Postural Shifts During Egg-Brooding and Their Impact on Egg Water Balance in Children's Pythons (*Antaresia childreni*)

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Abstract

Parental care typically consists of distinct behavioral components that are balanced to address the multiple needs of offspring. Female pythons exhibit post-oviposition parental care in which they coil around their parchment-shelled eggs throughout incubation (40–80 d). Subtle postural shifts during egg-brooding facilitate embryonic gas exchange but may entail hydric costs to the clutch. This study used a simple behavioral model to (1) further quantify the costs and benefits of specific parental behaviors to developing offspring and (2) determine the influence that developmental stage and relative clutch mass have on parental behavior. Although previous research has demonstrated that egg-brooding as a whole reduces clutch water loss, we hypothesized that egg-brooding female pythons specifically adopt a tightly coiled posture to conserve embryonic water, but must make postural adjustments to enhance gas exchange between the clutch and nest environments at the cost of increased clutch water loss. We measured rates of water loss in brooding Children's pythons (*Antaresia childreni*) and their respective clutches (i.e., brooding units) and monitored changes in brooding posture. We conducted serial trials to elucidate the effect of developmental stage on postural adjustments and water loss. Results demonstrated that the proportion of time females spent in a tightly coiled posture was inversely related to mean water loss from the brooding unit. Analyses indicated that slight adjustments in posture led to bursts in brooding unit water loss. Indeed, brooding unit water loss during postural adjustments was significantly higher than during tight coiling. These findings imply that python egg-brooding provides an adjustable diffusive barrier that leads to discontinuous gas exchange, which minimizes clutch water loss. Because females with larger relative clutch masses spent more time tightly coiled, egg-brooding female pythons may use a 'water first' strategy in which they intentionally conserve clutch water at the cost of reduced embryonic respiratory gas exchange.

Introduction

Parental care is an ecologically significant behavior adopted by many vertebrates, especially endothermic ones, and has thus received considerable attention (Clutton-Brock 1991; Farmer 2000). Parental care provides offspring with many requirements for development, such as water balance, energy provisioning, and temperature regulation (Clutton-Brock 1991; Deeming, 2004). Because the needs of

offspring are numerous and complex, parental care tends to comprise a repertoire of behaviors with each specific behavior probably associated with both costs and benefits to the offspring. For example, both nest attendance and food acquisition represent parental care behaviors in many birds; however, each has its own cost and benefit to the offspring (Clutton-Brock 1991). Furthermore, the relative importance and subsequent dedication to a specific parental behavior can vary with such factors as environmental temperature (Weston & Elgar 2005), availability of food (Dewey & Kennedy 2001), and age of offspring (Clutton-Brock 1991; Cezilly et al. 1994).

While widespread among endotherms and common in fish and amphibians, parental care is limited among reptiles (Clutton-Brock 1991). Among most squamates (lizards and snakes), parental care is usually restricted to pre-birth or pre-hatching events which include but are not limited to nest-site selection, altered thermoregulation during pregnancy, and deposition of energy-dense yolk reserves that can be utilized after hatching or birth (Shine 1983; Clutton-Brock 1991; Somma 2003). One exceptional, yet understudied, case of squamate post-oviposition parental care is the egg-brooding behavior exhibited by all pythons (family Pythonidae), wherein the female coils around her clutch during incubation (Vinegar et al. 1970; Slip & Shine 1988; Pearson et al. 2003; Fig. 1). Reptile eggs are greatly affected by changes in developmental temperature (Angilletta et al. 2000; Birchard 2004), and brooding

Fig. 1: A female *Antaresia childreni* brooding her clutch in the opening adjustment (OA) posture. Note that even in the OA posture, there is very close association between the female and her clutch. Photograph by D. F. DeNardo.

provides a buffer against changes in environmental temperature. In fact, some pythons use facultative thermogenesis to raise clutch temperature as much as 7°C above ambient temperature (Vinegar et al. 1970; Slip & Shine 1988).

