

## Parental behavior in pythons is responsive to both the hydric and thermal dynamics of the nest

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Accepted 31 January 2010

### SUMMARY

Parental behavior contributes to the success of a diverse array of taxa, and female-only nest attendance is particularly widespread. Python egg-brooding behavior is an intriguing example of female-only nest attendance because it significantly influences several critical developmental variables, namely embryonic predation, hydration, respiration and temperature. During brooding, females predominately adopt a tightly coiled posture that reduces the exchange of heat, water vapor, O<sub>2</sub> and CO<sub>2</sub> between the nest and clutch environment, which benefits egg water balance at the cost of respiration. To determine the plasticity of this important behavior, we manipulated nest temperature and humidity while monitoring nest–clutch thermal, hydric and respiratory relationships to test the hypothesis that female Children’s pythons (*Antaresia childreni*) modify their egg-brooding behavior due to an interaction between environmental thermal and hydric conditions. During moderate and high nest humidity treatments (23 and 32 g m<sup>-3</sup> H<sub>2</sub>O, respectively), females spent more time coiling tightly when the nest was cooling than when it was warming, which benefited clutch temperature. However, brooding females in low-humidity nest environments (13 g m<sup>-3</sup> H<sub>2</sub>O) showed a high frequency of tight coiling even when the nest was warming; thus, nest temperature and humidity had an interactive effect on egg-brooding behavior in support of our hypothesis. Our results also suggest that certain egg-brooding behaviors (i.e. postural adjustments) are more energetically costly to females than other behaviors (i.e. tight coiling). In sum, we provide empirical support for the adaptive plasticity of python egg-brooding behavior, which offers insight into the general significance of female-only nest attendance in animals.

Key words: adaptive plasticity, life history trade-offs, metabolism, parental care, snake, thermoregulation, water balance.

### INTRODUCTION

Parental care is instrumental to the success of a diverse array of taxa, and its broad evolutionary significance has been the motivation for substantial investigation (Williams, 1966; Trivers, 1972; Clutton-Brock, 1991; Gross, 2005). Among other benefits, nest-attending parents can increase their fitness by reducing embryonic predation [frogs (Townsend, 1986)], improving egg water balance [skinks (Somma, 1989)], thermoregulating embryos [bumblebees (Heinrich, 1979)], promoting embryonic respiration [fish (Lissaker and kVarnemo, 2006)], reducing pathogen infiltration of eggs [crickets (West and Alexander, 1963)] and provisioning offspring with food [birds (Clutton-Brock, 1991)]. As a result of this wide assortment of benefits, nest-attending behavior is taxonomically widespread (Clutton-Brock, 1991). While birds typically use bi-parental care, female-only parental care is the predominant mode of care in other internally fertilizing vertebrates [e.g. reptiles and mammals (Clutton-Brock, 1991)], including species within major taxa in which external fertilization predominates [i.e. fish and amphibians (Gross and Shine, 1981)], as well as terrestrial arthropods (Zeh and Smith, 1985).

Within the broad context of female-only nest attendance, python egg brooding has recently proven itself as a useful and relevant parental care model. Python egg brooding is simple, yet it affects several critical and quantitatively measurable developmental variables, including embryonic predation (Madsen and Shine, 1999), thermoregulation (Vinegar et al., 1970; Stahlschmidt and DeNardo, 2009a), water balance (Aubret et al., 2005a; Lourdais et al., 2007; Stahlschmidt et al., 2008) and respiration (Stahlschmidt and DeNardo, 2008). While the benefits of python egg brooding have

been well established, egg brooding limits nest–clutch respiratory gas exchange, which entails short-term metabolic and long-term phenotypic costs to offspring (Stahlschmidt and DeNardo, 2008; Stahlschmidt and DeNardo, 2009b). Thus, python egg brooding can be used to examine the physiological trade-offs of parental care.

