Effect of nest temperature on egg-brooding dynamics in Children's pythons

Zachary R. Stahlschmidt *, Dale F. DeNardo

School of Life Sciences, Arizona State University, Tempe, AZ 85287-4601, United States

ARTICLE INFO

ABSTRACT

Parental care meets several critical needs of developing offspring. In particular, egg brooding in pythons is an exceptional model for examining environmental influences on specific parental behaviors because brooding behaviors are dynamic yet simple and modulate embryonic temperature, respiration, and water balance. We used captive Children's pythons (*Antaresia childreni*) to assess their facultative endothermic capability, the influence on egg-brooding behaviors of the gradient between the nest temperature (T_{nest}) and clutch temperature (T_{clutch}), and the effect of these behaviors on the developmental micro-environment. We monitored maternal egg-brooding behavior, rates of brooding unit (i.e., female and associated clutch) respiratory gas exchange, T_{nest}, T_{clutch}, and intra-clutch oxygen tension (PO_2clutch) during acute changes among four incubation temperature conditions: constant preferred temperature (31.5 °C); 'cooling' ($T_{nest} < T_{clutch}$); constant cool temperature (25.6 °C); and 'warming' ($T_{nest} > T_{clutch}$). We demonstrated that *A. childreni* are not facultatively endothermic because brooding unit temperature coefficient (Q_{10}) for V_{O2} and V_{CO2} was similar to other ectothermic boid snakes (1.9–5.7) and T_{clutch} conformed to T_{nest} at the constant, cool temperature treatment. Females coiled tightly around eggs more often during cooling than during warming. Further, the amount of time that females spent tightly coiled during warming significantly affected the T_{nest}–T_{clutch} gradient. Together these results indicate that, although female *A. childreni* are not facultatively endothermic, they are capable of assessing the T_{nest}–T_{clutch} gradient and making behavioral adjustments to enhance the thermal micro-environment of their developing offspring.

1. Introduction

Parental care is a widespread behavior adopted by many vertebrates, especially endothermic species, and has thus received considerable attention [1,2]. Parental care meets many critical needs of the offspring during development, such as water balance, energy, and temperature regulation [1,3]. Therefore, parental care usually consists of a suite of distinct parental behaviors that can vary in relative importance with environmental factors like temperature [4], availability of food [5], season [6], and hydric conditions [7].

Although common in fishes and amphibians, parental care is relatively limited among reptiles [1,8]. One exceptional, yet understudied, case of post-oviposition parental care among squamates (lizards and snakes) is egg-brooding behavior exhibited by all python species (family Pythonidae), in which the female coils around her clutch during incubation [9–11]. Python egg brooding is a dynamic process, wherein the brooding female spends most of the time tightly coiled around her eggs, but periodically loosens her coils [12]. This balance between tight coiling and postural adjustments during brooding limits embryonic water loss while allowing sufficient respiratory gas exchange between the clutch and nest environment [12,13]. However, despite a more than 5-fold increase in respiratory gas exchange requirements by developing

embryos during incubation, female Children's pythons (*Antaresia childreni*) do not increase the frequency or duration of postural adjustments with incubation stage [12,13]. This lack of an adjustment to the changing metabolic needs of the embryos suggests that parental behaviors of female pythons are fixed or that behavior is dependent on competing critical incubation variables (e.g., respiration and water balance).

In some pythons (e.g., *Python molurus* and *Morelia spilota*), females are facultatively endothermic during egg brooding and this enhanced metabolic capability provides considerable thermal benefits to the developing embryos [9,14]. However, not all pythons are facultatively endothermic, and the extent to which egg brooding alone provides a thermal benefit through insulation or thermal inertia has not been quantified. Additionally, nest temperature can influence a female python's decision whether or not to brood her eggs for the full incubation period [15]. Although nest-attending bumble bees (*Bombus bifarius nearcticus*) [16] and hooded plovers (*Thinornis rubricollis*) [4] modulate their parental behaviors in response to nest temperature (T_{nest}), it is not known if T_{nest} alters the frequency or duration of postural adjustments of egg-brooding pythons.

