The insulation properties of the nest are responsible for much of its beneficial qualities (Walsberg and King, 1978b; Lombardo et al., 1995), and while the nest is the most common form of insulation, some parents provide insulation behaviorally (e.g., huddling, brooding) (Jouventin, 1975; Heinrich, 1981).

In addition to behavioral mechanisms for regulating the developmental thermal environment, many oviparous species provide heat to the eggs. Birds and some insects use physiological mechanisms to warm their eggs. Birds combine a high basal metabolic rate with a highly vascularized and poorly insulated incubation patch that provides an effective surface for the conductance of body heat to the eggs (Jones, 1971), increasing the rate of heat transfer through the patch when eggs are cool (Korhonen, 1991). Bees (Apidae) and wasps (Vespidae) use asynchronous muscular contractions to generate heat (Seeley and Heinrich, 1981). Animals without the physiological means of heating their eggs can still provide heat behaviorally by leaving their eggs to bask and then returning the acquired heat to the clutch (Shine, 2004).

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PYTHONIDAE

Pythons (Squamata: Pythonidae) have been demonstrated to be useful models for the study of thermoregulatory benefits associated with parental care for several reasons (Stahlschmidt and DeNardo, 2010). They exhibit female-only parental care, which is the most common form of parental care (Grossman and Shine, 1981). Pythons also brood their eggs with limited movememnt, making them easy to monitor in the laboratory, and they exhibit dynamic, quantifiable behaviors (Stahlschmidt and DeNardo, 2009). Finally, a range of thermoregulatory processes occurs among species within the family, making it possible to correlate the thermoregulatory efforts and capabilities with organismal and ecological variables.

All species of python are oviparous and females brood their eggs after ovipostion (Stahlschmidt and DeNardo, 2010). Python eggs are particularly susceptible to the effects of temperature. When eggs are incubated at suboptimal temperature ($< 30°C$), offspring show increased developmental abnormalities, reduced post-hatching growth, reduced survivorship, and smaller size (Burmese python (*Python molurus*): Vinegar et al., 1973; diamond python (*Morelia spilota spilota*): Harlow and Grigg, 1984; water python (*Liasis fuscus*): Shine et al., 1997).

Female pythons use nest site selection to enhance the thermal environment (Slip and Shine, 1997) and provide supplemental heat to their clutch by basking (Alexander, 2007). Optimizing the thermal benefits of the nest, however, can

incur other costs. For example, female water pythons (*L. fuscus*) that choose

burrow nests that have decreased thermal conductance and low convection also

have higher risks of varanid predation (Madsen and Shine, 1999). Females also

modify their coiling behavior and thus their thermal conductance in response to

environmental temperatures (Stahlschmidt and DeNardo, 2009). In addition to

these behavioral thermoregulatory mechanisms, some species physiologically

thermoregulate their clutch through facultative thermogenesis (Stahlschmidt and

DeNardo, 2010).

Facultative thermogenesis in pythons has been the subject of controversy

for almost 200 years. In 1832, the French scientist Lamarre-Piquot issued a report

of shivering behavior in a single Burmese python that included his belief that the

female was producing heat (Dowling, 1960). Although the French Academy

rejected his report as "false and hazardous," subsequent studies in the last 50

years have demonstrated conclusively that Burmese pythons heat their clutches up

to 7.2°C above ambient temperature (Dowling, 1960; Hutchison et al., 1966; Van

Mierop and Barnard, 1978).

Facultative thermogenesis by pythons during brooding has been used to

support the reproductive model of the evolution of endothermy (Farmer, 2000).

This model posits that endothermy evolved through two steps (Farmer, 2000).

First, females developed limited endothermy that enabled them to enhance the

developmental environment of the offspring. Second, females further enhanced

their metabolic rate to support increased foraging behavior to enhance energy

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provisioning to the offspring. While the reproductive model is the only model that provides a stepwise explanation for the evolution of endothermy, it fails to recognize that this model requires a critical pre-step. In order for limited endothermic potential to benefit the offspring, the female and offspring must have an extended interaction. Since it is unlikely that such an association would have evolved in tandem with thermoregulation, there must exist a pre-existing relationship where female attendance provides benefits to the offspring independent of endothermy. While previous studies have demonstrated such benefits of egg brooding in pythons (e.g., clutch water balance and thermoregulation via female-modulated adjustments in clutch insulation), no data currently exist on the cues that initiate and maintain brooding in pythons. In Chapter 1, I present the results of an investigation of brooding cues in the Children's python (*Antaresia childreni*). These results show that females are similar to solitary birds, being driven to brood largely by internal mechanisms.

In Chapter 2, I expand on existing literature on Burmese python facultative thermogenesis and the thermal cue females use when thermoregulating. Similar to previous studies, I found that the rate of muscular contractions and the metabolic rate increased with decreasing temperature until reaching a maximum at ~25°C. Interestingly, I found that females seemed to be cueing on their own body temperature, which could have detrimental consequences to the offspring as the clutch temperature becomes increasingly uncoupled from the female's body temperature. Thus at the lowest temperature, a

female's clutch experienced an environment almost three degrees cooler than her body temperature.

While knowledge of facultative thermogenesis in Burmese pythons is growing, little is known about other species of pythons. While facultative thermogenesis is widely attributed to pythons in general, brooding thermogenesis of pythons other than Burmese pythons has received little attention. Pythons are basal, old world snakes that vary dramatically in size, ranging from the 0.6 m pygmy python (*A. perthensis*) to the 10 m reticulated python (*P. reticulatus*). The 33 species (Rawlings et al., 2008) are divided into two major groups, the Afro-Asian pythons that range from western Africa to southern Asia and the Indo-Australian pythons that range from northern Indonesia to southern Australia. Facultative thermogenesis has only been confirmed in one other species, the diamond python (*Morelia spilota spilota*), while its absence has been documented in seven species (Stahlschmidt and DeNardo, 2010). Data from additional species is gravely needed to better understand phylogenetic, morphological, and ecological attributes that lead to thermogenic capability. Data are completely lacking on four of the nine major phylogenetic branches that comprise the family (Rawlings et al., 2008). Chapter 3 presents the results of an investigation into facultative thermogenesis in six species of python chosen to represent key branches within the family. The results suggest that python facultative thermogenesis is likely rare within the Pythonidae in that only one of the six species showed any indication of thermogenic capability during brooding.

These results beg the question of why python thermogenesis is so rare, given the substantial benefits it is assumed to provide offspring (Vinegar, 1973; Shine et al., 1997). This question is difficult to investigate in living animals; many species are unavailable and there is no way to induce or shutdown thermogenesis of brooding females. Thus, Chapter 4 investigates the energetic demand and thermal consequences of varying brooding conditions using an artificial snake and egg system, in which the artificial snake's degree of thermogenesis is experimentally set. The results show that thermogenesis is probably prohibitive in small snakes due to their low insulation, but that nest site selection can significantly increase the benefits of thermogenesis. Additionally, thermogenesis, clutch size, and nest site selection have complex effects on the mean and variance of incubation temperature, both of which affect offspring fitness (Shine et al, 1997; Angilletta and Sears, 2003).

In sum, my dissertation provides considerable insight into understanding facultative thermogenesis of pythons and thus better understanding potential driving forces for the evolution of endothermy. Future work can build on my dissertation by focusing on the internal mechanisms regulating python brooding behavior and the ecological factors that explain the distribution of python thermogenesis. Future work that focuses on the hormonal mechanisms responsible for facultative thermogenesis should also prove valuable.

Chapter 2: Do brooding pythons recognize their clutches? Investigating external cues for offspring recognition in the Children's python, *Antaresia childreni*

SUMMARY

Parental care provides substantial benefits to offspring but exacts a high cost to parents, necessitating the evolution of offspring recognition systems when the risk of misdirected care is high. In species that nest, parents can use cues associated with the offspring (direct offspring recognition) or the nest (indirect offspring recognition) to reduce the risk of misdirected care. Pythons have complex parental care, but a low risk of misdirected care. Thus, we hypothesized that female Children's pythons (*Antaresia childreni*) use indirect cues to induce and maintain brooding behavior. To test this, we used a series of five clutch manipulations to test the importance of various external brooding cues. Contrary to our hypothesis, we found that female *A. childreni* are heavily internally motivated to brood, needing only minimal external cues to induce and maintain egg brooding behavior. Females were no more likely to brood their own clutch in the original nest as they were to brood a clutch from a conspecific, a pseudo-clutch made from only the shells of a conspecific, or their clutch in a novel nest. The only scenario where brooding was reduced, but even then not eliminated, was when the natural clutch was replaced with similarly sized stones. These results suggest that egg recognition in pythons is similar to that of solitary-nesting birds, which have similar nesting dynamics.

INTRODUCTION

Parental care provides substantial benefits to offspring in the forms of energy

provisioning, thermal regulation, water balance, and protection (Clutton-Brock

1991; Deeming 2004), and such offspring benefits have led to parental care being

widespread across taxa. However, costs to the parent are substantial, making it

critical for the parent to identify its offspring (Waldman 1988). This is

particularly important, since parents often leave their offspring to return at a later

time (e.g., foraging behavior). When offspring are nest bound, parents can use

cues from either the offspring (direct offspring recognition) or from the nest

environment (indirect offspring recognition) to correctly allocate care.

The evolution of offspring recognition is driven by two nesting scenarios

(Huang & Pike 2010). Direct offspring recognition is expected to occur in species

with a high risk of misdirected care, such as exists in colonial nesting species

(Medvin & Beecher 1986; Pitcher et al. 2012) or species with high rates of nest

parasitism (Stokke et al. 2007). When such selection pressures are weak or absent,

indirect offspring recognition is expected to predominate, as it does in solitary

nesting birds (Waldman 1988), fish (Perrone Jr. & Zaret 1979), and amphibians

(Stynoski 2009). Each type of offspring recognition may entail a different set of

proximate mechanisms (i.e., external and internal cues) regulating the behavior.

External cues that induce and maintain parental care may be visual

(Underwood & Spencer 2006), auditory (Illmann et al. 2002), chemical (Reebs &

Colgan 1992), or a combination of these (Wolski et al. 1981). In direct

recognition systems, these cues may be produced by the offspring or applied to the offspring by the parent (Gubernick 1980), but parents utilizing indirect recognition systems often rely on features of the nest environment (Waldman 1987). However, regardless of the source, external cues are likely supplemental to strong internal stimuli to provide parental care (Rothstein 1975; Peterson 2000).

While post-paritive (i.e., after oviposition or birth) parental care is atypical of reptiles, it has been documented in numerous species (reviewed in Greene et al. 2002; Somma 2003; Stahlschmidt & DeNardo 2010). Where it does occur, little is known about offspring recognition. The few existing studies on the subject, focusing on scincid lizards, suggest that indirect offspring recognition is important in reptile parental care (Vitt & Cooper, Jr. 1989; Huang & Pike 2011). Pythons provide the most complex form of post-paritive parental care described among reptiles in that females tightly coil around their clutch typically until hatching. During this time, pythons use behavioral adjustments to influence embryonic temperature, hydration, and respiratory gas exchange (Aubret et al. 2005; Stahlschmidt & DeNardo 2008; Stahlschmidt et al. 2008, 2010).

While a considerable amount of information has been gathered regarding these physiological trade-offs, little is known about the proximate mechanisms that regulate these parental behaviors, including recognition of the offspring. Brooding pythons may periodically leave their clutch temporarily to thermoregulate (Shine 2004; Stahlschmidt, pers. comm.), thus necessitating some degree of clutch recognition. However, since python nests are solitary and the

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offspring are immobile during this time, there is minimal risk of a female python

misdirecting her care. We hypothesized that female pythons possess a strong drive

to coil on their clutches, and that clutch recognition would be predominantly

indirect. That is, as with many solitary-nesting birds, female pythons would

recognize their nesting site, but have limited ability to distinguish specifics of

their clutch.

We explored the extent to which post-oviposition Children's pythons

(*Antaresia childreni*) recognize their clutches by performing a series of

experimental clutch-nest substitutions. We predicted that an altered nest site

would reduce or eliminate brooding behavior, but that a female would not be able

to differentiate her clutch from other similar stimuli (e.g., another female's

clutch).

MATERIALS AND METHODS

Husbandry

We used reproductive female Children's pythons (*Antaresia childreni,* n = 7)

from a captive colony at Arizona State University (ASU). *Antaresia childreni* are

medium-bodied (< 1 m; 500 g) snakes native to rocky areas in the wet-dry tropics

of northern Australia (Wilson & Swan 2008). Females brood their clutches (egg

count = 8 ± 1 eggs, clutch mass = 83.3 ± 8.4 g, Stahlschmidt et al. 2011)

throughout incubation (approximately 50 days). While brooding, females adjust

their posture frequently and exhibit exploratory behavior (Stahlschmidt et al.