We have shown in Children’s pythons [*Antaresia childreni* (Gray 1842)] that python egg brooding is dynamic. During egg brooding, females predominately adopt a tightly coiled posture that reduces the exchange of heat, water vapor, O<sub>2</sub> and CO<sub>2</sub> between the nest and clutch environment that benefits water balance at the cost of embryonic respiration (Stahlschmidt and DeNardo, 2008; Stahlschmidt and DeNardo, 2009a; Stahlschmidt et al., 2008). Hence, females periodically adjust their egg-brooding postures to facilitate nest–clutch gas (O<sub>2</sub> and H<sub>2</sub>O vapor) exchange to benefit respiration at the cost of embryonic water balance (Stahlschmidt and DeNardo, 2008; Stahlschmidt et al., 2008).

While postural adjustments are used to balance the various embryonic needs even under static environmental conditions, incubation conditions vary over time (e.g. diel temperature shifts). Thus, it would seem beneficial to the developing offspring if females adjusted the timing and duration of postural adjustments based on environmental inputs. However, while embryonic oxygen consumption increases dramatically over the course of development, female *A. childreni* do not alter the relative frequency or duration of their postural adjustments, which results in developmental hypoxia that reduces offspring size, speed and strength (Stahlschmidt and DeNardo, 2008; Stahlschmidt et al., 2008). Contrarily, we have recently shown that egg-brooding females are capable of assessing

the nest–clutch temperature gradient, and they make behavioral adjustments to enhance the thermal micro-environment of their developing offspring. That is, females coil tightly around eggs less often when the nest is warming compared with when it is cooling (Stahlschmidt and DeNardo, 2009a).

Because of the contrasting results found in female response to variation in environmental temperature and respiratory gas concentrations, we designed an experiment to evaluate female response to another critical variable associated with brooding – water balance. Thus, we designed a multifactorial experiment to assess the relative importance of environmental temperature and hydric condition, two variables critically important to embryonic python development (Shine et al., 1997; Lourdais et al., 2007). We manipulated nest temperature and humidity while monitoring nest–clutch thermal, hydric and respiratory relationships to test the hypothesis that females modify their egg-brooding behavior due to an interaction between environmental thermal and hydric conditions. Our results will specifically provide further understanding of the dynamic nature of python egg brooding and, in general, build upon existing knowledge of the environmental influences on parental behaviors.

## MATERIALS AND METHODS

### Study species and reproductive husbandry

For this study, we used a long-term captive colony of *A. childreni* maintained at Arizona State University (ASU), Tempe, AZ, USA. *Antaresia childreni* are non-venomous, constricting snakes that inhabit rocky areas in northern Australia (Wilson and Swan, 2003). Husbandry and breeding of the animals followed that described previously (Lourdais et al., 2007). All procedures used in this study were approved by the ASU Institutional Animal Care and Use Committee (protocol #08-967R).

As described previously (Stahlschmidt et al., 2008; Stahlschmidt and DeNardo, 2009a), we housed brooding females in substrate-free, Teflon-coated 1.9 l chambers a few days prior to oviposition through the completion of all experimental trials to minimize disturbance, avoid clutch abandonment and ensure the exchange of respiratory gases (i.e. H<sub>2</sub>O vapor, O<sub>2</sub> and CO<sub>2</sub>) to and from the brooding unit (i.e. female and associated clutch). When not being used in an experimental trial, these brooding chambers were kept in an environmentally controlled room that had a 14 h:10 h L:D photo regime and maintained temperature at the species' preferred developmental temperature (31.5±0.3°C) (Lourdais et al., 2008) to preclude the need for any behavioral thermoregulation by females. Also, we delivered 20–40 ml min<sup>-1</sup> of hydrated air [absolute humidity=25.1–26.7 g m<sup>-3</sup>; relative humidity (RH)=80–85%] to each brooding chamber using methods described previously (Stahlschmidt et al., 2008; Stahlschmidt and DeNardo, 2009a). At oviposition, we briefly removed each female from her clutch to determine clutch size, clutch mass and female post-oviposition mass.