While the effects of temperature on development have been the best documented, other factors, such as water balance (Shine & Brown 2002) and gas exchange (Kam 1993; Warburton et al. 1995), also influence development and offspring quality. While the porosity of the nest allows for gas exchange (Walsberg 1980) during egg-brooding in birds, the tightly coiled nature of egg-brooding pythons limits gas exchange. Thus python brooding can conserve embryonic water (Lourdais et al. 2007) but limit oxygen availability to the embryos (Stahlschmidt & DeNardo 2008). However, python egg-brooding behavior is dynamic and involves two primary postures: tight coiling and postural adjustment (Stahlschmidt & DeNardo 2008). While postural adjustments serve to reduce intra-clutch hypoxia (Stahlschmidt & DeNardo 2008), they may impose a hydric cost to the parchment-shelled eggs. In this study, we document the occurrence of postural shifts by egg-brooding pythons and correlate those movements with changes in water loss from the brooding unit (i.e., female and associated clutch).

Python egg-brooding provides a simple model that can be used to quantitatively assess the implications of distinct parental care behaviors on physiologic parameters that are critical to embryonic development (e.g., temperature, CO_2, O_2, and H_2O). We used serial physiologic and behavioral observations of egg-brooding female Children's pythons (*Antaresia childreni*) to address several predictions. First, we predicted that tight coiling greatly reduces water loss rate of brooding units. Second, periodic postural adjustments facilitate rapid exchange of all gases including water vapor between the clutch and the nest environment. Support for these two predictions would provide further evidence that python egg-brooding serves as an adjustable diffusive barrier that minimizes clutch water loss while maintaining a viable clutch gaseous micro-environment. Previous work has shown that artificially increasing clutch size in ball pythons (*Python regius*) leads to decreased hatching success, presumably due to critically high water loss through the parchment-shelled eggs (Aubret et al. 2003). Thus, we predict that clutch characteristics such as size, absolute mass, and relative mass (i.e., clutch mass divided by maternal mass) are positively correlated with brooding unit water loss.

Methods

Study Animals and Maintenance

Antaresia childreni are medium-sized (up to 1.2 m snout-vent length, 600 g body mass), constricting snakes that inhabit rocky areas in northern Australia (Wilson & Swan 2003) and do not use facultative endothermy during brooding (DeNardo & Stahlschmidt, unpubl. data). *Antaresia childreni* nest at the end of a warm dry season (Jul.–Sep., Australian winter). Compared to the buried eggs of other squamates, maternally brooded python eggs have little, if any, contact with the substrate.

The snakes we used for this study are part of a long-term captive snake colony maintained at Arizona State University (ASU). A few days prior to oviposition, we moved gravid pythons into dual-ported, Teflon-coated, 1.9-l cylindrical brooding chambers which we then placed in an environmental chamber that had a 14:10 L:D photoperiod and maintained temperature at $30.5 \pm 0.3°C$ (which approximates the species' preferred incubation temperature, Lourdais et al. 2008). Females were not provided food or drinking water from this point until the end of the incubation to the best mimic natural conditions and minimize disturbance associated with feeding the females and cleaning any excrement from the chamber. At oviposition, we briefly removed females and their clutches from their respective brooding chambers to determine clutch size, clutch mass, and female post-oviposition mass. Each female re-coiled on her clutch within minutes of returning to her brooding chamber. Chambers were opaque on the bottom and sides, but the tops were transparent to allow observation. To prevent desiccation during non-experimental periods, we supplied each brooding chamber with 20–40 ml/min of hydrated air (absolute humidity, AH = $25.1–26.7 \text{ g/m}^3$; relative humidity, RH = 80–85%) using a valve-controlled aeration system that combined saturated air (bubbled through a water-filled column) with dry air. We housed animals in these chambers throughout the study to minimize disturbance.