The relationship between temperature and egg-brooding behavior is of great interest because reptile eggs are greatly affected by changes in developmental temperature [17,18]. For example, water python (*Liasis fuscus*) eggs incubated in ecologically relevant diel temperature cycles (i.e., 27.1°–32.9 °C and 24.3°–32.9 °C) exhibit reduced developmental,

morphological, and locomotor performance metrics relative to those reared in warm, constant thermal regimes (i.e., 32.0 °C) [19]. Thus, our study aimed to (1) confirm that our study species, A. childreni, is non-endothermic, (2) identify behavioral or physiological responses elicited by changes in the thermal environment of the nest, and (3) determine the extent to which egg brooding influences clutch temperature during shifts in nest temperature. We simultaneously monitored maternal behavior, rates of brooding unit (i.e., female and associated clutch) respiratory gas exchange, nest temperature (T_{nest}), clutch temperature (T_{clutch}), and intra-clutch oxygen tension (PO_2clutch) during four incubation temperature conditions: constant preferred temperature (31.5 ± 0.1 °C) [20]; 'cooling' ($T_{nest} < T_{clutch}$); constant cool temperature (25.6 ± 0.1 °C); and 'warming' ($T_{nest} > T_{clutch}$) (Fig. 1).

In this study, we tested a hypothesis based on embryonic thermoregulation to explain female brooding behavior. We propose that egg-brooding pythons will alter their posturing patterns to enhance the thermal environment of their developing embryos. We predict that female pythons will be more tightly coiled around their eggs during the cooling phase to reduce heat loss from the clutch, and less tightly coiled during the warming phase to facilitate embryonic heat gain from the warming nest environment. Because there will be no heat loss or gain from the clutch when nest temperature is constant (assuming A. childreni are not facultatively endothermic), we predict that behavior variables would not significantly differ between the two constant temperature conditions despite their nearly 6 °C difference. Alternatively, our null hypothesis is that temperature, differential or absolute, does not influence egg-brooding behavior. Support for null hypothesis would imply that python egg-brooding behavior patterns are fixed and the female is incapable of sensing or responding to changes in environmental conditions. Results can clarify the adaptive significance of python egg brooding, a parental care model that can elucidate the proximate factors involved in more complicated nest-attending parental care systems (e.g., rodents, birds, crocodilians, teleost fish, and social insects).

2. Materials and methods

2.1. Study species and reproductive husbandry

We used a long-term captive colony of A. childreni maintained at Arizona State University (ASU) for this study. A. childreni are non-venomous, constricting snakes that inhabit rocky areas in northern
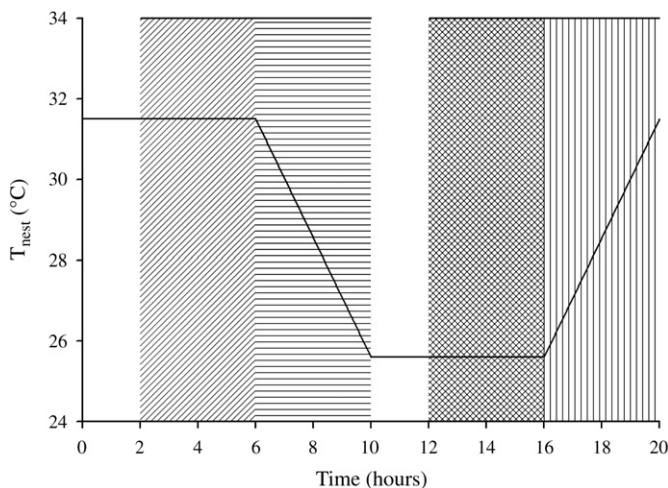

Fig. 1. Nine A. childreni brooding units were monitored during a sequence of four experimental temperature conditions: constant preferred incubation temperature (diagonal shading); cooling (horizontal shading); constant cool temperature (cross-hatched shading); and warming (vertical shading). We did not use the first 2 h of data for the two constant temperature conditions in order to allow time for stabilization of that condition.

Australia [21]. Husbandry and breeding of the animals followed that described previously [22]. All procedures used in this study were approved by the ASU Institutional Animal Care and Use Committee (protocol #05-792R).

As described previously [12], a few days prior to oviposition, we moved each gravid python into a Teflon-coated 1.9 l brooding chamber that was opaque on the bottom and sides, but transparent on the top to allow observation. Because clutches of naturally brooding pythons have minimal (if any) contact with substrate [21] and because substrate would provide a sink for gases that would interfere with data interpretation, females and their resultant clutches were not provided any substrate. We placed brooding chambers in an environmental chamber that had a 14:10 L:D photo regime and maintained temperature at 31.5 ± 0.3 °C to preclude the need for any behavioral thermoregulation by females. Additionally, we plumbed brooding chambers with two 3-way stopcocks on opposite sides of the chamber and used a valve-controlled aeration system that combined vapor-saturated air (via bubbling air through a water-filled hydrating column) with dry air to deliver 20–40 ml•min^{-1} of hydrated air (absolute humidity, AH = 25.1–26.7 g•m^{-3}; relative humidity, RH = 80–85%) to each brooding chamber. We housed brooding females in these chambers prior to and during experimental trials to minimize disturbance and avoid clutch abandonment. At oviposition, we briefly removed each female from her clutch to determine clutch size, clutch mass, and female post-oviposition mass.