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2008), and it is thought that they periodically bask as other pythons have been documented to do in natural environments (Slip & Shine 1988). Towards the end of gravidity (approximately the last 10 days), females were maintained in 1.9 L cylindrical brooding containers supplied with hydrated air within an environmental chamber maintained at $30.5 \pm 0.3°C$ (approximating the species' preferred incubation temperature; Lourdais et al. 2008) and a 14:10 L:D photoperiod. Females oviposited within the brooding container.

Experimental substitutions

Female *A. childreni* began their experimental trials within two days of oviposition. During the trials, females were maintained in a temperature controlled ($31.5 \pm 1°C$) chamber with the lights off. Each female was put through a series of five trials in random order, with one control trial and four trials in which one variable in the nest-clutch environment was substituted. To make a substitution, the female was temporarily removed from her nesting container and placed in a holding container while the manipulation was being made. Each substitution was completed within 2 min of opening the brooding female's nesting container. Trials were conducted in a temperature controlled chamber ($31.5 \pm 1°C$) with the lights off and lasted 8 h. During the trial, the female's behavior was recorded using infrared cameras and time lapse videography (Ganz CTR-030NC-2 Infrared Camera, CBC Corp., Torrance, CA; SSC-960 VHS VCR, Samsung, Seoul, Korea). After each trial, the female was placed with her clutch in her

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original nesting container, and she was allowed to brood undisturbed for at least 24 h. Behavior was categorized as either brooding (coiled around the clutch with little to no movement) or exploratory (>1/3 of the female's body moving). For each trial, we recorded the time until the female began brooding and the total time spent brooding. Each trial is described below.

Replacement of the clutch with a conspecific clutch

The clutch of the female in the trial was replaced with a random clutch from a non-study female that had laid within five days of the female in the trial. The conspecific clutch was removed from its female immediately prior to the substitution and placed within the trial female's nest container in approximately the same position as the original clutch. All females were able to achieve a tight coil around conspecific clutches despite differences in clutch size (2.1 ± 0.6 eggs; maximum difference = 5 eggs) and mass (33.4 ± 9.9 g; maximum difference = 63 g).

Replacement of the clutch with an odor-cleansed pseudoclutch

A pseudoclutch was prepared from a clutch of a female not included in these trials. A small opening ($\sim$1 cm^2) was made in each egg, and the contents were drained without altering the shape of the clutch. The empty shells were then washed twice in distilled water (dH$_2$O) for 5 min, followed by a 5 min hexane wash (Mallinnckrodt Baker Inc., Paris, KY), and then another dH$_2$O wash for 3

min. Sterilized forceps were then used to fill each wet eggshell with thin cotton strands (Safeway 100% pure Jumbo Cotton Balls, Safeway Inc., Pleasanton, CA) that had been soaked in a 70% mixture of Plaster of Paris (DAP Inc., Baltimore, MD), and the entire clutch was dried at 58°C for 3 h (Isotemp Oven Model 203, Fisher Scientific, Pittsburgh, PA). For the trials, the female's clutch was replaced with the pseudoclutch as described for the conspecific clutch replacement. The same pseudoclutch was used for all females, but, between uses, the pseudoclutch was rinsed for 30 sec in hexane, followed by 30 sec in dH_2O, and then dried.

Replacement of the clutch with a stone clutch

The stone clutch was intended as a negative control, to provide an object similar in shape to a clutch but without any chemical or tactile cues inherent to actual eggs beyond the general shape. The stone clutch was prepared by selecting six smooth stones that were approximately the same dimensions as *A. childreni* eggs. They were washed for 5 min in dH_2O, followed by a 5 min hexane wash, and another 5 min wash in dH_2O. They were cemented into a clutch formation using a 70% mixture of Plaster of Paris. For the trials, the natural clutch was replaced with the stone clutch as described for the conspecific clutch replacement. To control for shape, the same stone clutch was used for all females, but, between uses, the stone clutch was rinsed for 30 sec in hexane, followed by 30 sec in dH_2O, and then dried.

Control manipulation

As a positive control, the brooding female underwent manipulations similar to those described for the clutch replacement trials, but, in this case, the female's clutch was simply handled and returned to the nesting container, and then the female was returned to the nesting container.

Statistical analysis

Statistical analyses on behavioral data were performed using GraphPad Prism vers. 4 (GraphPad Software Inc., San Diego, California). Percent data were arcsine transformed. Comparisons among treatments were made using repeated measures analysis of variance (rmANOVA) with the level of statistical significance set at $\alpha < 0.05$. Data are presented as mean $\pm$ SEM.

RESULTS

All females brooded to some extent in all trials. In the positive control trials, females quickly initiated brooding around their clutches (1.7 ± 0.5 min), while females took significantly longer to initiate brooding around the stone clutch (34.3 ± 18.5 min; $F_{4,24} = 1.64$, p = 0.035; Fig. 2.1a). The times it took females to initiate brooding in a new container (9.2 ± 2.5 min), on another female's clutch (6.2 ± 2.3 min), and on the pseudoclutch (8.6 ± 4.4 min) was not significantly different than the time it took to initiate brooding of their own clutch in their original container.

Similarly, females in their original container and with their own clutch spent the vast majority of time (93 ± 3 %) brooding their eggs and spent significantly less time brooding the stone clutch (43 ± 16 %; $F_{4,24} = 4.34$, p = 0.009; Fig. 2.1b). The percent of time that a female spent brooding in a new nest (73 ± 13 %), on another female's clutch (78 ± 11 %), and on the pseudoclutch (70 ± 14 %) was not significantly different than the time the female spent on her own clutch in her original container.

DISCUSSION

Female Children's pythons demonstrated a very limited ability to distinguish their clutch from other clutches. In fact, only the replacement of her clutch with similarly shaped stones significantly reduced, but still did not eliminate, brooding efforts (Fig. 2.1). Females also showed no reduction in brooding effort in a new nest environment. These results suggest that external cues for clutch recognition are of limited importance in Children's python brooding behavior. Instead, it is likely that strong internal cues (presumably hormonal) provide a resilient drive to brood and only minor external cues are needed to initiate and maintain brooding behavior, at least for the short duration used in this study.

Although tactile cues are likely important, this experiment cannot eliminate the possibility that females are providing the clutch and/or nest with secretions from their skin. During early reproduction, female garter snakes (*Thamnophis sirtalis*) produce skin secretions that attract males, stimulate male sexual behavior,

and enable males to trail them (Mason et al. 1990; LeMaster & Mason 2001). It is possible that skin secretions continue to be produced post-parturition, but while skin secretions may aid in maintenance of brooding or returning to brood a clutch, it cannot explain a female's willingness to brood an artificial clutch that she had never been exposed to previously.

The lack of a reduction in brooding after changing the nesting container suggests that the nest does not provide an indirect cue. However, this experiment cannot eliminate the possibility that some spatial aspect of the nest environment acts as a brooding cue because all containers had the same dimensions. It is possible that females use some form of spatial orientation to familiarize themselves with their original nest environment. Such a spatial cue may be important for females navigating back to a dark subterranean nest after basking. Alternately, brooding may result from multiple cues such that the presence of her clutch was sufficient to stimulate brooding despite the loss of an indirect cue from her nest environment. It would have been informative to utilize an additional treatment group where females were presented with a conspecific clutch in a novel nest container, as such a manipulation would replace both direct and indirect cures from the female's brooding environment.

The type of parent-offspring recognition a species uses suggests different evolutionary pathways (Huang & Pike 2010). Indirect offspring recognition in a species may imply that parental care was driven by nest-site defense. For example, python parental care could have initially consisted of simply lying in

close proximity to the clutch to deter predation. In this scenario, females would have originally cued on features within the nest environment and maintained this cue as the behavior became more complex and associated with offspring development. Our results are interesting as they suggest that at some point female *A. childreni* may have transferred the brooding cue from the nest environment directly to the clutch, perhaps as their parental care became more associated with physiological benefits (e.g., hydric). Additionally, with this increase in parental care complexity, females developed strong internal cues to motivate them to brood with only limited external stimuli.

In summary, our results suggest that offspring recognition in pythons is similar to that of solitary-nesting birds, at least during the egg-brooding phase. In both cases, females care for immobile offspring that are isolated from other conspecific offspring. Additionally, in both cases albeit more so in birds, the female periodically leaves her brood. Given these similar nesting dynamics, it is not surprising that both taxa show strong drives to brood with only limited ability to specifically recognize their eggs. As a result, these parents are vulnerable to misidentifying their offspring if the general nesting conditions are maintained. Nest parasitism has been well-documented in birds (Payne 1977). Nest parasitism has not been documented in snakes and is unlikely under natural conditions due to the fact that snakes oviposit their entire clutch at one time. However, female pythons readily accept eggs that are experimentally added to alter clutch size

(Aubret et al. 2003). Given the limited power of external cues, future studies

should investigate internal cues that might drive brooding behavior in pythons.
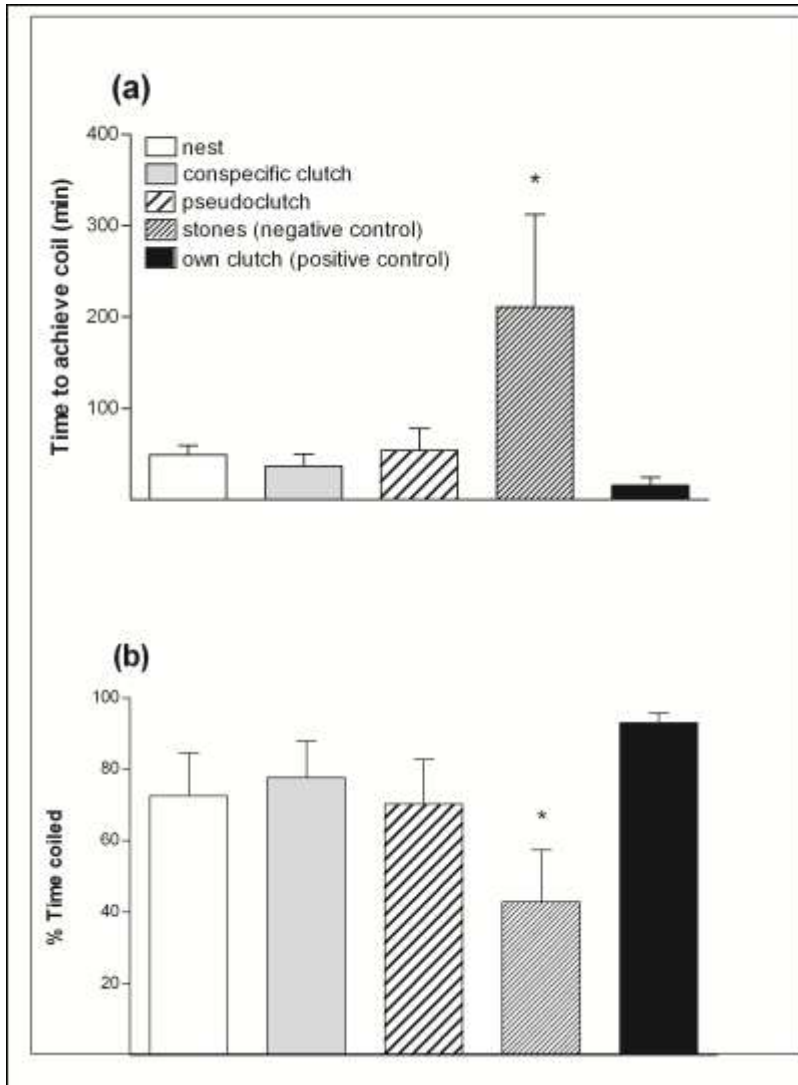
Fig. 2.1. Effects of treatment on brooding female Children's pythons (*Antaresia childreni*). Female Children's pythons (n = 7) (a) took longer to achieve an initial coil ($F_{4,24} = 1.64$, p = 0.035) and (b) spent significantly less time brooding ($F_{4,24} = 4.34$, p = 0.009) the stone clutch, but there was no significant difference among any of the other nest-clutch substitution. Asterisks indicate statistical differences using rmANOVA at a significance level of p = 0.05. Values are presented as mean ± SEM.