Experimental Procedure

We put each brooding unit through a sequence of respiro-hygrometric trials at 30.5°C during two periods of post-oviposition development (i.e., day of incubation divided by total incubation duration): the first week after laying (EARLY, mean = 16% of post-oviposition) and between 38- and 40-d post-laying (LATE, mean = 73%). This allowed us to elucidate the effect that embryonic development and, possibly, increased clutch metabolism had on postural adjustments. To avoid disturbance, we monitored trials in darkness with an infrared camera (EC-PC-Cam; Elyssa Corp., Briarcliff Manor, NY, USA) and image recording software (v2.0.13, ImageSalsa, Data Crunch Technologies, Folsom, CA) in real-time. We collected brooding images and respirometric data for 12 h, starting >60 min after the beginning of the trial. We imposed this delay to account for any female-metabolic effect of disturbance associated with starting the trial. We humidified influent air to 23.5 g/m^3 absolute humidity (i.e., 73% RH at 30.5°C) by combining dry CO_2-free air (CDA 1112; PureGas, Broomfield, CO, USA) with vapor-saturated CO_2-free air (bubbled through a water-filled column). We made continuous, automatic adjustments to the fluxes of the respective gases via a feedback-controlled system that included an input–output datalogger (23X; Campbell Scientific Instruments, Logan, UT, USA), precision hygrometer (RH100; Sable Systems, Las Vegas, NV, USA), and mass flow controller (Unit Instruments, Inc., Yorba Linda, CA, USA). We maintained flow of the humidified air to the experimental brooding chamber at 560 ml/min with a mass flow controller. Given this flow rate and the functional brooding chamber volume (i.e., 1.9 l minus brooding unit volume), the 99% equilibration period was approx. 9 min (Lasiewski et al. 1966). We passed air exiting the brooding chamber (effluent) through a hygrometer (RH200; Sable Systems) and then dried it with anhydrous $CaSO_4$ before flowing it through a CO_2 analyzer (LI-6252; Li-Cor Biosciences, Lincoln, NE, USA) and an O_2 analyzer (FC-1B; Sable Systems). We analyzed a baseline sample immediately before and after brooding trials to determine the composition of the influent. We compared H_2O, CO_2, and O_2 levels of influent and effluent air samples (recorded using a 21X datalogger; Campbell Scientific Instruments) to obtain an accurate, minute-to-minute assessment of each brooding unit's contribution to changes in the fractions of those three gases. For a summary of how we converted these raw data to rates of H_2O loss ($\dot{V}_{H_2O}$), and oxygen consumption ($\dot{V}_{O_2}$), and carbon dioxide production ($\dot{V}_{CO_2}$), see Equations 1–7 in Walsberg & Hoffman (2006).

To estimate total water loss and metabolic gas exchange of non-brooded clutches, we removed each clutch from its respective mother after the brooding trial. We weighed each clutch, counted viable eggs, and then placed the clutch into a 1.3-l test chamber. Then, we supplied each clutch chamber with the

same influent described above (i.e., 0% CO_2, 21% O_2, 23.5 g/m^3 AH, 560 ml/min), waited for the effluent dew point to stabilize (25–60 min), and determined clutch $\dot{V}_{H_2O}$ as above. Next, we used closed-system respirometry to determine clutch metabolic rate. We sealed the clutch chamber, passed dried air samples from the chamber ($T_{initial}$ and T_{final}) through an O_2 analyzer (S-3A; Applied Electrochemistry, Inc., Sunnyvale, CA, USA) and a CO_2 analyzer (FoxBox-C; Sable Systems), and used Equations 5, 6, and 11 in Vleck (1987) to determine $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. We then returned each clutch to its respective mother and moved them back to the temperature controlled housing chamber. We repeated the entire process 30–32 d later for LATE incubation measurements.

Analyses of Egg-Brooding

We recorded egg-brooding behavior from a viewpoint directly above the brooding female. We took digital still images every 5 s and analyzed them after all trials were completed. We determined tight brooding to be when a female was motionless and tightly coiled around her clutch to the point where none of the clutch was visible. We considered postural adjustments as individual behavioral events only if they were >30 s removed from any other postural adjustment. We categorized subtly distinct postural adjustments into three simple types: (1) non-opening adjustments (NA) were those in which motion was detected but no part of the clutch was visibly exposed, (2) opening adjustments (OA) involved visible exposure of the clutch, lasted <5 min, and did not entail a female's snout breaching the perimeter of her outermost coil, and (3) exploratory adjustments (EA) were postural adjustments that also involved visible exposure of the clutch. However, they were characterized by lasting more than 5 min or including a female's snout breaching the perimeter of her outermost coil. Also, during many EA bouts, the female inserted her head between her eggs and her coils, suggesting a different behavioral motivation (e.g., possibly egg inspection) than that of OA.