### Experimental procedure

To test our hypothesis, we evaluated real-time egg-brooding behavior, rates of brooding unit oxygen consumption ( $\dot{V}_{O_2}$ ), carbon dioxide production ( $\dot{V}_{CO_2}$ ) and water loss ( $M_{H_2O}$ ), nest temperature ( $T_{nest}$ ) and clutch temperature ( $T_{clutch}$ ) of eight *A. childreni* brooding units [female mass (means ± s.e.m.): 359.5±20.4 g; clutch size: 9±1 eggs; absolute clutch mass: 101.6±7.3 g; relative clutch mass (i.e. 100 × clutch mass / maternal mass): 28.4±1.7%] using a flow-through system. We evaluated each brooding unit during consecutive 4-h treatments of four temperature conditions [31.5°C, cooling

(1.4°C h<sup>-1</sup>), 26.0°C, and warming (1.4°C h<sup>-1</sup>)], and we repeated these trials at three influent humidity conditions assigned in random order [dew points (DP) of 31°C, 25°C and 16°C (13, 23 and 32 g m<sup>-3</sup> H<sub>2</sub>O, respectively)] (Fig. 1). Because field data do not exist for *A. childreni* nests, we used a thermal regime that represents a contracted diel thermal cycle from nests of water pythons, *Liasis fuscus*, which are sympatric with *A. childreni* (Madsen and Shine, 1999). Further, we used humidity information from a database maintained by the Commonwealth of Australia's Bureau of Meteorology to create ecologically relevant nest humidity treatments. Previously, we determined that *A. childreni* brooding behavior does not have a diel cycle (Stahlschmidt et al., 2008), so the effect of the sequential treatments would not be confounded by time of day. Also, as egg-brooding behavior does not change over incubation (Stahlschmidt and DeNardo, 2008; Stahlschmidt et al., 2008), we conducted all trials <1 week 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 (Stahlschmidt et al., 2008; Stahlschmidt et al., 2009a).

We conducted experimental trials in a modified 150 l thermoelectric cooler controlled by a datalogger (21X, Campbell Scientific Instruments, Logan, UT, USA) that produced the thermal cycle. We created influent air of known humidity by bubbling building supply air through a heated water column and then sending the air through a condensation chamber held at the desired DP (e.g. 16°C, 25°C or 31°C DP). For the 31°C DP trials, the condensation chamber temperature mimicked that of the trial thermal cycle to maintain a saturated influent air without condensation on the eggs (Fig. 1). We verified the humidity of influent air with a precision hygrometer (RH100, Sable Systems, Las Vegas, NV, USA) positioned immediately upstream of the brooding chamber. We maintained an influent flow rate of 500 ml min<sup>-1</sup> with an upstream pressure regulator, an adjustable rotameter and a flow tube (FL-344, Omega Instruments, Stamford, CT, USA) that was calibrated under experimental conditions.

We analyzed a baseline sample of influent air immediately before and after brooding trials and averaged the two to determine the O<sub>2</sub> and CO<sub>2</sub> concentrations of influent air (the difference between initial and final baseline samples was negligible at 0.0042±0.00081%). Baseline air and air exiting each chamber (effluent air) was passed through a precision hygrometer (RH300, Sable Systems) and dried

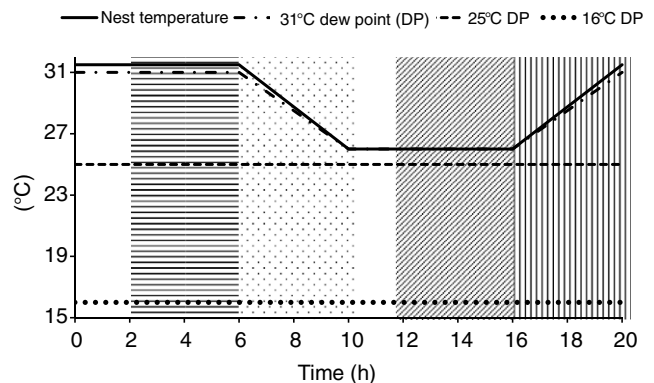


Fig. 1. Eight *Antaresia childreni* brooding units were monitored during a continuous sequence of four experimental temperature conditions [constant preferred incubation temperature (horizontal shading), cooling (dotted shading), constant cool temperature (diagonal shading) and warming (vertical shading)], and we repeated these trials under three humidity conditions [31°C dew point (DP) or vapor-saturated, 25°C DP and 16°C DP].