2.2. Experimental procedure

To test our hypothesis, we evaluated real-time egg-brooding behavior, rates of brooding unit (i.e., female and respective clutch) oxygen consumption ($\dot{V}_{O2}$) and carbon dioxide production ($\dot{V}_{CO2}$), T_{nest}, T_{clutch}, and PO_2clutch of nine A. childreni brooding units (mean female mass = 334.9 ± 11.0 g, mean clutch size = 10 ± 1 eggs, mean absolute clutch mass = 125.3 ± 9.7 g, mean relative clutch mass (i.e., 100•clutch mass / maternal mass) = 37.9 ± 3.5%) using a flow-through system during a 16-h sequence through four temperature conditions: 31.5 °C, cooling (1.5 °C h^{-1}), 25.6 °C, and warming (1.5 °C h^{-1}) (Fig. 1). Because field data do not exist for A. childreni nests, we created a thermal regime that represents a contracted diel cycle of the temperatures measured in the nests of L. fuscus [15], a python species sympatric with A. childreni (Fig. 1). We conducted all trials 5 to 7 d post-oviposition. To avoid disturbance, we monitored trials in darkness with an infrared camera and recorded real-time video for later analysis of brooding behavior variables as previously described [12,13].

Experimental trials were conducted in a modified 150 l cooler controlled by a datalogger (21X, Campbell Scientific Instruments, Logan, UT) that produced the thermal cycle. As previously described [12,13], influent air of known gas composition and flow rate was created by combining dry, acapnic air (CDA 1112, PureGas, Broomfield, CO) with water vapor-saturated air (produced by bubbling dry air through a water-filled hydrating column) using a feedback-controlled system. Resulting influent air was humidified to a 25.0 °C dewpoint and maintained at a flow rate of 560 ml•min^{-1} with a mass flow controller (Unit Instruments, Inc., Yorba Linda, CA) that we calibrated before the study using soap-film flow meters. We analyzed a baseline sample of influent air immediately before and after brooding trials and averaged the two to determine the composition of influent air (the difference between initial and final baseline samples was negligible at 0.0039 ± 0.00078%). Air exiting each chamber (effluent air) was passed through a precision hygrometer (RH200, Sable Systems, Las Vegas, NV), and dried by anhydrous $CaSO_4$ before flowing through a CO_2 analyzer (LI-6252, Li-Cor Biosciences, Lincoln, NE) and an O_2 analyzer (FC-1B, Sable Systems, Las Vegas, NV) that we calibrated to the manufacturer's specifications prior to experimental use. During trials, we recorded the O_2, CO_2, and H_2O concentrations of effluent air every minute using a Campbell 23X datalogger. We converted these

raw data to $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ using Eqs. (1)–(6) in Walsberg and Hoffman [23]. This flow-through respirometry system has demonstrated an accuracy of 0.4% in determining steady-state $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ [24].

We measured P_{O2}clutch in real-time for the duration of trials with a fiber optic O_2 probe and meter (OxyMini, World Precision Instruments, Sarasota, FL), and we recorded resultant data every minute with the 23X datalogger. We inserted a 4 mm diameter O_2 probe through a port in the floor of each brooding chamber 1.5–2 cm into the intra-clutch space. Under the conditions used, the O_2 probe had an accuracy of 0.19 kPa, a resolution of 0.06–0.12 kPa, a response time of 40 s, and consumed no O_2. Prior to each trial, we used the two-point (i.e., 0% and 20.95% O_2) calibration procedure recommended by the O_2 probe manufacturer. We fixed a Type-T thermocouple to the O_2 probe, interfaced it with the 23X datalogger, and used it to measure real-time T_{clutch}. We positioned another Type-T thermocouple 1–2 cm inside each brooding chamber's influx port, interfaced it with the 23X datalogger, and used it to measure T_{nest} in real-time. Together, this allowed us to determine the T_{nest}–T_{clutch} gradient (i.e., the difference between mean T_{nest} and mean T_{clutch}) at each temperature condition.