Postural adjustments were immediately followed by an increase in $\dot{V}_{H_2O}$ by the brooding unit (Fig. 2). To calculate the mean $\dot{V}_{H_2O}$ of the 'tight' posture for a given trial, we removed any behavior-induced peaks in $\dot{V}_{H_2O}$ (i.e., duration of postural adjustment + 13-min equilibration period) and averaged the remaining real-time $\dot{V}_{H_2O}$. To calculate the $\dot{V}_{H_2O}$ during an 'adjusting' posture, we divided the volume of H_2O lost during the postural adjustment (V_a) by
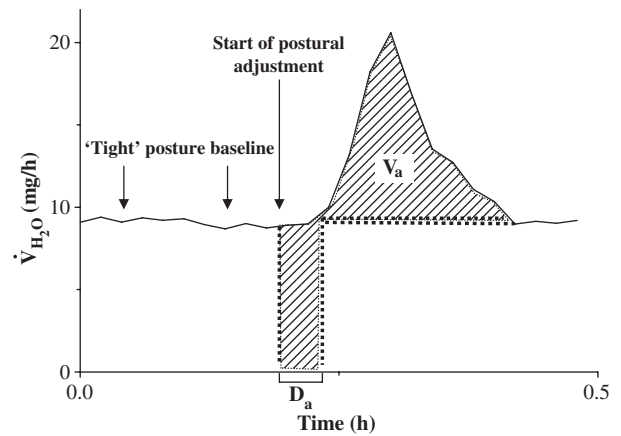
Fig. 2: Sample graph of a single adjustment-induced spike in brooding unit $\dot{V}_{H_2O}$. D_a (bracketed component of the x-axis) is the duration of the postural adjustment, while V_a (entire shaded area) is the volume of H_2O exchanged during the postural adjustment.

the duration of the adjustment (D_a) (Fig. 2). We calculated the mean $\dot{V}_{H_2O}$ of 'adjusting' posture by averaging the postural adjustment rates of a given trial. Often, several postural adjustments would take place during the 13-min equilibration period; thus, we could not distinguish the physiologic impact of individual adjustments in many instances.

Statistical Tests

For statistical tests, we used the JMP IN statistical package (version 5.1.2; SAS Statistical Institute, Inc., Cary, NC, USA). In EARLY–LATE and $\dot{V}_{H_2O,tight}$–$\dot{V}_{H_2O,adj}$ comparisons, we used paired t-tests and used log_{10}- or arcsine-transformations to normalize any non-normal data. To test the relationships of variables within individuals (e.g., clutch characteristics, water loss, and brooding behavior), we used simple linear regression analysis. All values are displayed as $\bar{x} \pm SE$. For negative results, we provide the results of a power analysis $(1 - \beta)$.

Results

Brooding Unit Characteristics

Among EARLY ($16 \pm 1\%$ post-oviposition development) and LATE ($73 \pm 1\%$ post-oviposition development) trials, maternal mass (341.9 ± 18.9 and 340.7 ± 18.7 g, respectively) was not significantly different (n = 7, $t_6 = -0.21$, p = 0.084, $1 - \beta = 0.086$). Also, clutch size was not significantly different among EARLY and LATE trials (10.4 ± 0.5 and 9.7 ± 0.6,

respectively; n = 7, t_6 = −2.0, p = 0.094, $1 − \beta$ = 0.79). However, due to a combination of removing dead eggs and mass lost as water, mean clutch mass was significantly less for the LATE trials (120.0 ± 7.4 vs. 109.8 ± 9.5; n = 7, t_6 = −2.7, p = 0.035). Hatching success was similar to that measured in previous python egg-brooding studies (80 ± 8%) (Aubret et al. 2003; Lourdais et al. 2007) and was not related to any maternal or clutch variables, as well as variables displayed in Tables 1 and 2 for either EARLY or LATE trials (R^2: $\bar{x} \pm SE$ = 0.16 ± 0.02, range = 0.004–0.53).