Table 1. The effects of temperature and humidity treatments on the percentage of time female *A. childreni* spent tightly coiled around their clutches (means  $\pm$  s.e.m.,  $N=8$ )

	Nest temperature condition				Nest-clutch temperature gradient		
	31.5°C	Cooling	26.0°C	Warming	Negative	Zero	Positive
Nest humidity							
16°C dew point (DP)	95.9 $\pm$ 1.4	95.8 $\pm$ 1.4	98.5 $\pm$ 0.3	94.7 $\pm$ 1.9	95.8 $\pm$ 1.4	97.2 $\pm$ 0.79	94.7 $\pm$ 1.9
25°C DP	91.5 $\pm$ 3.7	95.6 $\pm$ 0.8	90.6 $\pm$ 6.8	82.3 $\pm$ 6.4	95.6 $\pm$ 0.8	91.1 $\pm$ 4.5	82.3 $\pm$ 6.4
31°C DP	92.2 $\pm$ 2.8	95.2 $\pm$ 0.9	91.1 $\pm$ 3.6	70.5 $\pm$ 9.7	95.2 $\pm$ 0.9	91.7 $\pm$ 2.4	70.5 $\pm$ 9.7
Nest-clutch vapor pressure gradient							
Low (<0.01 kPa)	92.2 $\pm$ 2.8	95.2 $\pm$ 0.9	90.9 $\pm$ 3.7	70.5 $\pm$ 9.7	92.2 $\pm$ 2.8	91.4 $\pm$ 4.1	70.5 $\pm$ 9.7
Medium (0.01–1.45 kPa)	91.5 $\pm$ 3.7	95.6 $\pm$ 0.8	98.5 $\pm$ 0.3	82.3 $\pm$ 6.4	95.6 $\pm$ 0.8	95.0 $\pm$ 1.7	82.3 $\pm$ 6.4
High (1.46–2.82 kPa)	95.9 $\pm$ 1.4	95.8 $\pm$ 1.4	*	94.7 $\pm$ 1.9	95.8 $\pm$ 1.4	95.9 $\pm$ 1.4	94.7 $\pm$ 1.9

\*Could not be determined because animals did not experience this treatment.

by anhydrous CaSO<sub>4</sub> before flowing through a CO<sub>2</sub> analyzer (LI-6252, Li-Cor Biosciences, Lincoln, NE, USA) and an O<sub>2</sub> analyzer (FC-1B, Sable Systems) that we calibrated to manufacturer specifications prior to experimental use. During trials, we recorded the O<sub>2</sub>, CO<sub>2</sub> and H<sub>2</sub>O concentrations of effluent air every minute using a Campbell 23X datalogger. We converted these raw data to  $\dot{V}_{O_2}$ ,  $\dot{V}_{CO_2}$  and  $M_{H_2O}$  using equations 1–7 in Walsberg and Hoffman (Walsberg and Hoffman, 2006). This flow-through respirometry system has a demonstrated accuracy of 0.4% in determining steady-state  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  (Walsberg and Hoffman, 2005).

To measure real-time clutch temperature ( $T_{clutch}$ ), we positioned a Type-T thermocouple 1–2 cm into the clutch using an access port in the bottom of the brooding chamber, and interfaced it with the 23X datalogger (Campbell Scientific Instruments). We positioned another Type-T thermocouple 1–2 cm inside each brooding chamber's influx port, interfaced it with the Campbell 23X datalogger, and used it to measure nest temperature ( $T_{nest}$ ) in real-time. Together, these temperatures allowed us to determine the  $T_{nest}-T_{clutch}$  gradient (i.e. the difference between mean  $T_{nest}$  and mean  $T_{clutch}$ ) at each treatment. Given the dynamic nature of our experimental design, we were able to determine the effect(s) of several thermal and hydric variables on egg-brooding behavior. In addition to  $T_{nest}$  condition (i.e. 31.5°C, cooling, 26.0°C and warming) and nest humidity (31°C, 25°C and 16°C DP), we evaluated the effect(s) of the  $T_{nest}-T_{clutch}$  gradient (i.e. negative, zero and positive) and the nest-clutch vapor pressure gradient [i.e. low (<0.01 kPa), medium (0.01–1.45 kPa) and high (1.46–2.82 kPa)] on brooding behavior. This multi-factor approach allowed us to determine the relative importance of each variable on brooding behavior.