2.3. Metabolic, thermal, and behavioral analyses

We used two methods to determine if *A. childreni* females were facultatively endothermic during egg brooding. The energetic cost of endothermy is substantially higher than that of ectothermy, and early stage *A. childreni* eggs comprise just one-third of brooding unit respiratory gas exchange [12]. Thus, in direct contradiction to the metabolic sensitivity to ambient temperature of obligate ectotherms, brooding unit $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ would be significantly higher when T_{nest} is 25.6 °C compared to when it is 31.5 °C if egg brooding *A. childreni* were facultatively endothermic. To indirectly determine the endothermic capability of egg-brooding *A. childreni*, we measured the temperature coefficient (Q_{10}) of metabolism, the factor by which $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ increase for every 10 °C rise in T_{nest}. In addition, we directly determined the females' abilities to regulate the temperature of their respective clutches when they experienced a cool temperature by measuring T_{clutch} when T_{nest} was held constant at 25.6 °C.

As described previously [12,13], we categorized egg brooding into several behaviors. We defined tight brooding to be when a female was motionless and tightly coiled around her clutch. We considered postural adjustments as individual behavioral events only if they were >30 s removed from another postural adjustment. Subtly distinct postural adjustments are categorized into three types. (1) Non-opening adjustments (NA) are those in which female movement is noted but none of the clutch is visibly exposed. (2) Opening adjustments (OA) involve female movement with visible exposure of some of the clutch, last less than 5 min, and do not entail a female's snout breaching the perimeter of her outermost coil. (3) Exploratory adjustments (EA) are postural adjustments that also involve visible exposure of the clutch; however, they last longer than 5 min or entail the female's snout breaching the perimeter of her outermost coil. EA are distinguished from OA because during EA females often insert their heads between their eggs and their coils suggesting a different behavioral motivation (e.g., possibly egg inspection) than that of OA. Although not noted in previous studies, we define loose coiling to be when a female is motionless and coiled around her clutch in such a manner that two or more eggs and an area of the clutch larger than the size of her head (3–4 cm²) is exposed.

2.4. Statistical analyses

Data met the appropriate statistical assumptions of parametric statistics or were transformed as necessary, and were analyzed using JMP IN (version 5.1.2, SAS Statistical Institute, Inc., Cary, NC). Significance was determined at $\alpha < 0.05$ for all tests. To determine the overall effect of treatment (i.e., temperature condition), repeated-measures analysis of variance (rmANOVA) tests were used. *Post-hoc* analyses used Bonferroni-corrected paired *t* tests. To determine the treatment effect between specific predicted pairs (e.g., tight coiling between constant 31.5 °C and 25.6 °C treatments), we used paired *t* tests. To test relationships within individuals, we used simple linear regression analysis. All results refer to comparisons within nine individuals (i.e., $n = 9$), and values are displayed as mean ± S.E.M.

3. Results

Respiratory exchange ratios (i.e., RER, $\dot{V}_{CO2}/\dot{V}_{O2}$) did not significantly differ ($t_8 = 0.76$; $P = 0.47$) between the constant 25.6 °C (RER: 0.79 ± 0.03) and 31.5 °C (RER: 0.82 ± 0.04) conditions. Mean rates of respiratory gas exchange for brooding units increased with T_{nest} (Fig. 2, Table 1). To further refute facultative endothermy by egg-brooding *A. childreni*, the T_{nest}–T_{clutch} gradient during the constant 25.6 °C phase was miniscule (i.e., 0.11 ± 0.02 °C).

Temperature condition significantly affected the amount of time that females spent tightly coiled around their clutches (Table 1). *Post-hoc* analyses revealed no difference for any metric of behavior between constant 25.6 °C and 31.5 °C conditions. However, females spent more time tightly coiled during cooling than during warming, while constant temperature conditions were intermediate (Fig. 3). Paired analysis ($t_8 = 3.3$; $P = 0.011$) revealed that females spent more time adjusting
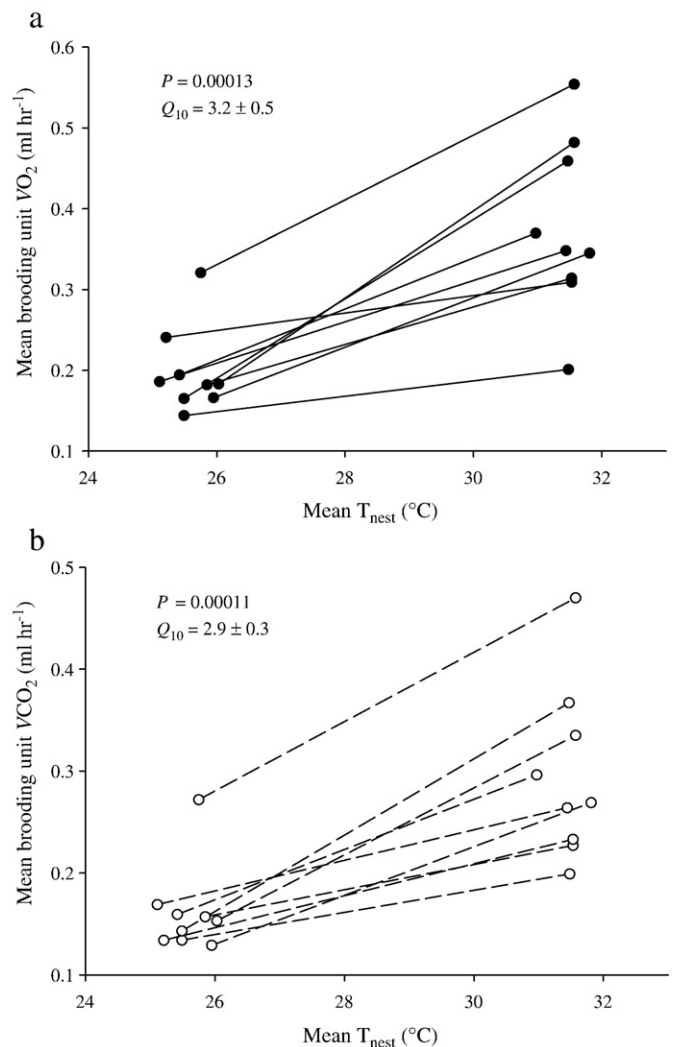