Table 1: Summary of behavioral analyses for EARLY and LATE trials ($\bar{x} \pm SE$; n = 7)

	EARLY	LATE	t_6	p-value	$1 − \beta$
Percentage of time spent tightly coiled	94.2 ± 1.2	94.7 ± 0.7	0.51	0.63	0.68
Non-opening adjustment (NA) rate (NA/h)	4.3 ± 0.5	5.0 ± 0.5	0.47	0.38	0.57
NA duration (s)	29 ± 3	23 ± 2	−1.6	0.0038	
Opening adjustment (OA) rate (OA/h)	0.6 ± 0.3	0.6 ± 0.3	0.52	0.62	0.63
OA duration (s)	84 ± 26	91 ± 17	0.26	0.80	0.81
Exploratory adjustment (EA) rate (EA/h)	0.05 ± 0.03	0.04 ± 0.02	−0.91	0.88	0.89
EA duration (s)	440 ± 20	373 ± 28	−0.80	0.81	1.0

Egg-Brooding Behavior

Females spent, on average, approx. 94% of brooding time tightly coiled around their eggs. Among the adjustment behaviors, there was a negative relationship between the frequency of a given adjustment behavior type and the mean duration of that type of adjustment (Table 1). The mean NA frequency was relatively high, but the mean duration of an NA was short (Table 1). Conversely, the mean EA frequency was roughly 100-fold less (Table 1). In fact, fewer than 43% of the 12-h trials contained an EA event. However, when EA occurred, the duration was long relative to the other types of postural adjustments (Table 1). Relative to NA and EA behaviors, OAs were intermediate in both frequency and duration.

Interestingly, mean NA duration was the only behavioral variable that was statistically different between EARLY and LATE trials (Table 1). Because the percentage of time spent adjusting included the total frequency and duration of postural adjustments, it was the most inclusive behavioral metric, and it was not significantly different between EARLY and LATE trials (EARLY: 5.8 ± 1.2%; LATE: 5.3 ± 0.7%, t_6 = −0.51, p = 0.63, $1 − \beta$ = 0.62).

### Brooding Unit and Clutch Physiology

Predictably, mean clutch $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ increased approx. 2.5-fold between trial periods (Table 2). We found a significant, negative relationship between mean brooding unit $\dot{V}_{H_2O}$ and the percentage of time

Table 2: Summary of physiologic data for EARLY and LATE trials ($\bar{x} \pm SE$; n = 7)

	EARLY	LATE	t_6	p-value	$1 − \beta$
Brooding unit (female coiled on eggs)					
Mass (g)	469.0 ± 21.8	451.6 ± 21.1	−2.8	0.033	
CO_2 production rate (ml/h)	9.11 ± 0.57	12.36 ± 0.52	4.7	0.0033	
O_2 consumption rate (ml/h)	12.28 ± 0.72	16.34 ± 0.69	6.8	0.0048	
Total H_2O loss rate (mg/h)	19.4 ± 3.0	19.8 ± 4.2	0.20	0.85	0.85
H_2O loss rate during tight coiling (mg/h)	17.5 ± 3.0	17.9 ± 3.9	0.27	0.79	0.79
H_2O loss rate during postural adjustment (mg/h)	44.6 ± 7.7	40.2 ± 5.4	−1.0	0.35	0.42
Clutch separated from female					
CO_2 production rate (ml/h)	3.32 ± 0.20	8.42 ± 0.64	8.6	0.00040	
O_2 consumption rate (ml/h)	4.3 ± 0.3	11.8 ± 1.1	8.2	0.00039	
H_2O loss rate (mg/h)	130.1 ± 14.3	162.3 ± 7.2	2.7	0.036	
Individual egg separated from female[a]					
Mass (g)	11.6 ± 0.9	11.3 ± 0.7	−2.0	0.097	0.11
CO_2 production rate (ml/h)	0.32 ± 0.02	0.87 ± 0.05	12	<0.0001	
O_2 consumption rate (ml/h)	0.40 ± 0.02	1.09 ± 0.07	11	0.00013	
H_2O loss rate (mg/h)	13.23 ± 1.00	16.92 ± 1.20	4.1	0.0090	

[a]Individual egg values were calculated by dividing clutch values by clutch size.

spent in the tightly coiled posture (Fig. 3). Also, $\dot{V}_{H_2O,tight}$ was significantly lower than $\dot{V}_{H_2O,adj}$ for brooding units during both EARLY and LATE trials (Fig. 4). The mean non-brooded clutch $\dot{V}_{H_2O}$ was higher in LATE trials compared to EARLY trials (Table 2). However, brooding unit $\dot{V}_{H_2O}$ did not significantly differ between EARLY and LATE trials, indicating that egg-brooding prevents the enhanced water loss from the eggs as development progresses.