As described previously, we categorized egg brooding into two behavior types that are strongly associated with nest-clutch thermal, hydric and respiratory dynamics (Stahlschmidt and DeNardo, 2008; Stahlschmidt and DeNardo, 2009a; Stahlschmidt et al., 2008). 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.

#### Statistical analyses

We verified that our data met the appropriate statistical assumptions of parametric statistics, or we transformed them as necessary. We analyzed all data with SPSS (version 15, SPSS, Inc., Chicago, IL, USA) and determined significance at  $\alpha < 0.05$  for all tests. To determine the independent and interactive effects of treatment (i.e. temperature condition,  $T_{nest}-T_{clutch}$  gradient, nest humidity and the nest-clutch vapor pressure gradient), we used two-factor repeated-measures analysis of variance (RMANOVA) tests. If sphericity was significant, we used Huynh-Feldt epsilon adjustments. We used Bonferroni-corrected paired *t*-tests for *post-hoc* analyses. To test relationships among individuals within treatments (e.g. the effect of tight coiling on brooding unit  $\dot{V}_{O_2}$  during 31.5°C temperature and 26°C DP conditions of the nest), we used simple linear regression analysis. We present all results as means  $\pm$  s.e.m., and they refer to comparisons among eight individuals (i.e.  $N=8$ ).

#### RESULTS

Coiling behavior was significantly influenced by all temperature and humidity treatments, and there was an effect of the temperature

Table 2. Repeated-measures analysis of variance results for the independent and interactive effects of temperature and humidity treatments on the percentage of time female *A. childreni* spent tightly coiled around their clutches ( $N=8$ )

Nest temperature condition and nest humidity	d.f.	<i>F</i>	<i>P</i>
Nest temperature condition	3	7.28	<0.01
Nest humidity	2	4.64	0.029
Temperature condition $\times$ nest humidity*	4.4	3.11	0.026
Nest-clutch temperature gradient and nest humidity			
Nest-clutch temperature gradient	2	8.32	<0.01
Nest humidity	2	5.32	0.019
Temperature gradient $\times$ nest humidity	4	3.99	0.011
Nest-clutch temperature and vapor pressure gradients			
Nest-clutch temperature gradient	2	8.88	<0.01
Nest-clutch vapor pressure gradient	2	5.20	0.020
Temperature gradient $\times$ vapor pressure gradient*	2.7	4.30	0.021

\*Huynh-Feldt epsilon adjusted.

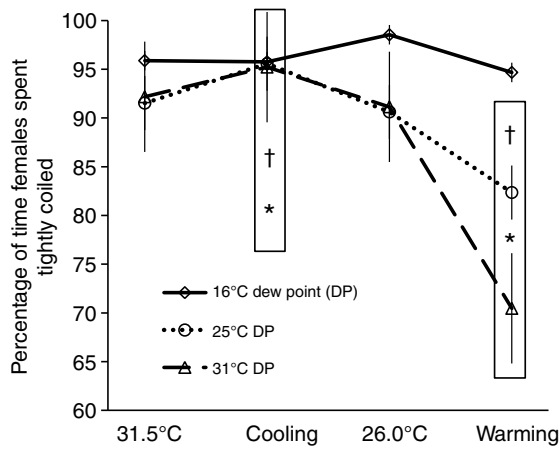


Fig. 2. The effect of humidity on the mean percentage of time female *Antaresia childreni* spent tightly coiled (% TC) during trials utilizing four sequential temperature conditions. Error bars represent s.e.m., and significant between-temperature differences for the 25°C DP and 31°C DP trials are denoted by boxed \* and †, respectively (N=8). Note the significant decrease in % TC during the warming stage for both the 25°C DP and 31°C DP trials but the constant high rate of % TC during the drier 16°C DP trials.