Chapter 3: Revisiting python thermogenesis: brooding burmese pythons

(*Python molurus*) cue on body, not clutch, temperature

SUMMARY

Previous studies have shown that brooding Burmese pythons, *Python molurus*, use endogenous heat production to buffer clutch temperature against sub-optimal 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, as well as muscle twitch rate and metabolic rate to evaluate the buffering capability of brooding thermogenesis as well as the thermal cues regulating thermogenesis. We found that, as expected, both muscle twitch rate and metabolic rate were negatively correlated with nest temperature. Furthermore, at nest temperature 6 °C below optimal developmental temperature, females maintained body temperature at the optimal temperature. However, while thermogenesis significantly increased clutch temperature, clutch temperature decreased with decreasing nest temperature. Our results confirm previously reported general patterns of facultative thermogenesis and, in addition, strongly suggest that females use core body temperature to regulate their thermogenic activity.

INTRODUCTION

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 better understanding 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). Post-oviposition, 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 sub-optimal for development (Van Mierop and Barnard, 1978; Slip and Shine, 1988), with most studies focusing on the Burmese python (*Python molurus*).

Previous studies agree on the general attributes of facultative thermogenesis in brooding *P. molurus*. 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. molurus* 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 was curvilinear and found females had an elevated metabolic rate and 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 fitness (Shine et al., 1997) and could indicate a role for melatonin in the behavior's regulation (reviewed by Lutterschmidt et al., 2008). These discrepancies challenge attempts to synthesize data and thus better understand

facultative thermogenesis in brooding pythons, particularly since all studies to date have relied on a single brooding female to generate most of the data.

We thus initiated a study of *P. molurus* 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 body temperature (T_b), $T_{\Delta cl}$, R_{tw} and carbon dioxide production ($\dot{V}_{CO_2}$) during brooding at three temperatures within the reported thermogenic range. Additionally, we wanted to directly measure T_b internally, something previously not done, and assess the presence of a diel cycle for each of the variables.

MATERIALS AND METHODS

Animals and maintenance

We used three gravid female *P. molurus* (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 surgically implanted with a small (~3 g) temperature logger (iButton, Maxim, USA) during early gravidity. The logger was programmed to record temperature every 45 min. Approximately one week before oviposition, the

gravid female was weighed and moved to a respirometry unit housed in a dark environmental chamber maintained at 31.5 ± 0.3 °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 air tight. 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 though 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 ($T_{\Delta x} = T_x - T_{env}$).

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 one hour before and after brooding by diverting supply air directly to the $CaSO_4$. During her first trial, female #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 post-experiment female mass. Using the same respirometry system, we collected non-brooding respirometric data from each female three 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^{-1}) were calculated by randomly selecting three non-overlapping 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 the R software package (R Development Core Team, 2011). Treatment results were analyzed using rmANOVA. In order 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 ± SEM, 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. 3.1A). Although the temperature differential was small (1.6 ± 0.1 °C; Fig. 3.1B) at the highest T_{env} (31.5 °C), the low variance in T_b and T_{env} data resulted in a statistically significant difference ($t(94) = 7.93$, $P < 0.0001$). 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 body temperature relatively constant, T_{cl} decreased proportionally to T_{env} ($F(2, 4) = 10.34$, $P = 0.026$; Fig. 3.1A); however, T_c at 31.5 °C was 32.7 ± 0.1 °C, closely approximating T_b. Although female mass and clutch size varied considerably (Table 3.1), 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. 3.1A).

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. 3.2). Linear regression analysis showed that R_{tw} had a significant negative slope ($\beta = -0.22$, $F(1, 7) = 12.46$, $P = 0.0095$) and 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 3.1), both of which had similar mass specific metabolic rates that followed an endothermic pattern (Table 3.1, Fig. 3.3), 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. 3.4), but statistical analyses revealed no significant hourly differences in mean $\dot{V}_{CO_2}$, mean R_{tw}, T_b, or T_{cl}. When measured three months post-brooding, females were not able to maintain a thermal differential with their environment. Non-reproductive females did not coil when placed into their previous brooding containers, and twitches were not present during any of the post-reproductive trials. Mean Q_{10} for non-reproductive females was 4.09 ± 0.06.

DISCUSSION

Our results confirm the general previously documented pattern of thermoregulation in brooding *P. molurus*. Our calculated temperature differentials were within the reported range, and, at our highest T_{env}, females maintained a $T_{\Delta b}$ of approximately 1 °C, supporting the most commonly reported brooding female preferred temperature of 33 °C (Hutchison et al., 1976). 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^{-1}-hr^{-1}, Q_{10} = 0.26: Hutchison et al., 1966; $\dot{V}_{O_2}$ = 154 ml-kg^{-1}-hr^{-1}, Q10 = 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 previously reported values (42 twitches-min^{-1}: Hutchison et al., 1966; 35 twitches-min^{-1}: 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 true difference in contraction rates. When females are brooding, two types of twitches can be observed: 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^{-1}.

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} significantly decreased with decreasing experimental temperature likely due to enhanced conductive heat loss (Fig. 3.1). This relationship 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 and delayed hatching up to 20 days and resulted in a significant decrease in recapture success (Shine et al., 1997), most likely due to decreased prey acquisition by the hatchlings (Madsen and Shine, 1998). Few studies have examined these effects in *P. molurus*, but *P. molurus* 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. 3.1), 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 non-shivering thermogenesis. Similar to all previous studies, we found a high correlation between R_{tw} and V_{CO_2} ($r^2 = 0.91$) despite our differences in R_{tw} compared to previous studies. This correlation has been used to argue against the presence of non-shivering 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. 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. molurus*. 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. 3.4), 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. 3.4) 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.

Table 3.1. Burmese python (*Python molurus*) female and clutch metrics. Body mass, snout-vent length (SVL), and clutch data for female Burmese pythons used in the experiment.

Python (ID #)	SVL (m)	Prelay mass (kg)	Postlay mass (kg)	Clutch mass (g)	Fertile eggs (#)	Total eggs (#)
1	2.6	11.8	7.3	2078	16	24
2	3.2	25.5	16.3	6108	39	42
3	3.0	20.9	14.5	5110	37	37
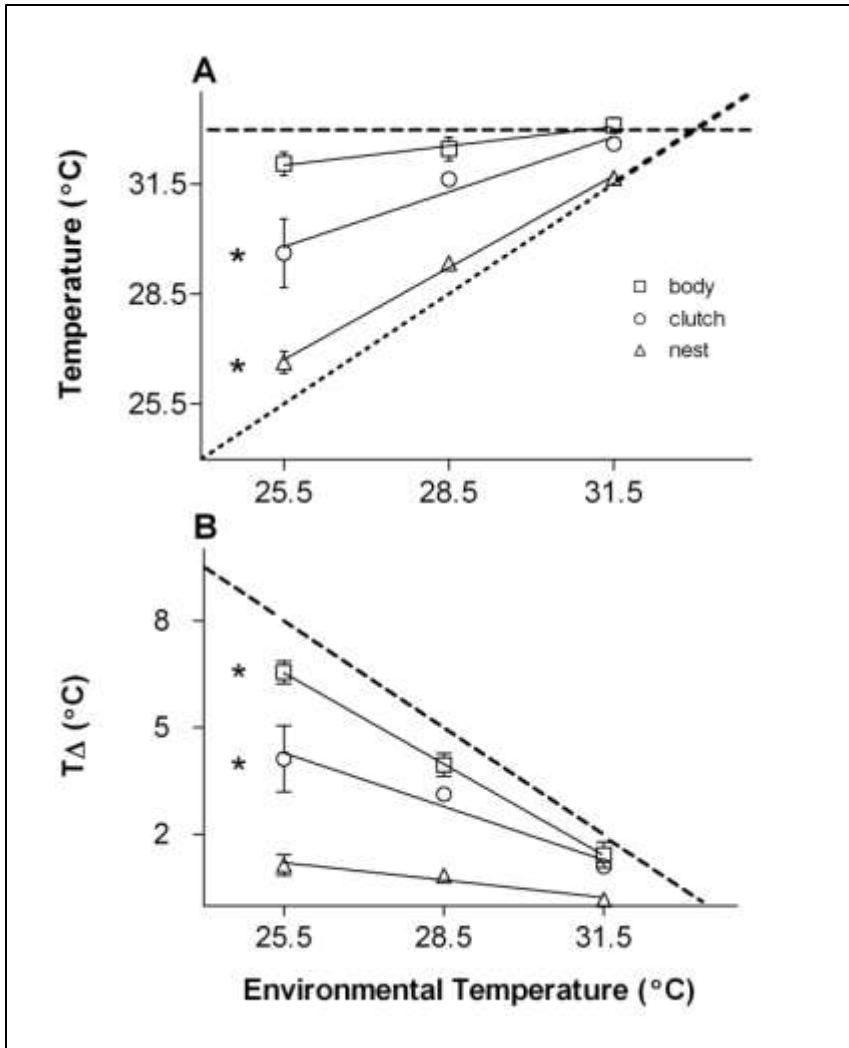
Fig. 3.1. Temperature differentials in brooding female Burmese pythons (*Python molurus*). The effect of environmental temperature on (A) the temperature of the female's body, clutch, and nest, and (B) temperature differentials between these three temperatures and the environmental temperature in female Burmese pythons (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 ± SEM.
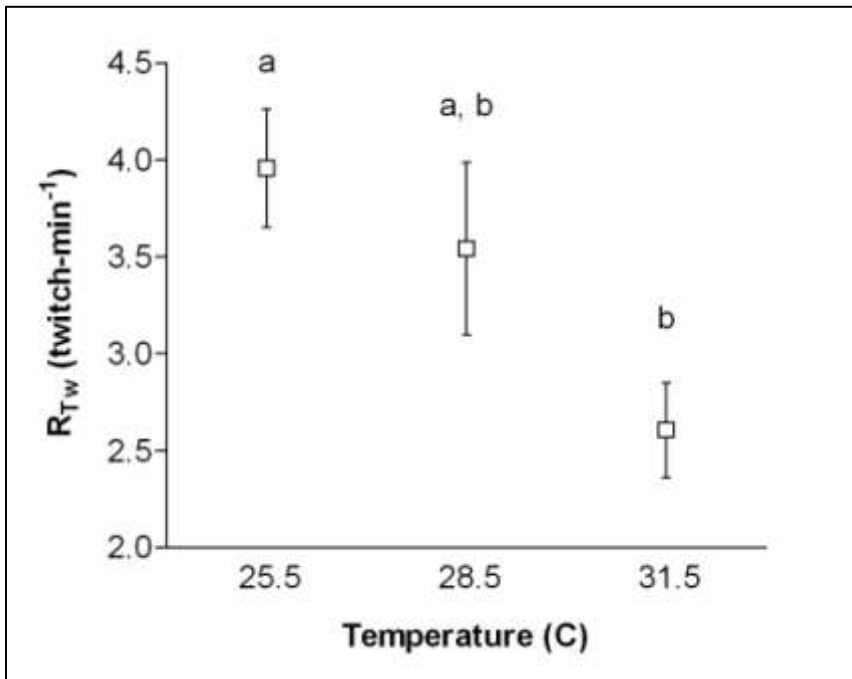
Fig. 3.2. Twitch rate in brooding female Burmese pythons (*Python molurus*). The effect of environmental temperature on twitch rate (R_{tw}) in brooding female Burmese pythons ($n = 3$). Letters indicate statistical differences among temperatures using rmANOVA. Values are presented as mean ± SEM.
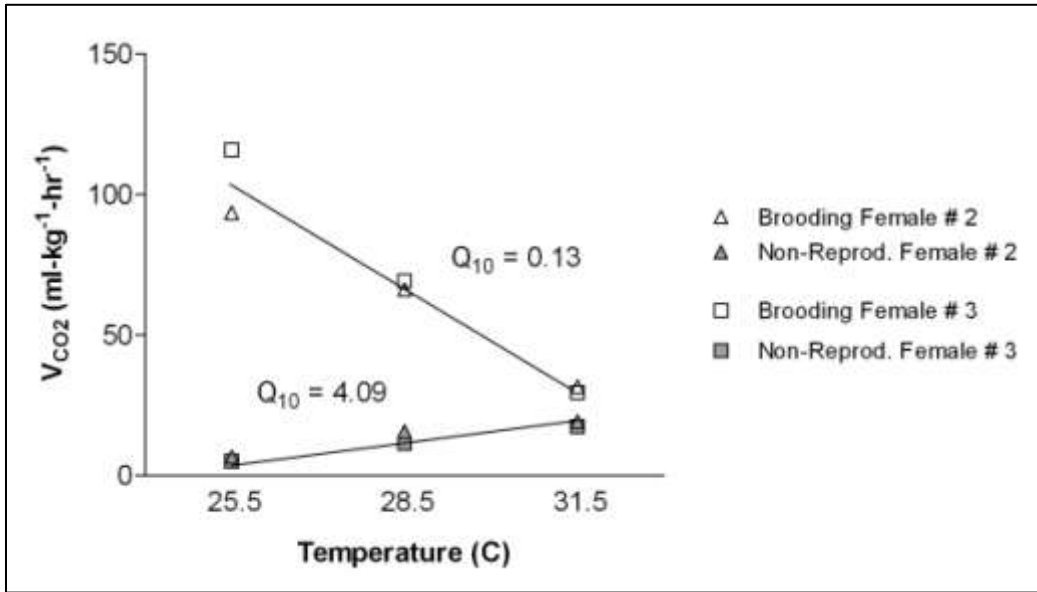
Fig. 3.3. Metabolic rates in two female brooding Burmese pythons (*Python molurus*). The effect of environmental temperature on mean metabolic rate ($\dot{V}_{CO_2}$) of two female Burmese pythons during brooding and after reproduction.
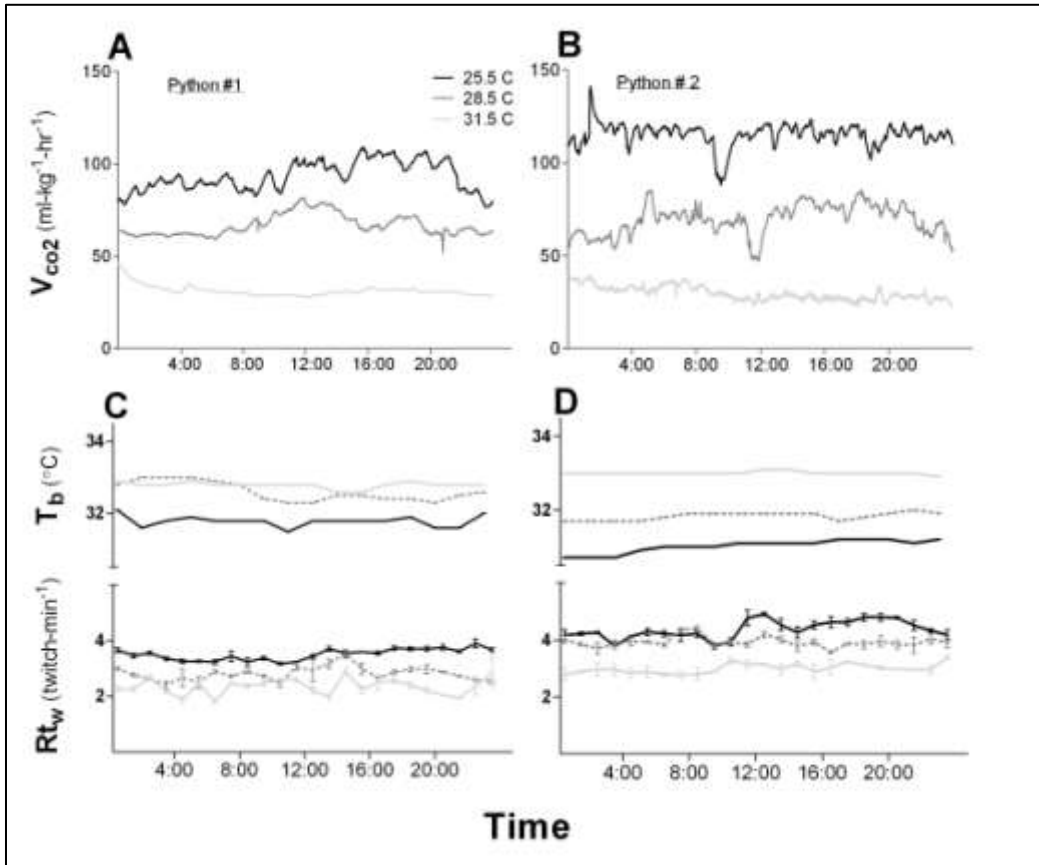
Fig. 3.4. Brooding metrics over a 24 h period in two Burmese pythons (*Python molurus*). Metabolic rate ($\dot{V}_{CO_2}$), female body temperature (T_b), and twitch rate (R_{tw}) over a 24 h period for two brooding female Burmese pythons at three environmental temperatures. Upper graphs (A, B) show 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 ± SEM.