Fig. 2. Effect of constant temperature condition on mean brooding unit. a) Oxygen consumption rate ($\dot{V}_{O_2}$) and associated temperature coefficient (Q_{10}); and b) carbon dioxide production rate ($\dot{V}_{CO_2}$) and associated Q_{10} ($n = 9$).

Table 1
Antaresia childreni maternal brooding behavior, nest-to-clutch thermal relations, respiratory gas exchange rates, and intra-clutch oxygen partial pressure during four temperature conditions (mean ± S.E.M.).

	31.5 °C	Cooling	25.6 °C	Warming	F	P
Time spent loosely coiled (%)	9.9 ± 7.3	3.1 ± 2.9	20.5 ± 12.5	15.9 ± 7.7	1.6	0.23
Time spent adjusting posture (%)	5.7 ± 1.5	4.4 ± 1.5	9.3 ± 2.6	18.9 ± 4.2	3.6	0.086
Non-opening adjustment (NA) rate (per h)	2.3 ± 0.4	2.5 ± 0.4	1.0 ± 0.2	3.0 ± 0.6	3.6	0.087
NA duration (s)	21.6 ± 5.7	15.3 ± 2.8	19.5 ± 6.2	18.2 ± 5.3	0.25	0.86
Opening adjustment (OA) rate (per h)	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	1.9 ± 0.4	2.3	0.18
OA duration (s)	49.3 ± 10.2	51.7 ± 9.6	44.0 ± 9.4	75.5 ± 20.0	1.2	0.38
Exploratory adjustment (EA) rate (per h)	0.4 ± 0.1	0.4 ± 0.3	0.5 ± 0.2	1.2 ± 0.3	2.0	0.22
EA duration (s)	247.0 ± 44.8	175.0 ± 38.8	649.0 ± 134.0	458.0 ± 117.8	0.15	0.93
O_2 consumption rate (ml·h^{-1})	22.5 ± 2.1	21.1 ± 1.9	11.9 ± 1.1	14.8 ± 2.1	30	<0.0001
CO_2 production rate (ml·h^{-1})	17.7 ± 1.7	15.6 ± 1.8	9.7 ± 0.9	13.1 ± 0.9	25	<0.0001
T_{nest}–T_{clutch} gradient (°C)	0.03 ± 0.01	− 1.52 ± 0.13	0.11 ± 0.02	1.42 ± 0.11	110	<0.0001
PO_2clutch (kPa)	18.78 ± 0.10	19.14 ± 0.09	19.53 ± 0.11	19.29 ± 0.13	71	<0.0001

their posture during warming (18.9 ± 4.2%) than during cooling (4.4 ± 1.5%). Because embryonic metabolic rate increases with temperature and females did not change their rate of ventilating behaviors between 25.6° and 31.5 °C, PO_2clutch was negatively related to T_{nest} (Table 1).

During warming, brooding behavior impacted developmental temperature since the amount of time that females spent tightly coiled was negatively correlated to the T_{nest}–T_{clutch} gradient ($R^2 = 0.45$; $F_{1,7} = 5.7$; $P = 0.049$). Absolute clutch mass was positively correlated to the absolute value of the T_{nest}–T_{clutch} gradient during both cooling ($R^2 = 0.77$, $F_{1,7} = 23$; $P = 0.0019$) and warming ($R^2 = 0.55$; $F_{1,7} = 8.5$; $P = 0.023$) conditions, demonstrating that the clutch itself provides thermal inertia. Relative clutch mass was also positively correlated to the T_{nest}–T_{clutch} gradient during both cooling ($R^2 = 0.53$; $F_{1,7} = 7.8$; $P = 0.027$) and warming ($R^2 = 0.52$; $F_{1,7} = 7.7$; $P = 0.028$) conditions.