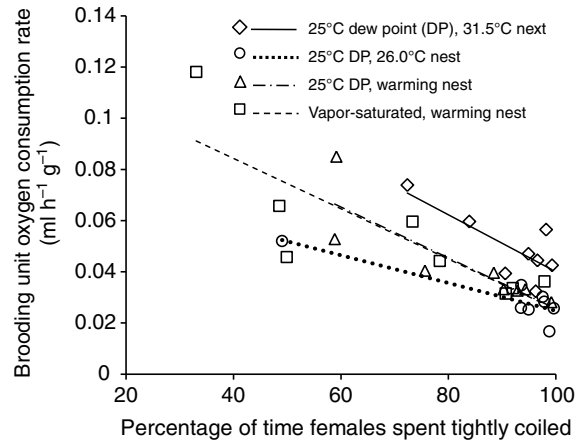


Fig. 3. Significant relationships between the amount of time female *Antaresia childreni* (N=8) spent tightly coiled (% TC) and brooding unit oxygen consumption rate. Relationships during other treatments were non-significant possibly due to low sample size and low among-individual variation in % TC (Tables 3 and 4).

× humidity interaction (Tables 1, 2; Fig. 2). Because animals did not experience a high nest–clutch vapor pressure gradient when nest temperature was 26.0°C, we could not determine the effects of the nest–clutch vapor pressure gradient + temperature condition on coiling behavior. *Post-hoc* analyses support previously reported findings in that brooding females held at a 25°C DP spent more time coiling tightly when the nest was cooling than when it was warming (Stahlschmidt and DeNardo, 2009a) (Fig. 2). We obtained similar results for brooding females during vapor-saturated nest conditions (i.e. the 31°C DP treatment, Fig. 2). However, this pattern did not persist under dry conditions, as brooding females at 16°C DP showed a high frequency of tight coiling even when the nest was warming (Fig. 2).

In agreement with a previous study (Stahlschmidt et al., 2008), tight coiling (TC) conserved embryonic water because the percentage of time that a female spent tightly coiled (% TC) was negatively related to brooding unit  $M_{H_2O}$  during 16°C DP and 25°C DP nest conditions (Table 3) [note: exposed python eggs lose water ~10-fold faster than females alone so the vast majority of brooding unit  $M_{H_2O}$  is derived from the eggs during postural adjustments

(Stahlschmidt et al., 2008)]. As in Stahlschmidt and DeNardo (Stahlschmidt and DeNardo, 2009a), % TC was significantly and positively related to mean  $T_{nest}-T_{clutch}$  gradient during warming at 25°C and 31°C DP treatments (Table 4). Low sample size and low among-individual variation in % TC may have contributed to the lack of significance during other warming and cooling treatments as % TC was positively, although non-significantly, related to mean  $T_{nest}-T_{clutch}$  gradient in all of these instances (Table 4).

Respiratory exchange ratios (i.e. RER,  $\dot{V}_{CO_2}/\dot{V}_{O_2}$ ,  $0.82\pm 0.04$ ) were not affected by temperature condition ( $F_{3,21}=1.5$ ,  $P=0.25$ ,  $1-\beta=0.33$ ), DP ( $F_{1,7}=0.011$ ,  $P=0.92$ ,  $1-\beta=0.051$ ) or the temperature × DP interaction ( $F_{2,8,19,5}=2.4$ ,  $P=0.11$ ,  $1-\beta=0.49$ ). Postural adjustments seemed to come with an energetic cost to females as tight coiling behavior was negatively related to brooding unit  $\dot{V}_{O_2}$  during several treatments (Fig. 3), particularly when among-individual variance of % TC was high (Tables 3 and 4). The disparity in brooding unit  $\dot{V}_{O_2}$  is primarily due to changes in maternal  $\dot{V}_{O_2}$  because early-stage *A. childreni* embryos are not O<sub>2</sub> limited and the clutch consumes ~50% less O<sub>2</sub> than the brooding female (Stahlschmidt and DeNardo, 2008; Stahlschmidt et al., 2008). Again, low sample size and low