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Chapter 4: How prevalent is facultative thermogenesis in the Pythonidae?

SUMMARY

Facultative thermogenesis is often attributed to pythons in general despite the limited comparative data available for the family. All species within the Pythonidae brood their eggs, but only two species are known to produce endothermic heat to enhance thermal regulation of the developing embryos. Additionally, several species are reported to not have thermogenic capability sufficient enough to influence the developmental environment. To further evaluate which pythons use facultative thermogenesis during brooding and to provide insight into potential phylogenetic, morphological, and ecological factors that influence thermogenic capability, we measured brooding metabolic rates and clutch temperature differentials in six python species. Four of the species (*Aspidites melanocephalus*, *A. ramsayi*, *Bothrochilus boa*, *Morelia viridis*) represent major phylogenetic branches which currently are devoid of data on the subject. We included *Morelia spilota cheynei* because it is a sister subspecies of *M. s. spilota* and *M. s. imbricata*, two subspecies with documented facultative thermogenesis. Previous studies of *Python regius* demonstrated an increase in metabolic rate of brooding female, but this response was limited and insufficient to influence clutch temperature. Thus, we repeated work on this species. We found no evidence of facultative thermogenesis in five species: *A. melanocephalus*, *A. ramsayi*, *M. viridis*, *M. s. cheynei*, and *P. regius*. However, we found that *B. boa* had a thermal metabolic sensitivity indicative of facultative

thermogenesis; however, its low metabolic rate and the absence of visible twitching makes substantial facultative thermogenesis questionable. We conclude that, despite being present in two distinct python lineages, facultative thermogenesis is relatively rare among the Pythonidae.

INTRODUCTION

Although it often requires a substantial expenditure of resources on the part of the parent, parental care is widespread because it confers numerous fitness benefits to offspring (e.g., increased survival and growth) (Clutton-Brock, 1991). An important type of parental care is thermoregulation of the developmental environment (Shine and Harlow, 1996; Ashmore and Janzen, 2003). Thermoregulation of the developmental environment has also been proposed as an initial step in the reproductive model of the evolution of endothermy (Farmer, 2000). Pythons (Squamata: Pythonidae) are unique among reptiles in providing ubiquitous parental care (Stahlschmidt and DeNardo, 2010), an important aspect this parental care being thermoregulatory behavior (Shine, 2004).

Pythons are ectothermic throughout most of their ontogeny, but during brooding the females of some species are known to increase their metabolic rate and exhibit muscular twitching, termed facultative thermogenesis (FT). Of the 34 extant python species, FT has been confirmed in only two species: the Burmese python (*Python molurus*), and the carpet python (*Morelia spilota*) (Vinegar et al., 1970; Slip and Shine, 1988). FT provides substantial heat to the clutch in these

species, supplementing the insulatory benefits associated with coiling around the eggs (Stahlschmidt et al., 2008). Female FT is inversely proportional to environmental temperature (T_{env}), with metabolic rate and muscular twitching increasing as temperature decreases below optimal developmental temperature up to maximum FT output when T_{env} approaches 24°C (Van Mierop and Barnard, 1978).

While studies of python FT provide insight into our understanding of developmental thermal biology and the evolution of endothermy, the occurrence of FT within the Pythonidae as a whole remains unclear (Stahlschmidt and DeNardo, 2010). Pythonidae is represented by 34 species distributed between two phylogenetic clades, an Afro-Asian clade and an Indo-Australian clade (Rawlings et al., 2008). The two species with confirmed FT come from the different clades - *P. molurus* within the Afro-Asian group and *M. spilota* within the Indo-Australian group (Fig. 4.1). Conversely, the absence of FT has been confirmed in species from three of the nine major branches that comprise the family: the reticulated python (*P. reticulatus*; Vinegar et al., 1970), the water python (*Liasis fuscus*; Stahlschmidt et al., unpub.), and the Children's python (*Antaresia childreni*; Stahlschmidt and DeNardo, 2009) (Fig. 4.1). Brooding metabolic data are currently absent for all other species including no representation from four of the major lineages.

Further complicating our understanding of FT within the Pythonidae, generalizations cannot be made to all species within a branch. In addition to the

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thermogenic Burmese pythons, the Afro-Asian group contains at least two species without FT, the rock python (*P. sebae*; Vinegar et al., 1970) and the ball python (*P. regius*; Ellis and Chappell, 1987). Interestingly, ball pythons do have elevated metabolic rates while brooding, but they do not produce enough heat to warm their clutch (Ellis and Chappell, 1987).

The aim of this study was to assess the presence of brooding FT in three of the remaining four branches within the Pythonidae. Our selected species were the black-headed python (*Aspidites melanocephalus*), the woma (*A. ramsayi*), the Bismarck ringed python (*Bothrochilus boa*), and the green tree python (*M. viridis*), the *A. melanocephalus* and *A. ramsayi* being nested within a single branch. We also selected the jungle carpet python (*M. spilota cheynei*), which is a sister subspecies to the diamond python (*M. s. spilota*) and the south-western carpet python (*M. s. imbricata*). FT has been documented in both of the latter subspecies (Slip and Shine, 1988; Pearson et al., 2003). *Morelia spilota* is widely distributed throughout Australia and thus experiences a great range with considerable variation in environmental temperature. *Morelia s. spilota* and *M. s. imbricata* are both from high latitudes, while *M. s. cheynei* is more tropical in distribution. Ball pythons (*Python regius*) were also included in an attempt to clarify the somewhat ambiguous prior results from this species.

MATERIALS AND METHODS

Animals

Data were collected on six species of python over two breeding seasons from 2007 to 2008: black-headed python (*Aspidites melanocephalus*), woma (*A. ramsayi*), Bismarck ringed python (*Bothrochilus boa*), jungle carpet python (*Morelia spilota cheynei*), green tree python (*M. viridis*), and ball python (*Python regius*). The first five species were borrowed from private snake breeders and housed at Arizona State University (ASU) during the two year period. Ball pythons were part of a long-term captive breeding colony at ASU.

Animals were housed individually in temperature controlled rooms (27 ± 1°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. During the non-breeding season (June to November), animals were fed weekly and provided water *ad libitum*. In November, animals underwent a six week cooling period in which we turned off the subsurface heating elements and ceased feeding. In January we turned on the subsurface heating elements and resumed feeding. After two weeks, we began rotating males through the females' cages. Breeding ended in February, and we began ultrasound scans (Concept/MCV, Dynamic Imaging, Livingston, Scotland) of females weekly to assess their reproductive progress.

We weighed gravid females one week prior to oviposition. For three of the species (*M. viridis*, *M. s. cheynei*, *P. regius*), pre-oviposition females were placed

in triple-ported, cylindrical containers (7.0 – 12.0 L), which we housed within a temperature controlled walk-in environmental chamber (T = 30.5 ± 0.3°C; 14:10 L:D photoperiod). The containers were tightly sealed and supplied with humidified air (20-40 ml/min; relative humidity (RH) = 80-85%). For the other three species (*A. melanocephalus*, *A. ramsayi*, *B. boa*), we placed similar brooding containers inside the pre-oviposition females' cages. These containers had openings bored into the lids that allowed females to enter the container and oviposit. Following oviposition, the lids were replaced with a solid lid and the females were moved to the environmental chamber and provided air as described above. All females oviposited in the containers between April and June. The oviposition chambers served as metabolic chambers during the study.

Measurements

Rates of O_2 consumption ($\dot{V}_{O_2}$; ml/h) and CO_2 production ($\dot{V}_{CO_2}$; ml/h) were collected from brooding females using an open-circuit respirometry system within seven days of oviposition. Supply air was scrubbed of CO_2 and water (CDA 1112, PureGas, Broomfield, CO), then routed through a mass flow controller (Unit Instruments, Inc., Yorba Linda, CA) before entering the metabolic chamber containing the brooding female. Efflux air from the metabolic chamber sequentially passed through a hygrometer (R2300, Sable Systems, Las Vegas, NV), a drying vial of $CaSO_4$, a CO_2 analyzer (LI-6252, Li-Cor Biosciences, Lincoln, NE), and an O_2 analyzer (FC-1B, Sable Systems, Las Vegas, NV). Air

flow was adjusted for each metabolic chamber to attain 99% equilibration times (Lasiewski et al., 1966) of less than 20 min. Flow rates ranged from $0.75 - 2.0$ L/min. Gas analyzers were calibrated weekly. Data were recorded on a 23X datalogger (Campbell Scientific Instruments, Logan, UT).

Females were acclimated for 12 h before beginning data collection. Steady state metabolic data were collected from each female over 12 h at two temperatures (25.5 and 31.5°C). $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated using the equations supplied by Walsberg and Hoffman (2006) using the lowest 20 min metabolic interval after subtracting clutch $\dot{V}_{O_2}$.