4. Discussion

Often referred to as shivering thermogenesis, facultative endothermy during egg brooding and its thermal benefits have been demonstrated in only two python species: *P. molurus* (17.1 kg body mass) [9] and *M.s. spilota* (6.6 kg body mass) [14]. We have clarified the temperature-related patterns of metabolism and behavior in egg-brooding *A. childreni*. The Q_{10} values for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in *A. childreni* brooding units (mean: 3.1; range: 1.9–5.7) approximate those of other, non-reproductive boa and python species (mean = 2.6) [25]. In further support of our prediction that *A. childreni* are not facultatively endothermic, we demonstrated that egg-brooding females were unable to maintain an elevated T_{clutch} when experiencing a constant cool T_{nest} (Table 1). In larger python species, endothermy comprises a substantial portion of females' energy expenditure while egg brooding at cool temperatures (*P. molurus*: 92%, *M.s. spilota*: 95%) [9,14]. Thus, the tremendous additional energetic cost of maintaining homeothermy at cool ambient temperatures for small-bodied pythons with high surface area-to-volume ratios and less reserve energy, such as *A. childreni*, may make facultative endothermy unfeasible.

Parental care provided by female pythons modulates critical and often competing developmental variables such as clutch temperature [9,10,15], water balance [12,22], and respiration [13]. A tightly coiled posture is central to regulating these variables and, thus, integral to the embryonic thermoregulation hypothesis we proposed and tested with *A. childreni*. Because the amount of time that females spent tightly coiled changed with shifts in temperature, the hard-wired (null) hypothesis was not supported (Table 1), which suggests that egg-brooding pythons use external information to enhance the embryonic micro-environment. Not unexpectedly, the ability of parents to use cues from the embryonic micro-environment for behavioral modification has been shown in other taxa as well [26,27]. In particular, nest-attending thermoregulatory behaviors are influenced by T_{nest} in a number of species, such as bumble bees (*B.b. nearcticus*) [16], greater snow geese (*Anser caerulescens atlantica*) [28], and hooded plovers (*T. rubricollis*) [4]. In fact, the embryonic thermoregulation hypothesis, which asserts that

egg-brooding behavior is influenced by the T_{nest}–T_{clutch} gradient, was supported by our results. Females coiled tightly more often during cooling and less often during warming (xFig. 3). In addition to the effect of temperature on the relative use of different brooding behaviors in the laboratory, free-ranging female *L. fuscus* use T_{nest} to determine the duration of egg brooding ('cool' nests: mean = 53.8 d; 'warm' nests: mean = 6.5 d) [15]. Thus, it appears that both short- (e.g., postural adjustments) and long-term (e.g., duration of clutch attendance) maternal decisions are influenced by thermal conditions in egg-brooding pythons.

In addition to incubation temperature, other variables are critical to embryonic development and could thus influence egg-brooding behavior in pythons. Prairie skinks (*Eumeces septentrionalis*) and Malayan pit vipers (*Calloselasma rhodostoma*) modulate their egg-attending behavior in response to shifts in incubation humidity [7,29]. Further, non-brooded *A. childreni* eggs are extremely sensitive to desiccation with relative humidity (RH) as high as 75–80% leading to no successful hatching of non-brooded eggs [22]. However, egg brooding increases hatching success at 75–80% RH to 80% [22]. By maintaining a constant 25.0 °C nest dewpoint during all of the temperature conditions, our study created high (i.e., 31.5 °C: 1.45 kPa) and low (i.e., 25.6 °C: 0.11 kPa) clutch–nest vapor pressure gradients. However, because both temperature and vapor pressure were altered between treatments, the influence of nest humidity on egg-brooding behavior was not directly tested. Future studies should hold T_{nest} constant and alter nest dewpoint to better examine the role of the clutch–nest vapor pressure gradient on egg-brooding behavior in pythons. Like python egg-brooding postural adjustments [13], fin-fanning parental behavior exhibited by some fish increases oxygen in the eggs' micro-environment [26,27]. However, while fin-fanning is negatively correlated with dissolved oxygen levels
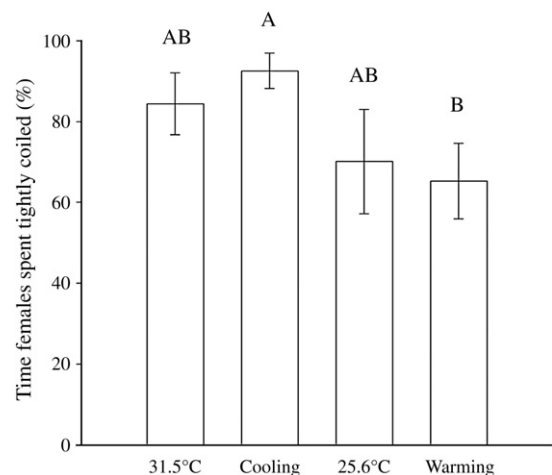