Table 3. Relationships between the amount of time female *A. childreni* (N=8) spent tightly coiled (% TC) and brooding unit water loss rates ( $M_{H_2O}$ , mg h<sup>-1</sup> g<sup>-1</sup>) during 16°C dew point (DP) and 25°C DP nest conditions

Treatments	R <sup>2</sup>	F	P	Equation	Variance of % TC
16°C DP					
31.5°C	0.59	8.6	0.026	$y=-0.0042x+0.47$	16.0
Cooling	0.54	7.0	0.039	$y=-0.0025x+0.30$	16.0
26.0°C	0.63	10.3	0.018	$y=-0.0136x+1.37$	0.70
Warming	0.80	23.5	0.0029	$y=-0.0019x+0.23$	30.0
25°C DP					
31.5°C	0.68	13.0	0.011	$y=-0.0025x+0.29$	84.5
Cooling	0.63	10.4	0.018	$y=-0.0027x+0.29$	18.6
26.0°C	0.73	16.2	0.0069	$y=-0.0014x+0.11$	287.6
Warming	0.55	7.3	0.036	$y=-0.0028x+0.30$	253.2

Note: relationships during 31°C DP are not presented because the nest–clutch vapor pressure gradient and, thus,  $M_{H_2O}$  approximated zero in most instances (i.e. the air was vapor-saturated or nearly so).

Table 4: Relationships between the amount of time female *A. childreni* (N=8) spent tightly coiled (% TC) and the mean nest-clutch temperature gradients during warming and cooling nest conditions

Treatments	R <sup>2</sup>	F	P	Equation	Variance of % TC
16°C Dew point (DP)					
Cooling	0.38	3.7	0.10	y=0.050x-5.80	16.0
Warming	0.20	1.5	0.26	y=0.028x-1.64	30.0
25°C DP					
Cooling	0.39	3.8	0.10	y=0.034x-4.17	18.6
Warming	0.66	11.5	0.015	y=0.013x-0.33	253.2
31°C DP or vapor-saturated					
Cooling	0.33	3.0	0.14	y=0.048x-5.44	11.2
Warming	0.61	9.4	0.022	y=0.0084x-0.029	571.3

among-individual variation in % TC may have contributed to the lack of significance during other treatments as % TC was similarly, although non-significantly, related to brooding unit  $\dot{V}_{O_2}$  in all of these instances (all non-significant  $R^2=0.19-0.48$ ;  $P=0.057-0.28$ ).

### DISCUSSION

We experimentally demonstrate that both  $T_{\text{nest}}$  and humidity conditions influence python egg-brooding behavior, and that these two effects significantly interact with one another. Also, in agreement with previous studies, the tightly coiled brooding posture reduced nest-clutch exchange of water vapor and heat, particularly during unfavorable thermal and hydric nest conditions (Stahlschmidt and DeNardo, 2009a; Stahlschmidt et al., 2008). Thus, python egg-brooding postural adjustments are functionally significant to embryonic thermoregulation and water balance because these movements modulate and respond to these two important developmental variables.

Not unexpectedly, many animals significantly invest into the thermoregulation and water balance of their offspring because these aspects are crucial to development and survival (Clutton-Brock, 1991). Although taxonomically and functionally (i.e. behaviorally and physiologically) diverse, parental care adaptations that enhance these variables tend to fall within two simple categories – buffering and provisioning. First, parents can improve the fitness of their offspring by simply providing resistance to embryonic water or heat loss through building nests and synthesizing eggshells or egg coats (Clutton-Brock, 1991). Second, parents can enhance embryonic thermoregulation and water balance by providing heat or water to their embryos. Examples include viviparity (i.e. live-bearing, which allows parents to directly regulate embryonic temperature and hydration), endothermic brooding and parental feeding (Clutton-Brock, 1991). To optimize offspring fitness, pythons use either buffering alone [e.g. *A. childreni* (Stahlschmidt and DeNardo, 2009a); this study] or both buffering and provisioning [e.g. *Python molurus* (Hutchison et al., 1966; Vinegar et al., 1970)].