We collected temperature data from the clutch (T_c), the metabolic chamber (T_n), and the environmental chamber (T_e) using type-T thermocouples feeding into the 23X datalogger. We measured T_c by inserting a thermocouple into the center of the clutch through a port in the floor of the metabolic chamber. T_n was measured by inserting a thermocouple into the influx port in the side of the chamber so that it protruded ~5 cm into the metabolic chamber. The floor port was sealed by a plastic stopper, and the influx port was sealed by the placement of the influx hose. T_e was measured by securing a thermocouple ~10 cm from the metabolic chamber.

Females were separated from their clutches after brooding data had been collected. Clutch metabolic data were collected at the same temperatures using closed respirometry. Single-ported, closed respirometry containers were air tight

and ranged in size from 1.2 – 6.7 L. We used 140 mL plastic syringes to draw an initial and final gas samples, then dried the air using $CaSO_4$ before passing it through an O_2 analyzer (S-3A, Applied Electrochemistry, Inc., Sunnyvale, CA). Oxygen consumption of the clutch (ml O_2/hr) was calculated as ($O_2\%_{final}$ - $O_2\%_{initial}$) X functional chamber volume / time.

Statistics

We used paired T-tests to test for differences in metabolic rate and ΔT (T_c – T_e) within each species with sufficient sample size. We used covariate analysis of variance (ANCOVA) with temperature as a covariate to test for differences among species. All statistics were performed in SPSS (SPSS Inc., Chicago, IL). All values are presented as mean ± SEM.

RESULTS

All females maintained a tight coil around their clutches during the experiment, and once removed from their clutches fed normally. While the coils of most females covered the majority of the clutch, female *B. boa* displayed an unusual coil in that the female was wrapped completely around the clutch (including under the clutch), leaving no parts of it exposed. We did not observe twitches on any female during or after the experiment. Female mass, clutch size, and clutch mass are presented in Table 4.1.

Brooding metabolic rates varied considerably within and among species (Table 4.2). Mass-adjusted $\dot{V}_{O_2}$ was significantly different among species at each temperature ($F_{5, 35} = 7.08$, $p < 0.001$). Within species mass-adjusted $\dot{V}_{O_2}$ was significantly different at the two temperatures for *A. melanocephalus* ($t(2) = 4.47$, $p = 0.046$), *A. ramsayi* ($t(2) = 5.72$, $p = 0.029$), and *M. s. spilota* ($t(2) = 4.56$, $p = 0.044$). Mean Q_{10} values for these species ranged from 2.2 ± 0.9 to 3.5 ± 1.5 (Table 4.2). *Python regius* had substantial variation in mass-adjusted $\dot{V}_{O_2}$. There was no significant difference in their mass-adjusted metabolic rates between the two temperatures ($t(5) = 2.23$, $p = 0.155$), but the mean Q_{10} value was 2.5 ± 1.3 (Table 4.2). Both *B. boa* had metabolic rates that were inversely related to T_e. $\dot{V}_{O_2}$ decreased from 41.1 and 102.4 ml/kg/h at 25.5°C to 33.9 and 72.3 ml/kg/h at 31.5°C, respectively ($Q_{10} = 0.8$ and 0.6). The single *M. viridis* had a Q_{10} of 3.5, increasing $\dot{V}_{O_2}$ from 18.2 to 38.7 ml/kg/h.

We were able to collect temperature data from all species except *B. boa* (Table 4.3). Due to the unusual nature of their coiling, it was not possible to insert thermocouples into their clutches. Additionally, these females are sensitive to any disturbance of their nesting environment and quickly abandon their clutches if disturbed. There were no significant differences between T_c and T_e within any of the other five species (Table 4.3). Calculated temperature differentials from these five species showed that ΔT_c was not significantly different from zero (Table 4.3).

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DISCUSSION

We are the first to report brooding metabolic rates in *A. melanocephalus*, *A. ramsayi*, *B. boa*, *M. s. cheynei*, and *M. viridis*. These species are nested within four of the nine major phylogenetic branches within the Pythonidae (Rawlings et al., 2008), thus our results combined with previously published data provide data on FT for all nine branches. While our study was limited by low sample size, the lack of data on brooding python metabolic rates and the difficulty in obtaining reproductive animals provides merit to this study. Our results suggest that all of these species, except perhaps *B. boa*, are not thermogenic during brooding, either metabolically (Table 4.2) or thermally (Table 4.3). In general, our results support the claim that facultative thermogenesis (FT) in pythons is likely rare.

The metabolic profile, indicative of FT, in our two *B. boa* was an unexpected result (Table 4.2). While an increased metabolic rate would produce more heat, we were unfortunately unable to collect temperature data from the clutch or nest due to the females' sensitivity to disturbance. No twitches were observed at any time during reproduction in either female, but this does necessarily imply that heat was not produced muscularly. Muscle contractions that produce heat do not necessarily produce visible muscle tremor (e.g., birds) (Hohtola, 2004). However, the small size of the females combined with their low power production (brooding max = 1 mW/kg at 25.5°C) seems unlikely to provide sufficient heat to sustain a

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temperature differential, unless the nest was insulated considerably. Little is known of the ecology of *B. boa*, but as one of the few fossorial pythons the effect of burrow insulation should not be discounted.

Female brooding *P. regius* have been shown to have higher mass adjusted metabolic rates than when non-reproductive and to respond to decreases in temperature with a 2- to 4-fold increase in $\dot{V}_{O_2}$, as well as being capable of a 12 fold increase (Ellis and Chappell, 1987). Our data from *P. regius* overall match these previously reported brooding $\dot{V}_{O_2}$ values, and our calculated mean Q_{10} (2.5 ± 1.3) was also not significantly different from zero, but this was largely due to the unusually high thermal sensitivity of a single female (Table 4.2). With this female excluded, the mean Q_{10} for this species would be 1.2 ± 0.2, matching the previous finding that this species is metabolically uncoupled from temperature during brooding (Ellis and Chappell, 1987). We also found no evidence that female *P. regius* are able to sustain a temperature differential (Table 4.3), emphasizing the question of the role of the increased metabolism during brooding.

Previous studies have shown that FT in pythons is phylogenetically separated by a large distance, being present in the genera *Python* and *Morelia* (Fig. 4.1). The metabolic profile of *B. boa*, which is situated between these two branches, suggests that decreased metabolic sensitivity during brooding may be an ancestral trait of the family. However, at least some pythons exhibit a large metabolic scope when feeding (Secor and Diamond, 1995), and python metabolism may be a labile

trait that has evolved an increased sensitivity to the reproductive state independently in several lineages but which has only evolved into full FT in species that can minimize the energetic costs or increase the benefits to the offspring.

The two species that do express full FT, *P. molurus* and *M. spilota*, have distributions at the northern and southern latitudinal boundaries of the family (Vinegar et al., 1970; Slip and Shine, 1988). FT has been well documented in the two subspecies of *P. molurus* (*P. m. bivittatus*, *P. m. molurus*), both of which are heavy bodied, thus reducing their thermal conductance and the costs of thermogenesis (Ellis and Chappell, 1987). However, we found no evidence of FT in *M. s. cheynei*. In contrast to *M. s. spilota*, in which FT has been confirmed, this subspecies's range does not extend south of northern Queensland (Wilson and Swan, 2000). Our results suggest that even within a species the energetic costs of FT may require substantial offspring benefits before it is viable.

Pythons have been used to support the reproductive model of the evolution of endothermy, whereby the production of heat during incubation initiated the evolution of a high basal metabolic rate (Farmer, 2000). While this model emphasizes the benefits to offspring that thermoregulation of the developmental environment can provide, it has been pointed out that the substantial costs of thermogenesis may be prohibitive (Angilletta and Sears, 2003). The rarity of python FT supports this caution, but as yet there has been no discussion of the methods brooding females use to reduce these costs. Brooding females can reduce

thermal conductance via nest site selection (Shine et al., 1997) and supplement

thermogenesis with basking (Slip and Shine, 1988). These factors need to be

quantitatively evaluated if python FT is to be useful in the discussion.

In sum, our results support the conclusion that most species of python are not

thermogenic and stress the importance of ecological factors in the full expression

of FT within the family. Our understanding of python FT, however, is severely

limited by our lack of understanding of the mechanisms underlying brooding

thermogenesis. Future research into the hormonal mechanisms underlying

thermogenesis would provide valuable information that could be linked to the

genetic underpinning of python brooding thermogenesis.

Table 4.1. Female and clutch metrics of six species of python. Female mass, clutch size, and clutch mass are presented for each individual in the experiment.

Species, n	Female mass (g)	Clutch size (eggs)	Clutch mass (g)
Aspidites melanocephalus, 3	2116	5	627.5
	1724	6	644.0
	3157	7	648.5
mean ± SEM	*2332 ± 427*	*6 ± 1*	*640.0 ± 6.4*
Aspidites ramsayi, 3	950	9	348.0
	1008	10	439.7
	866	2	384.2
mean ± SEM	*942 ± 41*	*10 ± 1*	*390.6 ± 26.7*
Bothrochilus boa, 2	1563	18	351.0
	1780	13	243.0
mean ± SEM	*1671*	*16*	*297.0*
Morelia spilota cheynii, 3	2100	16	636.0
	2200	19	760.3
	1422	11	479.5
mean ± SEM	*1907 ± 244*	*15 ± 2*	*625.3 ± 81.2*
Morelia viridis, 1	1000	13	126.0
Python regius, 6	1017	5	323.7
	641	3	121.7
	734	4	212.0
	934	4	241.2
	1137	7	722.0
	1593	6	303.2
mean ± SEM	*1009 ± 138*	*5 ± 1*	*294.1 ± 95.7*

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Table 4.2. Metabolic metrics in six species of python. Thermal sensitivity (Q_{10}), respiratory exchange ratio (RER), total $\dot{V}_{O_2}$ (ml/hr), and mass specific $\dot{V}_{O_2}$ (ml/hr/kg) are presented for each female in the experiment. Clutch $\dot{V}_{O_2}$ was subtracted from total $\dot{V}_{O_2}$ prior to calculations.

| Species, n | Q_{10} | 25.5°C | | |
		RER	V_{O_2} (ml/hr)	V_{O_2} (ml/hr/kg)
Aspidites melanocephalus, 3	4.8	0.7	28.0	13.2
	1.2	0.6	50.8	29.5
	1.4	0.7	38.8	12.3
mean ± SEM	*2.5 ± 1.2*	*0.6 ± 0.1*	*39.2 ± 6.6*	*18.3 ± 5.5*
Aspidites ramsayi, 3	3.6	0.7	24.4	25.7
	2.1	0.8	14.8	14.6
	2.3	0.7	19.0	21.9
mean ± SEM	*2.7 ± 0.5*	*0.7 ± 0.1*	*19.4 ± 2.8*	*20.8 ± 3.2*
Bothrochilus boa, 2	0.7	0.7	64.3	41.1
	0.6	0.7	182.3	102.4
mean	*0.6*	*0.7*	*123.3*	*71.8*
Morelia spilota cheynii, 3	1.0	0.8	122.1	58.1
	3.8	0.7	66.5	30.2
	5.5	0.7	25.1	17.7
mean ± SEM	*3.4 ± 1.3*	*0.7 ± 0.1*	*71.2 ± 28.1*	*35.3 ± 12.0*
Morelia viridis, 1	3.5	0.9	18.2	18.2
Python regius, 6	1.4	0.6	12.3	12.1
	1.6	0.8	13.6	21.1
	0.4	0.4	25.1	34.2
	1.0	1.4	28.3	30.3
	1.6	0.9	29.2	25.7
	8.9	1.6	14.0	8.8
mean ± SEM	*2.5 ± 1.3*	*0.9 ± 0.2*	*20.4 ± 3.3*	*22.0 ± 4.1*

| Species, n | 31.5°C | | |
	RER	V_{O_2} (ml/hr)	V_{O_2} (ml/hr/kg)
Aspidites melanocephalus, 3	0.8	71.5	33.8
	0.8	56.1	32.5
	1.3	47.8	15.1
mean ± SEM	*1.0 ± 0.2*	*58.5 ± 7.0*	*27.2 ± 6.0*
Aspidites ramsayi, 3	0.4	52.8	55.5
	0.9	23.2	23.0
	0.7	31.1	35.9
mean ± SEM	*0.7 ± 0.1*	*35.7 ± 8.8*	*38.1 ± 9.4*
Bothrochilus boa, 2	0.7	53.0	33.9
	0.8	128.7	72.3
mean	*0.7*	*90.8*	*53.1*
Morelia spilota cheynii, 3	0.7	125.3	59.6
	0.7	148.8	67.6

		0.8	69.7	49.0
mean ± SEM		*0.7 ± 0.1*	*114.6 ± 23.4*	*58.7 ± 5.4*
Morelia viridis, 1		0.7	38.7	38.7
Python regius, 6		1.1	15.0	14.7
		0.9	17.9	27.9
		1.1	13.8	18.8
		1.8	28.0	30.0
		0.7	38.2	33.6
		0.7	51.8	32.5
mean ± SEM		*1.0 ± 0.2*	*27.5 ± 6.5*	*26.3 ± 3.2*

Table 4.3. Temperature metrics in six species of python. Environmental temperature (T_e), metabolic chamber temperature (T_n), clutch temperature (T_c), and ΔT are presented for each female in the experiment.