Aside from a negative relationship with clutch size in LATE trials [$R^2 = 0.63$, $F(1,5) = 8.2$, $n = 7$, $p = 0.035$], mean brooding unit $\dot{V}_{H_2O}$ was not significantly related to our other predicted independent
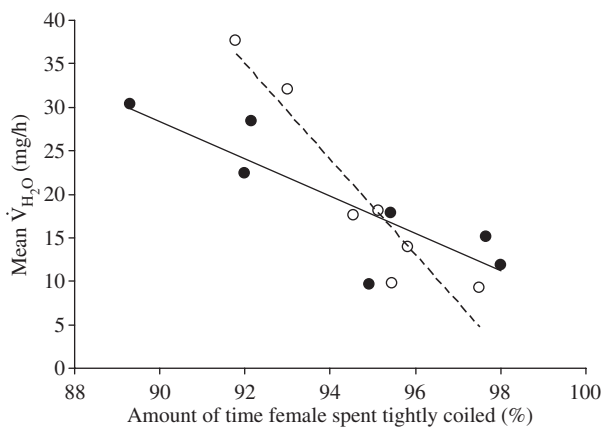
Fig. 3: Relationship between the percentage of time spent in the tightly coiled posture and mean brooding unit $\dot{V}_{H_2O}$ for EARLY [solid line; $y = -2.1x + 220.7$, $R^2 = 0.73$, $F(1,5) = 13$, $n = 7$, $p = 0.014$] and LATE [dashed line; $y = -5.5x + 541.1$, $R^2 = 0.89$, $F(1,5) = 40$, $n = 7$, $p = 0.0014$] trials.
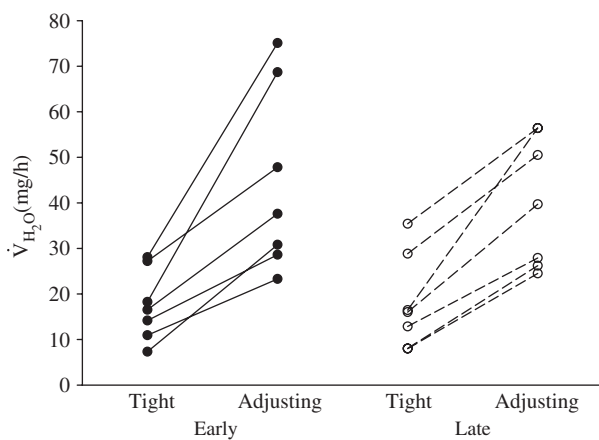
Fig. 4: Comparison between brooding unit $\dot{V}_{H_2O,tight}$ and $\dot{V}_{H_2O,adj}$ for EARLY and LATE trials (EARLY, $t_6 = 4.7$, $p = 0.0034$; LATE, $t_6 = 7.1$, $p = 0.0004$, $n = 7$). Each line connects the tight and adjusted values of an individual female.
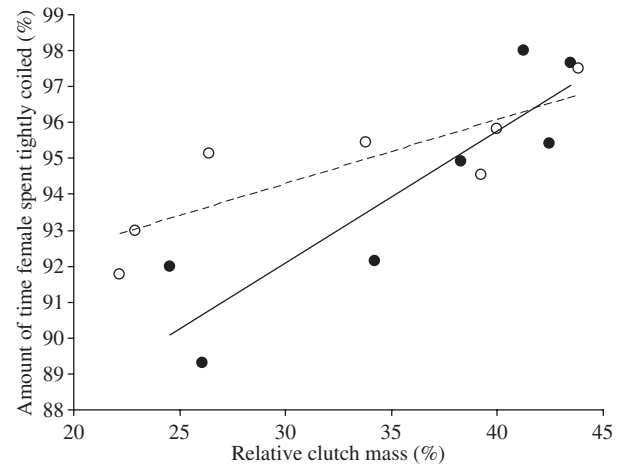
Fig. 5: Relationship between relative clutch mass (clutch mass divided by maternal mass) and the percentage of time spent in the tightly coiled posture for EARLY [solid line; $y = 36.6x + 81.1$, $R^2 = 0.79$, $F(1,5) = 19$, $n = 7$, $p = 0.0074$] and LATE [dashed line; $y = 32.5x + 86.8$, $R^2 = 0.71$, $F(1,5) = 12$, $n = 7$, $p = 0.018$] trials.