Given embryos' sensitivity to incubation conditions (Deeming and Ferguson, 1991; Deeming, 2004), parental behaviors are often modified in response to environmental thermal or hydric dynamics. For example, ambient temperature ( $T_a$ ) influences the percentage of time female little stints (*Calidris minuta*) spend brooding and foraging (i.e. females brood more when  $T_a$  is low) (Tulp et al., 2009). To reduce egg desiccation, female prairie skinks (*Eumeces septentrionalis*) increase their egg-brooding behaviors when the nest substrate is relatively dry (Somma and Fawcett, 1989). In addition to shifts in brooding behaviors, several reptile species demonstrate adaptive nest site selection related to thermal or hydric conditions [e.g. lizards (Shine and Harlow, 1996), turtles (Belinsky et al., 2004), snakes (Brown and Shine, 2004)]. The role of hygrosensation in

adaptive nest site selection is taxonomically widespread. For example, Montell demonstrated that fruit flies (*Drosophila melanogaster*) enhance the fitness of their offspring by preferentially ovipositing on moist substrate (Montell, 2008). Our results indicate that python egg-brooding behavior is adaptively plastic in response to both nest thermal and hydric dynamics.

While highly beneficial to the offspring, parental care generally entails substantial energy-related costs to the parent(s) (Clutton-Brock, 1991). In pythons, egg brooding is generally accompanied by lost foraging time and anorexia (Madsen and Shine, 1999; Aubret et al., 2005b). Accordingly, egg brooding obligates significant epaxial muscle atrophy and reduces contraction strength in female *A. childreni* under laboratory conditions (O. Lourdaïs and D.F.D., unpublished). However, other research suggests that brooding-related maternal costs are minimal (Aubret et al., 2005b). Interestingly, some water pythons nest in root boles where they brood their eggs for the duration of incubation (mean: 58 days) while other females nest in more thermally stable varanid burrows and only brood their eggs for the first week of incubation (Madsen and Shine, 1999). Females that use root boles and thus brood throughout incubation have reduced reproductive body condition and survival rate relative to those females that nest in burrows (Madsen and Shine, 1999).

Similar to large-scale decisions (i.e. to brood or not to brood the clutch), finer-scale egg-brooding decisions (i.e. the frequency of postural adjustments) also entail energetic costs. In fact, of the brooding units in this study, the one with the most behaviorally active female (i.e. lowest % TC) consumed >3-fold more  $O_2 g^{-1}$  body mass than the brooding unit that had the least active female when the nest was warming and vapor-saturated. The energetic cost of postural adjustments is probably relatively low compared with the energy demands of other aspects of parental investment (e.g. yolk deposition and lost foraging time) but warrants further investigation.

To conclude, our results combined with those of previous studies (e.g. Madsen and Shine, 1999) demonstrate that female pythons make both large-scale and fine-scale parental decisions. In addition to deciding where to brood and for how long, female pythons can also alter their brooding behavior. Thus, while providing a less complex form of parental care compared with that of mammals and birds, female pythons assess and respond to specific nest conditions to optimize the developmental environment experienced by their offspring. Although python egg brooding is emerging as a simple yet valuable parental care model, many critical questions regarding this system remain unanswered. Future research should focus on the proximate sensory mechanisms of egg-brooding decision-making such as neuronal pathways [e.g. transient receptor potential channels (Romanovsky, 2007; Montell, 2008)] and hormonal regulation (e.g. estradiol, progesterone, thyroxin and prolactin). Additionally, while challenging, field studies that integrate the

various levels of parental decision-making (e.g. nest site selection, brooding duration and postural adjustment frequency) would provide critical insight.

### ACKNOWLEDGEMENTS

We would like to thank Glenn Walsberg and Jake Brashears for technical support and the National Science Foundation (IOS-0543979 to D.F.D. and a Graduate Research Fellowship to Z.R.S.) for financial support. We are grateful to Ernest Nigro for attentive animal husbandry and those who helped with the preparation of the manuscript, including Kevin McGraw and two anonymous reviewers.

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