Species, n	25.5°C			
	T_e (°C)	T_n (°C)	T_c (°C)	ΔT (T_c-T_e)
Aspidites melanocephalus, 3	25.5	25.7	25.5	-0.07
	26.3	26.3	26.2	-0.08
	25.7	25.4	25.7	-0.07
mean ± SEM	*25.8 ± 0.2*	*25.8 ± 0.3*	*25.8 ± 0.2*	*-0.1 ± 0.3*
Aspidites ramsayi, 3	26.1	27.1	26.2	0.17
	25.4	25.8	26.6	1.19
	25.4	24.9	25.5	0.04
mean ± SEM	*25.6 ± 0.2*	*26.0 ± 0.6*	*26.1 ± 0.3*	*0.5 ± 0.6*
Morelia spilota cheynei, 3	25.5	25.9	25.6	0.07
	25.8	26.3	26.0	0.20
	25.5	25.7	25.3	0.67
mean ± SEM	*25.6 ± 0.1*	*26.0 ± 0.2*	*25.6 ± 0.2*	*0.3 ± 0.2*
Morelia viridis, 1	26.0	26.0	26.3	0.27
Python regius, 6	24.4	25.7	26.1	1.77
	26.3	25.7	25.8	-0.47
	26.3	26.1	26.1	-0.20
	25.9	26.0	26.4	0.45
	26.2	25.8	25.7	-0.59
	25.0	25.8	25.3	0.32
mean ± SEM	*25.7 ± 0.3*	*25.9 ± 0.1*	*25.9 ± 0.1*	*0.2 ± 0.4*

Species, n	31.5°C			
	T_e (°C)	T_n (°C)	T_c (°C)	ΔT (T_c-T_e)
Aspidites melanocephalus, 3	31.9	31.9	31.6	-0.28
	32.4	32.2	32.2	-0.18
	31.5	32.3	31.3	-0.14
mean ± SEM	*31.9 ± 0.3*	*32.1 ± 0.1*	*31.7 ± 0.3*	*-0.2 ± 0.3*
Aspidites ramsayi, 3	32.9	31.5	32.2	-0.69
	31.1	33.9	32.7	1.56
	31.4	33.9	32.3	0.86
mean ± SEM	*31.8 ± 0.6*	*33.1 ± 0.8*	*32.4 ± 0.1*	*0.6 ± 0.8*
Morelia spilota cheynii, 3	31.3	31.9	31.6	0.31
	31.6	31.7	31.7	0.08
	31.3	31.6	31.4	0.10
mean ± SEM	*31.4 ± 0.1*	*31.7 ± 0.1*	*31.6 ± 0.1*	*0.2 ± 0.1*
Morelia viridis, 1	31.8	31.4	31.9	0.13
Python regius, 6	31.3	31.5	31.7	0.41
	31.3	31.4	31.3	0.09
	31.2	31.3	31.4	0.17
	31.5	31.8	31.5	-0.02
	31.9	31.3	31.9	-0.08

| | 31.8 | 32.5 | 32.1 | 0.33 |
| *mean ± SEM* | *31.5 ± 0.1* | *31.6 ± 0.2* | *31.7 ± 0.1* | *0.2 ± 0.2* |

Fig. 4.1. Phylogeny of facultative thermogenesis in pythons. Strict consensus tree adapted from Rawling et al. (2008) showing the presence (T) or absence (NT) of facultative thermogenesis in pythons.

Chapter 5: Why aren't all pythons facultatively thermogenic? Insight from a thermogenic pseudoserpent

SUMMARY

Parental care of eggs is energetically costly particularly if the clutch is warmed. While pythons present an interesting model of facultative thermogenesis, little has been done regarding the tradeoffs between the energetic costs to the female and the thermal benefits to the developing offspring. We designed an artificial snake able to maintain the temperature of artificial clutches under variable power loads. This allowed us to estimate the energetic costs of maintaining the clutches at a preferred temperature given unlimited power, half power, or no power, thus mimicking homeothermy, half homeothermy, and ectothermic conditions. We manipulated clutch size (5, 10, 15 eggs), diel cycle (2, 4, 6 °C), and insulation (with and without) at each of these power levels. We found no significant effect of clutch size on either energetic or thermal costs. Energetic costs increased with the amplitude of the diel cycle and decreased with the addition of insulation. Thermal benefits to the clutch decreased with the amplitude of diel cycle. Interestingly, thermal benefits also increased with the addition of insulation. We discuss these results within the context of the reproductive model of the evolution of endothermy.

INTRODUCTION

The maintenance of high and relatively constant temperature by metabolic means (endothermy) constitutes a major innovation that appeared independently in two

vertebrate lineages: birds and mammals (Bennett and Ruben, 1979). The extreme complexity and cost of endothermy suggests that there must have been substantial selective advantages driving the evolution of endothermy (Bennett and Ruben, 1979; Koteja, 2000). While one can postulate advantages to the ability to maintain high temperature, it is unclear what selective forces may have initiated the evolution of endothermy (Hayes and Garland, 1995; Ruben, 1995; Koteja, 2000).

The evolution of endothermy has generated considerable scientific controversy and several hypotheses have been advanced (Ruben, 1995; Hayes and Garland, 1995). These include thermoregulation *per se* (Heinrich, 1977; Crompton et al., 1978), 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 endothermy into two steps. Transitory endothermy was first selected at low costs to provide embryos with a developmental environment that had a relatively high mean temperature and low variation. From this point, additional benefits associated with the evolution of endothermy, 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 "definitive" endothermy as displayed by birds and mammals. The reproductive model is distinct from previous theories in

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that endothermy is primarily beneficial to the progeny rather than the adult (Farmer, 2000).

The reproductive model has been highly 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 suggest not only 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 (Shine et al., 1997; Andrews et al., 2000). 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).

Not surprisingly, empirical tests of any evolution of endothermy model are rare since few extant organisms provide an opportunity to assess the selective advantages of limited endothermy. The unique characteristics of pythons where females of some species are facultatively thermogenic during egg brooding (Vinegar et al., 1970), has been used to support arguments for and against the reproductive model (Farmer, 2000; Angilletta and Sears, 2003). However, little work has been conducted to estimate the relationship between the energetic costs to the female and the thermal benefits to the developmental environment, as well as how biotic and abiotic factors related to the brooding event may influence these costs and benefits. In fact, while python brooding is widely mentioned in

discussions of examples of spatially or temporally limited endothermy, the fact is, while all python species brood their eggs, only a few are known to have significant thermogenic capability (Stahlschmidt and DeNardo, 2010).

Since 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 (for a review, see Stahlschmidt and DeNardo, 2009). 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 energy demands required of facultative thermogenesis under various environmental and clutch conditions, and (2) determine the thermal consequences of the various conditions when thermogenic capability was absent or limited. Specifically, we ran thermometric trial sets that included three thermogenic potentials (homeothermy, ectothermy, and an intermediate condition). We ran these three condition sets under multiple diel cycle regimens (2, 4, 6 °C) and multiple clutch sizes (5, 10, and 15 eggs). Additionally, we performed the clutch size trials in both non-insulated and insulated nest conditions. As heat production was a product of the power supplied to the pseudoserpent, for each trial condition we were able to directly estimate the energetic costs and thermal consequences on 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 pythons (*Antaresia childreni*). The body of

the pseudoserpent was constructed by inserting a 130 cm spring into a 135 cm

length of polyethylene 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 Ω, All Electronics

Corporation, Van Nuys, CA, USA) in series with sufficient distance between

them so 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 shrink. The resistors were

then inserted into the body of the pseudoserpent. A #3 rubber stopper was inserted

into each end of the tubing, with the resistor string wire exiting through a small

(~3mm) hole in each of the stoppers. Additionally, a syringe needle (18 G, 2.5

cm) was stuck through one of the stoppers and then had a stopcock attached to it.

The stopcock and needle were used for filling the pseudoserpent with water

before the start of each trial set.

The complete pseudoserpent was then coiled into a beehive posture typical of

a brooding female and secured in that position using fine wire. To mimic the seal

typically provided by the snake's head, we secured a piece of polyethylene 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 filled any small gaps between coils with pieces of synthetic 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 that of a live *A. childreni* female brooding her eggs (Fig. 5.1).

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 differential between T_{Int} and the target incubation temperature (T_{Set}, 30.5°C, which approximates the preferred incubation temperature of *A. childreni*, Lourdais et al., 2008).

Trials

Trials were conducted in an environmental 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 of chamber temperature (T_{Env}) via a 3-thermocouple array that surrounded the pseudoserpent

approximately 10 cm from the pseudoserpent. The environmental chamber was provided with a small fan to prevent thermal heterogeneity within the chamber.

In addition to chamber temperature, the datalogger also recorded input from a thermocouple at the center of the clutch (T_{Cltch}) and from a 3-thermocouple array which measured female-clutch interface temperature (T_{Int}) at three points. During trial sets with nest insulation, T_{env} was measured just outside the insulating box, and we added a 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_{Cltch}, 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 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_{Cltch} 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). The first 8 h of day 3 were used as an equilibration period to assure starting temperatures were the same for all three power condition. After the 8 hr equilibration period, we recorded all temperatures for a continuous 24 hr period.

Diel cycle amplitude trials – Trial sets were run 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). Three trial sets were run

at each of the three diel cycles, and clutch size for these trials was 10 eggs.

Clutch size trials – Trial sets were run in triplicate for clutch sizes of 5, 10, and 15

eggs.

Insulation trials – Trial sets were similarly run in triplicate for clutch sizes of 5,

10, and 5 eggs; but, for these trial sets, we placed the pseudoserpent within a

rectangular box (24 x 17 x 16 cm) constructed by pinning a single layer of shade

cloth (Synthesis Commercial 95, Arizona Sun Supply Inc., Phoenix, AZ) to the

six sides of a wooden frame. We determined the thermal conductivity (k, W/°C-

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

environmental 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 placed a constant voltage of 1.6 W across the resistor and allowed the

system to reach steady state conditions (~1.5 h). We used the equation k = (Q x

Δd)/(A x ΔT) to calculate the thermal conductivity of the system, where Q = heat

flow in watts, Δd = the thickness of the shade cloth, A = the surface area of the

box, and ΔT = 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

the homogeneity of the thermal environment. For our material, k = 0.6 ± 0.2

W/°C-m^2 with no air circulation and 2.0 ± 0.2 W/°C-m with air circulation. While

the clutch size trial sets mimicked the situation of brooding a clutch in open space

on the surface, these trials provided insulation similar to that which might be

provided if a female broods her eggs in a more restricted environment (e.g.,

within a burrow chamber).

Data analysis

Data were analyzed using the GLM Procedure Univariate analysis of variance

(ANOVA) with a Type III model in SPSS (SPSS Inc., Chicago, IL). Independent

factors were: the type of diel cycle (2, 4, 6°C), the clutch size (5, 10, 15 egg), and

insulation (no insulation, one layer). The amount of allowable power (full, half,

zero) was used as a covariate in the analysis. All data were tested for parametric

assumptions, and percent data were arcsine transformed to achieve normality

before being analyzed. All data are presented as mean ± SEM.

RESULTS

Energetic costs

The power used by the pseudoserpent during the trials reflected the energetic

costs of maintaining T_{Cltch} above T_{Env} (Table 5.1). The mean power was the

average power recorded over a 24 h run and reflected average metabolic rate a

female would have to sustain. The maximum recorded power reflected the

maximum metabolic rate a female would have to provide. Total power consumed

measured the total cost to the female over a single day. All power metrics increased proportional to the amplitude of the diel cycle (mean: $F_{2,63} = 20.072$, P < 0.001; maximum: $F_{2,63} = 17.309$, P < 0.001; total: $F_{2,63} = 20.072$, P < 0.001). The addition of insulation had no effect on the maximum recorded power ($F_{1,63} = 0.631$, P = 0.430), but significantly decreased mean and total power ($F_{1,63} = 26.316$, P < 0.001; $F_{1,63} = 26.316$, P < 0.001 respectively). Clutch size had no significant effect on any power metric (mean: $F_{2,63} = 0.383$, P = 0.684; maximum: $F_{2,63} = 0.066$, P = 0.937; total: $F_{2,63} = 0.383$, P = 0.684).