variables, i.e., clutch mass and relative clutch mass (R^2-range: 0.032–0.52; p-range: 0.066–0.71). However, relative clutch mass was related to the amount of time females spent tightly coiled in both EARLY and LATE trials (Fig. 5).

Discussion

Our data further demonstrate that python egg-brooding is dynamic, combining a predominant tight-coiling behavior with varying types of postural adjustments that differ in frequency and duration. Several results from our study suggest that through these postural adjustments egg-brooding provides an adjustable diffusive barrier between the clutch and the nest environment that limits the exchange of water vapor. Postural adjustments were tied to bursts in $\dot{V}_{H_2O}$ (Fig. 2), as well as $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ by the brooding unit.

Despite 30+ d between our EARLY and LATE trials, brooding female *A. childreni* did not show a reduction in body mass, and this result is consistent with published data for other egg-brooding pythons (Aubret et al. 2005b; Stahlschmidt & DeNardo 2008). These results suggest that python egg-brooding, unlike parental care behaviors of many other vertebrate taxa, entails very little direct energy costs. However, this does not imply that egg-brooding comes without costs to females. Madsen & Shine (1999) found that female water pythons (*Liasis fuscus*) that brooded their eggs for the entire incubation were in poorer

body condition and were less likely to reproduce the following year compared to females that abandoned their clutches early in incubation. Thus, egg-brooding may impose an energy cost through the loss of foraging time.

Our data support those of Lourdais et al. (2007) in demonstrating that brooding, as a whole, drastically reduces clutch water loss in *A. childreni*. However, our finding that $\dot{V}_{H_2O,tight}$ is approx. two- to threefold $<\dot{V}_{H_2O,adj}$ for brooding units demonstrates that tight brooding specifically is important to egg water balance. In further support, the proportion of time spent tightly coiling was inversely correlated to mean brooding unit $\dot{V}_{H_2O}$ (Fig. 3).

In contradiction of our third prediction and previous research in *P. regius* (Aubret et al. 2003), *A. childreni* brooding unit $\dot{V}_{H_2O}$ was not affected by female and clutch characteristics. The discrepancy between our results and those of Aubret et al. (2003) may result from the fact that clutch size variation was a natural non-manipulated variable in our study, while Aubret et al. (2003) manipulated clutch size by adding or subtracting eggs. Nonetheless, prior to manipulation, *P. regius* in Aubret et al. (2003) had considerably larger relative clutch masses than *A. childreni* [*P. regius*: 51.7 ± 2.9% at oviposition; *A. childreni*: 35.8 ± 2.9% (EARLY) and 32.8 ± 3.3% (LATE)], possibly challenging the female's ability to fully encompass her clutch. Regardless, it is noteworthy that *A. childreni* females with larger relative clutch masses adopt a tightly coiled posture more often than females with smaller relative clutch masses, possibly representing some compensatory behavior to limit water loss from the larger clutch (Fig. 5).