Fig. 3. Effect of temperature condition on the amount of time females spent tightly coiled ($n = 9$).

[27], egg-brooding female *A. childreni* do not alter their tight coiling behavior as incubation progresses and as embryonic metabolic rate increases substantially [12,13]. As a result, oxygen concentration within the clutch diminishes as incubation progresses and the low PO_2 entails short-term costs (i.e., reduced embryonic metabolism) [13] and long-term costs (i.e., smaller, weaker offspring) [30]. The longer term effects of such hypoxic conditions remain unknown and warrant study. Thus, although water balance, respiration, and thermoregulation are each important to development, temperature appears to be the primary variable upon which python egg-brooding behavior is regulated.

The role of temperature in regulating parental behaviors is not unexpected since metrics critically related to offspring fitness in reptiles (e.g., hatchability and hatchling growth rate), as well as sex determination in many species, are significantly affected by changes in incubation temperature [18,31]. We have quantified the degree of thermal buffering between the clutch and nest environment provided by egg brooding. Though not dramatic, the mean (1.47 °C) and maximum (3.39 °C) $T_{nest}-T_{clutch}$ gradient during warming and cooling periods likely have a significant impact on development. For example, incubation duration is temperature dependent and considered to be the strongest indicator of neonate survival in free-ranging *L. fuscus* [15]. Also, incubation duration is significantly affected by a 1 °C change in incubation temperature in our colony of *A. childreni* (e.g., incubation at 30.5 °C compared to 31.5 °C extends incubation duration an average of 6 days; Stahlschmidt and DeNardo, unpublished). Thus, the ability of egg-brooding females to evaluate the $T_{nest}-T_{clutch}$ gradient to regulate behavior and enhance T_{clutch} could significantly benefit developing offspring. Although clutch mass better explains the variance in the $T_{nest}-T_{clutch}$ gradient during changes in T_{nest}, we have demonstrated that shifts in egg-brooding behavior during warming also significantly impact the $T_{nest}-T_{clutch}$ gradient in *A. childreni*.

Our results have further defined the physiological effects of T_{nest} on python brooding units, the role of the $T_{nest}-T_{clutch}$ gradient on egg-brooding behaviors, and the influence of these behaviors on the developmental micro-environment. The thermally, physiologically, and behaviorally quantifiable nature of the python egg-brooding system allowed us to perform a multi-faceted assessment of a simple parental care model. Farmer's reproductive model postulates that parental care played an integral role in the transitions from obligate ectothermy to facultative endothermy to obligate endothermy [2]; thus, a better understanding of python egg brooding may elucidate the role of parental care in the evolution of endothermy. Future studies should continue to examine the costs and benefits associated with python egg brooding for both ectothermic and facultatively endothermic species. Ecologically, further field studies are necessary to clarify the role of relevant factors (e.g., relative clutch characteristics, the $T_{nest}-T_{clutch}$ gradient, and nest humidity) on maternal python decision-making.