Interface temperature

The interface temperature (T_{Int}) measured the temperature between the inner surface of the pseudoserpent and the clutch (Table 5.2). The pseudoserpent was programmed to control T_{Int}. As T_{Int} closely mirrored the pseudoserpent's temperature, it reflected a brooding female python's ability to regulate her own temperature. We calculated the mean interface differential ($\Delta_{Int} = T_{Int} - T_{Env}$), the maximum recorded Δ_{Int}, and the percent of time Δ_{Int} was equal to or greater than 0.5 °C (Table 5.2). We also calculated the variance of T_{Int} (Table 5.3). The amplitude of the diel cycle significantly increased all Δ_{Int} metrics except the percent of time T_{Int} was below T_{Set} (mean: $F_{2,63} = 17.559$, P < 0.001; maximum: $F_{2,63} = 7.064$, P = 0.002; variance: $F_{2,63} = 10.252$, P < 0.001; percent: $F_{2,63} = 2.682$, P = 0.076). The addition of insulation had no effect on the mean or maximum Δ_{Int} (mean: $F_{1,63} = 0.727$, P = 0.397; maximum: $F_{1,63} = 2.979$, P =

0.089), but significantly increased the variance and percent of time T_{Int} was below T_{Set} ($F_{1,63} = 21.403$, $P < 0.001$; $F_{1,63} = 4.403$, $P = 0.040$ respectively). Clutch size had no significant effect on any metric of T_{Int} (mean: $F_{2,63} = 0.106$, $P = 0.899$; maximum: $F_{2,63} = 0.105$, $P = 0.901$; variance: $F_{2,63} = 1.447$, $P = 0.243$; percent: $F_{2,63} = 0.874$, $P = 0.422$).

Clutch temperature

Clutch temperature (T_{Cltch}) was recorded at the center of the clutch. We calculated mean Δ_{Cltch} ($\Delta_{Cltch} = T_{Env} - T_{Cltch}$), the maximum recorded Δ_{Cltch}, and the percent of time Δ_{Cltch} was equal to or greater than 0.5 °C (Table 5.2), as well as the variance of Δ_{Cltch} (Table 5.3). Similar to T_{Int} metrics, the amplitude of the diel cycle significantly increased all Δ_{Cltch} metrics except the percent of time T_{Cltch} was below T_{Set} (mean: $F_{2,63} = 7.681$, $P = 0.001$; maximum: $F_{2,63} = 17.541$, $P < 0.001$; variance: $F_{2,63} = 10.766$, $P < 0.001$; percent: $F_{2,63} = 0.736$, $P = 0.483$). The addition of insulation significantly increased all clutch metrics (mean: $F_{1,63} = 9.600$, $P = 0.003$; maximum: $F_{1,63} = 11.346$, $P = 0.001$; variance: $F_{1,63} = 4.692$, $P = 0.029$; percent: $F_{1,63} = 40.120$, $P < 0.001$). Clutch size had no significant effect on any clutch metric (mean: $F_{2,63} = 0.464$, $P = 0.631$; maximum: $F_{2,63} = 0.117$, $P = 0.890$; variance: $F_{2,63} = 1.514$, $P = 0.228$; percent: $F_{2,63} = 2.192$, $P = 0.120$).

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DISCUSSION

Facultative thermogenesis in the Children's python

We designed an artificial snake model (pseudoserpent) to estimate the costs associated with reproductive endothermy in pythons. These costs can be partitioned into the energetic costs an endothermic female incurs while supplying heat to her clutch and the thermal costs to the clutch when that heat is insufficient to maintain an optimal developmental temperature. For facultative thermogenesis to be a viable form of parental care, the decrease in thermal costs to the offspring must outweigh the increase in energetic costs to the female (Angilletta and Sears, 2003).

The fully endothermic pseudoserpent was able to maintain the temperature of the clutch within an optimal range (Table 5.2), but doing so required significant amounts of energy (Table 5.1). At the 6 °C amplitude with a 10 egg clutch, the pseudoserpent used 148 ± 4 kJ over the 24 h run (Table 5.1). If this is extrapolated to a 600 g female Children's python (*Antaresia childreni*), she would have to use 235 g of fat over the average 60 day brooding period, or 39% of her body mass. If she were to maintain the maximum power we recorded during the entirety of the same run, she would have to use 469 g fat, or 78% of her body mass, over the 60 day brooding period. In contrast, a brooding, facultatively thermogenic 16 kg Burmese python (*Python molurus*) maintaining a 6 °C gradient averages approximately 772 kJ/day (21 g fat/day) (Chapter 3), which over a 60 d period would require a total of 1.2 kg of fat, only 7.5% of her body mass.

These differences can be attributed to the high thermal conductivity of smaller females. Using the maximum recorded power and the temperature differential the pseudoserpent maintained at that power, we calculate the thermal conductivity of the pseudoserpent to be 0.63 ± 0.1 W/kg-°C, closely matching the thermal conductivity of 0.65 W/kg-°C reported for ball pythons (*P. regius*) (Ellis and Chappell, 1987). In contrast, the two thermogenic species, the *P. molurus* and the diamond python (*Morelia spilota*), are large and have reduced thermal conductivity (0.27 and 0.114 W/kg-°C respectively).

Environmental conditions reduced these costs, the greatest reduction in energetic cost being due to a decrease in the diel cycle. During the 2 °C diel cycle, the cost of heating a 10 egg clutch for 60 days decreased to 84 g of fat, or 14% of body mass, while the addition of a single layer of insulation during the 6 °C diel cycle still required 165 g of fat, or 28% of body mass. We estimated the thermal conductivity of the insulated nest environment under experimental conditions to be 2.0 ± 0.3 W/°C-m. The thermal conductivity of soil can range widely, from 0.3-4 W/°C-m depending on the type of soil and the amount of moisture, but the thermal conductivity of dry soil is approximately 1 W/°C-m (Somerton, 1992). As the energy required to maintain a gradient is directly proportional to the thermal conductivity, a burrow with this conductivity would reduce by half the energetic costs in our model, but this would still require the loss of 84 g of fat (14% body mass) during a 60 day brooding period.

The substantial energetic costs incurred by the pseudoserpent did reduce the thermal costs to the clutch by reducing Δ_{Cltch}, the percent of time the clutch spent at suboptimal temperatures (Tables 5.2), and the variance in T_{Cltch} (Table 5.3). When the pseudoserpent was supplied with zero power, mimicking the fully ectothermic condition, the mean Δ_{Cltch} increased by approximately 3 °C and the maximum Δ_{Cltch} increased by approximately 5 °C during the 6 °C diel cycle (10 egg clutch, Table 5.2). Additionally, the clutch was below optimal developmental temperature almost 100% of the time (Table 5.2) and the variance of T_{Cltch} increased by approximately 3.5 °C (Table 5.3).

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 Δ_{Cltch}, with survival rates quickly approaching zero when $\Delta_{Cltch} > 3$ °C (Vinegar, 1973). In the ectothermic water python (*Liasis fuscus*), however, egg survival seems robust to daily temperature fluctuations (maximum $\Delta_{Cltch} = 8$ °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).

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 no data currently exist on the relation between 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

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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 thermogenesis would require prohibits them from being facultatively thermogenic.

Clutch size and interface temperature

Clutch size had no significant effect on any energetic or thermal metric (Tables 5.1, 5.2, 5.3). This result suggests that the increased thermal inertia of larger clutches is not a factor in determining species limitations on clutch size, which may be driven instead by the ability of the female to coil around the clutch. Although unexpected, this result supports 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 results of Chapter 3 suggest that brooding *P. molurus* regulate their own body temperature during facultative thermogenesis and only indirectly regulate clutch temperature. Similarly, the pseudoserpent increased heat production in proportion to the magnitude of Δ_{Int}. As the thermocouples were in direct contact with the inner surface of the pseudoserpent, Δ_{Int} was presumably largely driven by the pseudoserpent's temperature. This had the counter-intuitive effect of increasing thermal costs to the clutch when insulation was added (Table 5.2). Under the insulated condition, the pseudoserpent consumed less power (Table

5.1) regulating its own temperature (Table 5.2), which thus decreased transference of heat to the clutch.

Relation to the reproductive model of endothermy

The reproductive model for the evolution of endothermy posits that transitory endothermy was initially selected for the benefits it provided to the developing offspring (Farmer, 2000). Transitory endothermy likely evolved through intermediate steps. In pythons this would have entailed small increases in thermogenesis that provided sufficient offspring benefits to drive full facultative thermogenesis. As much of the debate regarding the reproductive model centers around how the costs of such limited states would be compensated 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 a fully thermogenic, 600 g female would have to expend during 60 days under a 6 °C diel cycle from 235 g of fat (39% body mass) to 165 g of fat (28% body mass). Limited thermogenesis greatly reduced the thermal costs to the clutch associated with the fully ectothermic state. Under a 6 °C diel cycle, the maximum Δ_{Cltch} and the percent of time the clutch spent below optimal

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developmental temperature decreased by 52% and 50% respectively. The mean Δ_{Cltch} decreased by 58%, and the variance of T_{Cltch} decreased by 73%. This trend repeated itself under the 2 °C and 4 °C diel cycles. Thus, a limited thermogenic female under a 2 °C diel cycle would only lose 62 g of fat (10% initial body mass) but still a 50-75% reduction in thermal costs. These results suggest that the evolution of limited endothermy would confer disproportionate benefits to the offspring in reduction of thermal costs, although the high thermal conductivity of a small species would likely still make the energetic cost prohibitive.

In sum, our results support the reproductive model for the evolution of endothermy when the energetic costs can be decreased, either environmentally or through a decrease in thermal conductivity. The increase in thermal costs caused by the layer of insulation, however, makes it clear that the regulation of thermogenesis must be taken into account in order to correctly evaluate the effects of environmental variables.

Table 5.1. Artificial snake power data. Maximum (mW), mean (J), and total power (kJ) used by an artificial snake during trials. Power supplied was the thermogenic limit put on the pseudoserpent (see text for details). We manipulated the amplitude of the diel cycle (2, 4, or 6 °C) as well as clutch size (5, 10, or 15 eggs), the latter in both insulated and non-insulated conditions (see text). All trials were run in triplicate and values are presented as   mean ± SEM.

		Power supplied	Max power (mW)			Mean power (J)			Total power (kJ)		
	Diel Cycle										
No Insulation, 10 egg	*2°C*	*Full*	1213	±	16	37	±	1	53	±	2
		Half	618	±	10	27	±	1	39	±	2
	4°C	*Full*	2323	±	18	70	±	1	100	±	1
		Half	1172	±	7	49	±	1	71	±	2
	6°C	*Full*	3410	±	51	103	±	2	148	±	4
		Half	1721	±	32	72	±	2	104	±	3
	Clutch Size										
No Insulation, 6 °C	*5 eggs*	*Full*	3802	±	29	76	±	1	109	±	1
		Half	1369	±	33	56	±	5	81	±	8
	10 eggs	*Full*	3903	±	72	84	±	1	121	±	1
		Half	1414	±	16	58	±	1	84	±	1
	15 eggs	*Full*	3960	±	98	86	±	5	123	±	8
		Half	1479	±	63	61	±	3	87	±	4
	Clutch Size										
Insulation, 6 °C	*5 eggs*	*Full*	3554	±	18	64	±	1	93	±	1
		Half	1112	±	14	45	±	1	65	±	2
	10 eggs	*Full*	3555	±	34	72	±	1	104	±	2
		Half	1162	±	36	51	±	0	73	±	0
	15 eggs	*Full*	3605	±	1	73	±	6	105	±	8
		Half	1166	±	51	51	±	4	73	±	6

Table 5.2. Artificial snake temperature data. Differences between set temperature of the pseudoserpent and both clutch and interface temperatures during trials. Power refers to the thermogenic limit put on the pseudoserpent (see text for details). We manipulated the amplitude of the diel cycle (2, 4, or 6 °C) as well as clutch size (5, 10, or 15 eggs), the latter in both insulated and non-insulated conditions (see text). All trials were run in triplicate, and values are presented as mean ± SEM.

	Power	Clutch Temperature (°C)			Interface Temperature (°C)		
		Mean ΔT	Max ΔT	% time ΔT ≥ 0.5°C	Mean ΔT	Max ΔT	% time ΔT ≥ 0.5°C
Diel Cycle - No Insulation, 10 egg							
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	38.9 ± 0.9	0.31 ± 0.01	0.92 ± 0.02	32.1 ± 0.9
	None	1.32 ± 0.07	2.23 ± 0.06	88.1 ± 8.4	1.12 ± 0.06	2.06 ± 0.05	73.1 ± 3.7
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	45.6 ± 2.2	0.59 ± 0.02	1.80 ± 0.05	41.6 ± 0.9
	None	2.22 ± 0.02	4.01 ± 0.02	91.0 ± 3.4	2.06 ± 0.05	3.92 ± 0.04	81.9 ± 1.8
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	47.9 ± 1.5	0.90 ± 0.01	2.73 ± 0.01	45.7 ± 0.2
	None	3.18 ± 0.01	5.87 ± 0.03	98.4 ± 2.5	3.04 ± 0.05	5.82 ± 0.02	87.0 ± 1.7
Clutch Size - No Insulation, 6°C Diel Cycle							
5 eggs	*Full*	0.73 ± 0.09	1.28 ± 0.61	78.6 ± 1.8	0.08 ± 0.01	0.24 ± 0.01	0.0 ± 0.0
	Half	1.52 ± 0.15	3.08 ± 0.05	88.7 ± 7.0	0.91 ± 0.15	2.57 ± 0.08	49.1 ± 9.3
	None	3.10 ± 0.16	5.75 ± 0.11	98.9 ± 1.9	2.73 ± 0.05	5.47 ± 0.09	81.7 ± 2.2
10 eggs	*Full*	0.69 ± 0.19	1.19 ± 0.17	76.1 ± 26.5	0.08 ± 0.01	0.44 ± 0.31	0.0 ± 0.0
	Half	1.48 ± 0.22	3.19 ± 0.29	84.7 ± 21.2	0.86 ± 0.03	2.56 ± 0.09	45.5 ± 0.5
	None	3.37 ± 0.14	5.88 ± 0.12	100.0 ± 0.0	2.95 ± 0.07	5.59 ± 0.07	89.1 ± 2.2
15 eggs	*Full*	0.73 ± 0.19	1.15 ± 0.30	72.7 ± 13.1	0.08 ± 0.01	0.25 ± 0.04	0.0 ± 0.0
	Half	1.48 ± 0.14	3.22 ± 0.19	81.4 ± 10.4	0.84 ± 0.03	2.56 ± 0.04	45.6 ± 1.0
	None	3.39 ± 0.07	5.94 ± 0.08	100.0 ± 0.0	3.01 ± 0.01	5.68 ± 0.03	90.7 ± 0.7
Clutch Size - Insulation, 6°C Diel Cycle							
5 eggs	*Full*	1.0 ± 0.4	2.0 ± 1.0	82.4 ± 0.06	0.3 ± 0.4	1.0 ± 1.3	14.5 ± 0.3
	Half	1.8 ± 0.7	3.9 ± 1.4	84.2 ± 0.04	1.4 ± 0.9	3.5 ± 1.6	50.1 ± 0.1
	None	9.1 ± 10.3	14.0 ± 14.0	100 ± 0.0	8.9 ± 10.6	13.8 ± 14.4	85.8 ± 0.1
10 eggs	*Full*	0.7 ± 0.2	1.2 ± 0.2	76.1 ± 0.3	0.08 ± 0.01	0.4 ± 0.3	0.1 ± 0.1
	Half	1.5 ± 0.2	3.2 ± 0.3	84.7 ± 0.2	0.86 ± 0.03	2.6 ± 0.09	45.5 ± 0.1
	None	3.4 ± 0.1	5.9 ± 0.1	100.0 ± 0.0	2.95 ± 0.07	5.6 ± 0.07	89.1 ± 0.1
15 eggs	*Full*	0.7 ± 0.2	1.2 ± 0.3	72.7 ± 0.1	0.08 ± 0.01	0.25 ± 0.03	0.0 ± 0.0
	Half	1.5 ± 0.1	3.2 ± 0.2	81.4 ± 0.1	0.84 ± 0.03	2.56 ± 0.04	45.6 ± 0.1
	None	3.4 ± 0.1	5.9 ± 0.1	100.0 ± 0.0	3.01 ± 0.01	5.68 ± 0.03	90.7 ± 0.1

Table 5.3. Artificial snake mean and variance data. Power supplied was the thermogenic limit put on the pseudoserpent (see text for details). We manipulated the amplitude of the diel cycle (2, 4, or 6 °C) as well as clutch size (5, 10, or 15 eggs), the latter in both insulated and non-insulated conditions (see text). All trials were run in triplicate and values are presented as mean ± SEM.

|		Power Supplied	Clutch Variance (°C)		Interface Variance (°C)	
	Diel Cycle					
		Full	0.001	± 0.001	0.000	± 0.000
	2°C	*Half*	0.105	± 0.008	0.123	± 0.005
		None	0.405	± 0.010	0.432	± 0.013
No Insulation, 10 egg		*Full*	0.002	± 0.001	0.001	± 0.000
	4°C	*Half*	0.419	± 0.047	0.470	± 0.024
		None	1.601	± 0.048	1.722	± 0.028
		Full	0.005	± 0.005	0.001	± 0.000
	6°C	*Half*	0.983	± 0.067	1.091	± 0.013
		None	3.608	± 0.059	3.840	± 0.037
	Clutch Size					
		Full	0.064	± 0.053	0.003	± 0.001
	5 eggs	*Half*	0.921	± 0.047	0.921	± 0.047
		None	3.276	± 0.241	3.276	± 0.241
No Insulation, 6 °C		*Full*	0.040	± 0.011	0.004	± 0.001
	10 eggs	*Half*	0.989	± 0.072	0.922	± 0.069
		None	3.063	± 0.043	3.397	± 0.152
		Full	0.074	± 0.046	0.003	± 0.001
	15 eggs	*Half*	1.022	± 0.060	0.886	± 0.048
		None	3.136	± 0.055	3.424	± 0.038
	Clutch Size					
		Full	0.104	± 0.079	0.023	± 0.012
	5 eggs	*Half*	0.920	± 0.081	0.896	± 0.075
		None	2.987	± 0.112	3.433	± 0.027
Insulation, 6 °C		*Full*	0.091	± 0.092	0.022	± 0.013
	10 eggs	*Half*	0.901	± 0.052	0.911	± 0.089
		None	2.866	± 0.141	3.223	± 0.176
		Full	0.064	± 0.013	0.019	± 0.009
	15 eggs	*Half*	0.691	± 0.544	0.639	± 0.540
		None	1.838	± 1.537	1.956	± 1.678
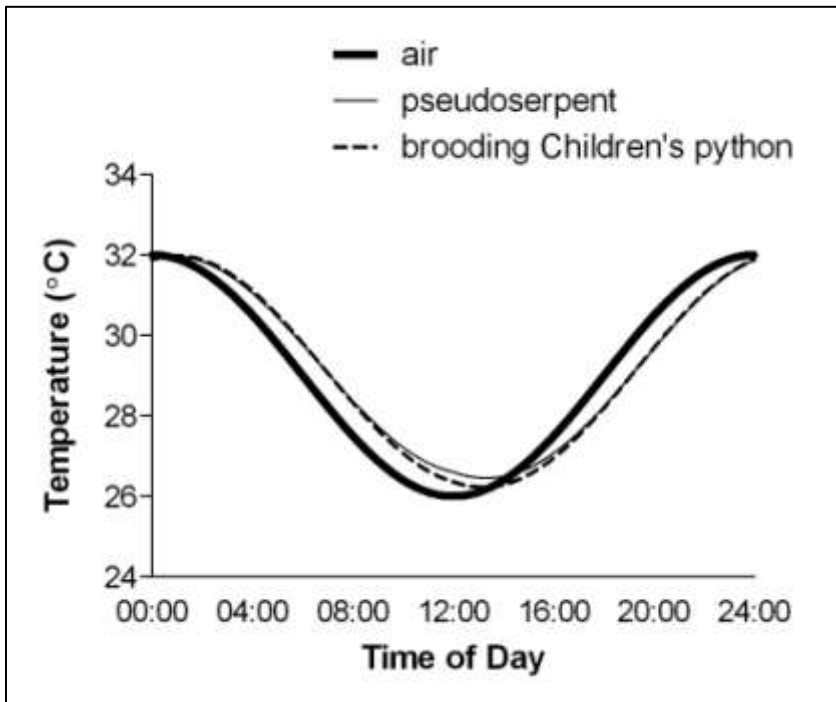
Fig. 5.1. Comparison of real and artificial snake. Comparison of clutch temperatures between a clutch being brooded by a female Children's python (*Antaresia childreni*) and an artificial clutch surrounded by a similarly sized pseudoserpent simultaneously maintained in a 6 °C diel cycle. Since *A. childreni* is ectothermic, the model was set to zero thermogenic potential to compare the insulating properties of the female and the pseudoserpent.
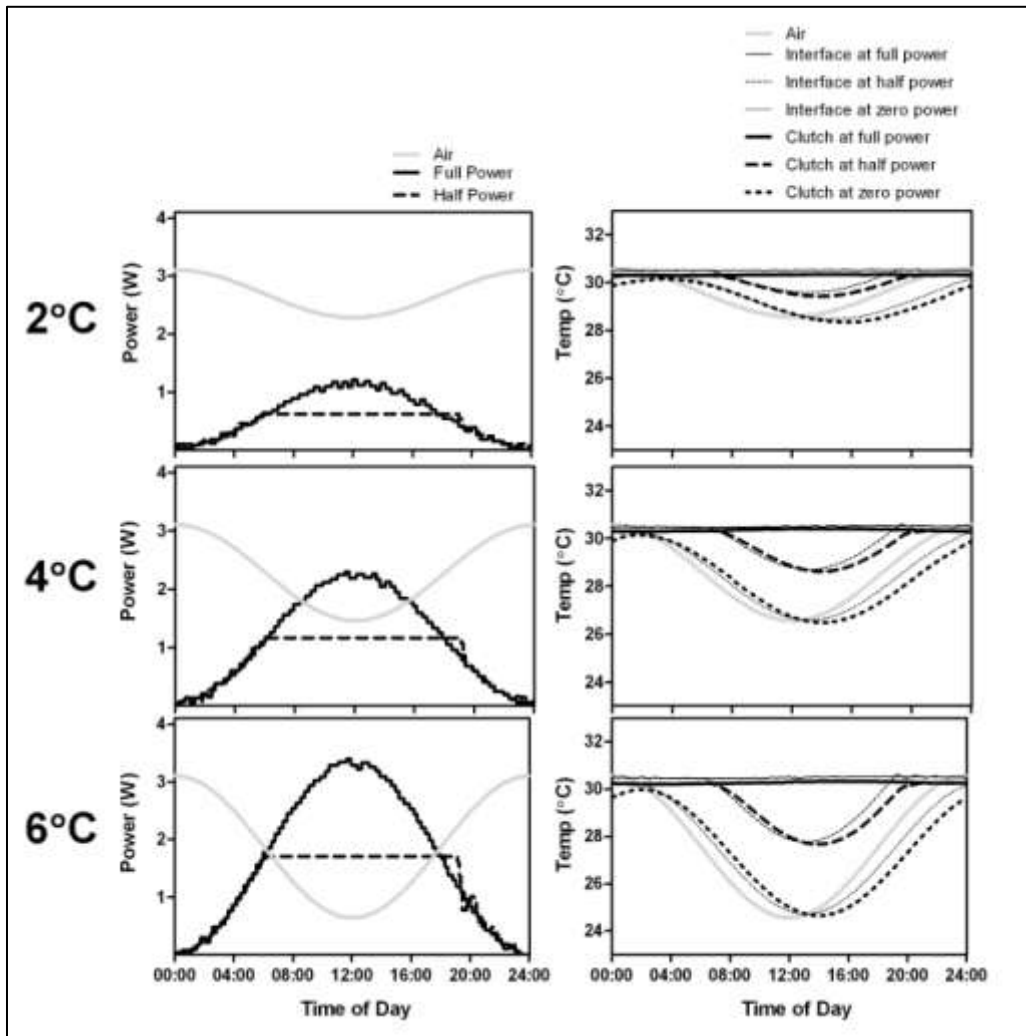
Fig. 5.2. Example of artificial snake trial. Example of power consumed and temperature data collected during trials using a pseudoserpent model with variable power potential (4 mW, half the maximum power required for clutch homeothermy, and no power) and variable thermal diel cycles (2, 4, and 6 °C) set to maintain interface temperature (the temperature at the contact zone between the pseudoserpent and artificial clutch) at 30.5 °C. Trials were run at different thermal diel cycles (amplitude of 2, 4, or 6 °C) and the pseudoserpent was programmed to have a heating potential of either power. Graphs on the left show the power used by the snake when it was provided full power potential and half power potential. Graphs on the right show the artificial clutch temperature (T_{Cltch}) and the temperature at the interface between the pseudoserpent and clutch (T_{Int}) during the same cycles.

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