Acknowledgements

References

[1] Clutton-Brock TH. The evolution of parental care. Princeton: Princeton University Press; 1991.
[2] Farmer CG. Parental care: the key to understanding endothermy and other convergent features in birds and mammal. Amer Nat 2000;155:326–34.
[3] Deeming DC. Reptilian incubation: environment and behaviour. Cambridge: Cambridge University Press; 2004.
[4] Weston MA, Elgar MA. Parental care in Hooded Plovers (*Thinornis rubricollis*). Emu 2005;105:283–92.
[5] Dewey SR, Kennedy PL. Effects of supplemental food on parental-care strategies and juvenile survival of northern Goshawks. Auk 2001;118:352–65.
[6] Magee SE, Neff BD. Temporal variation in decisions about parental care in bluegill, *Lepomis macrochirus*. Ethology 2006;112:1000–7.
[7] Somma LA, Fawcett JD. Brooding behaviour of the prairie skink and its relationship to the hydric environment of the nest. Zool J Linn Soc 1989;95:245–56.
[8] Somma L. Parental behavior in Lepidosaurian and Testudinian reptiles: a literature survey. Malabar: Krieger Publishing Company; 2003.
[9] Vinegar A, Hutchison VH, Dowling HG. Metabolism, energetics, and thermo-regulation during brooding of snakes of genus Python (Reptilia, Boidae). Zoologica 1970;55:19–48.
[10] Slip DJ, Shine R. The reproductive biology and mating system of diamond pythons, *Morelia spilota*. Herpetologica 1988;44:396–404.
[11] Pearson DM, Shine R, Williams A. Thermal biology of large snakes in cool climates: a radio-telemetric study of carpet pythons (*Morelia spilota imbricata*) in south-western Australia. J Therm Biol 2003;28:117–31.
[12] Stahlschmidt ZR, Hoffman TCM, DeNardo DF. Postural shifts during egg-brooding and their impact on egg water balance in Children's pythons (*Antaresia childreni*). Ethology 2008;114:1113–21.
[13] Stahlschmidt ZR, DeNardo DF. Alternating egg-brooding behaviors create and modulate a hypoxic developmental micro-environment in Children's pythons (*Antaresia childreni*). J Exp Biol 2008;211:1535–40.
[14] Harlow P, Grigg G. Shivering thermogenesis in a brooding diamond python, *Morelia spilotes spilotes*. Copeia 1984;4:959–65.
[15] Madsen T, Shine R. Life history consequences of nest-site variation in tropical pythons. Ecology 1999;80:989–97.
[16] O'Donnell S, Foster RL. Thresholds of response in nest thermoregulation by worker bumble bees, *Bombus bifarius nearcticus* (Hymenoptera: Apidae). Ethology 2001;107:387–99.
[17] Angilletta MJ, Winters RS, Dunham ED. Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. Ecology 2000;81:2957–68.
[18] Birchard GF. Effects of incubation temperature. In: Deeming DC, editor. Reptilian incubation: environment and behaviour. Cambridge: Cambridge University Press; 2004. p. 103–24.
[19] Shine R, Madsen TRL, Elphick MJ, Harlow PS. The influence of nest temperatures and maternal brooding on hatchling phenotypes of water pythons. Ecology 1997;78:1713–21.
[20] Lourdais O, Heulin B, DeNardo DF. Thermoregulation during gravidity in the Children's python (*Antaresia childreni*): a test of the preadaptation hypothesis for maternal thermophily in snakes. Biol J Linn Soc 2008;93:499–508.
[21] Wilson S, Swan G. Reptiles of Australia. Princeton: Princeton Field Guides; 2003.
[22] Lourdais O, Hoffman TCM, DeNardo DF. Maternal brooding in the Children's python (*Antaresia childreni*) promotes egg water balance. J Comp Physiol B 2007;177:569–77.
[23] Walsberg GE, Hoffman TCM. Using direct calorimetry to test the accuracy of indirect calorimetry in an ectotherm. Physiol Biochem Zool 2006;79:830–5.
[24] Walsberg GE, Hoffman TCM. Direct calorimetry reveals large errors in respiro-metric estimates of energy expenditure. J Exp Biol 2005;208:1035–43.
[25] Chappell MA, Ellis TM. Resting metabolic rates in boid snakes: allometric relationships and temperature effects. J Comp Physiol B 1987;157:227–35.
[26] Green BS, McCormick MI. Oxygen replenishment to fish nests: males adjust brood care to ambient conditions and brood development. Behav Ecol 2005;16:389–97.
[27] Lissaker M, Kvarnemo C. Ventilation or nest defense—parental care trade-offs in a fish with male care. Beh Ecol Sociobiol 2006;60:864–73.
[28] Poussart C, Gauthier G, Larochelle J. Incubation behaviour of greater snow geese in relation to weather conditions. Can J Zool 2001;79:671–8.
[29] York DS, Burghardt GM. Brooding in the Malayan pit viper, *Calloselasma rhodostoma*: temperature, relative humidity, and defensive behavior. Herp J 1988;6:210–21.
[30] Stahlschmidt ZR, DeNardo DF. Obligate costs of parental care to offspring: Egg brooding induced hypoxia creates smaller, slower, and weaker python offspring. Biol. J. Linn. Soc. Lond. in press. doi:10.1111/j.1095-8312.2009.01280.x.
[31] Valenzuela N. Temperature-dependent sex determination. In: Deeming DC, editor. Reptilian incubation: environment and behaviour. Cambridge: Cambridge University Press; 2004. p. 211–26.