The idea that egg-brooding may provide an adjustable diffusive barrier is not novel. Several researchers have suggested that some birds are capable of behaviorally modifying nest humidity to benefit their eggs (Rahn et al. 1977; Morgan et al. 1978). For example, water to humidify the nest environment could be provided by the nest-attending parent via respiratory and cutaneous water loss rather than by diffusion from the eggs. Most avian eggs lose 20–22% of their mass through water loss during the incubation period (Deeming & Ferguson 1991), so an increase in nest humidity by the parent could significantly benefit offspring (Rahn et al. 1977; Morgan et al. 1978). However, Walsberg (1980, 1983) rejected this notion that bird parental behavior significantly regulates egg dehydration due in large part to the combination of low resistance and high convection characterized by most avian nests. Additionally, prairie skinks, *Eumeces septentrionalis* and *P. regius* exhibit nest-attending

behaviors that significantly reduce egg mortality due to desiccation (Somma & Fawcett 1989; Aubret et al. 2005a). However, in none of these studies have the effects on water loss rates of specific behavioral components of parental care been quantified nor has the potential impact of the behaviors on embryonic CO_2 and O_2 exchange been explored. Thus, to the best of our knowledge, python egg-brooding represents the only established example of dynamic parental care by vertebrates that significantly mediates the fluxes of water vapor, CO_2, and O_2 in the developmental environment.

When compared to other parchment-shelled squamate eggs, *A. childreni* eggshells have very high conductance to water vapor (Lourdais & DeNardo, unpubl. data) that appears to be related to maternal egg-brooding (but which led to the other is unclear). High eggshell conductance to gases (e.g., O_2, CO_2, and H_2O vapor) or 'thinning' probably occurs concurrently with longer durations of egg retention and prior to the evolution of viviparity (live-bearing) (Packard et al. 1977; Shine 1985). Interestingly, *A. childreni* lay eggs which contain embryos at stage 35 of 40 (Lourdais et al. 2008) whereas other squamates lay eggs near stage 30 and rarely beyond stages 32–33 (Shine 1983; Andrews & Mathies 2000). Thus, maternal egg-brooding coupled with eggshell thinning may serve as either a pre-adaptation to viviparity or an alternative reproductive strategy to viviparity.

The discontinuous gas exchange exhibited by some insects is somewhat analogous to the described python egg-brooding system. Some insects utilize a discontinuous gas exchange cycle (DGC) and periodically close their spiracles, which is a highly effective barrier to diffusion between their endotracheal systems and the external environment (Lighton 1996; Quinlan & Gibbs 2006). DGC have been confirmed in 59 species, primarily within the Hymenoptera and Coleoptera (Quinlan & Gibbs 2006) and may have evolved as a means to reduce evaporative water loss (Buck 1958; Chown & Davis 2003). When spiracles are closed (C), spiracular water loss is reduced and internal CO_2 concentration is elevated. This 'C' phase of the typical DGC is comparable to the tight postural phase exhibited by brooding pythons, although pythons in the tightly coiled posture do not completely prevent gas exchange between the clutch and nest environments. In DGC, spiracles are periodically fluttered or opened (O) to allow respiratory gas exchange at the expense of a short-term increase in water loss. The 'O' phase in DGC is similar to the postural adjustment phase of python egg-brooding (Fig. 2).

Researchers have generally concluded that spiracle control during DGC is regulated by CO_2 detection (Kaars 1981; Harrison et al. 1995). Similar to other species, python egg $\dot{V}_{CO_2}$ increases substantially throughout the incubation period. However, as in Stahlschmidt & DeNardo (2008), nearly all metrics of python brooding behavior showed no change between EARLY and LATE trials. Thus, it appears that the frequency and duration of postural adjustment respond to neither changes in embryonic metabolic rate nor developmental stage.

Because water loss rate across the eggshell increases as incubation proceeds, an increase in the frequency or duration of postural adjustments in response to increased embryonic metabolism might lead to fatal egg desiccation. Indeed, the absence of maternal egg-brooding is lethal to *A. childreni* embryos in conditions of relative humidity as high as 75–100% (Lourdais et al. 2007). Thus, egg-brooding female pythons may use a 'water first' strategy in which they intentionally conserve clutch water at the cost of hypoxic clutch conditions that can lead to reduced embryonic metabolism (Stahlschmidt & DeNardo 2008). Larger clutches have higher $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ than smaller clutches and likely would require more frequent postural adjustments to maintain normal respiratory gas exchange. Instead, females with larger relative clutch masses more frequently adopted a tightly coiled posture (Fig. 5) in support of the 'water first' strategy.

To summarize, we have demonstrated that python egg brooding consists of distinct behaviors that significantly affect egg water balance. Combined with previous data, it appears that maternal egg-brooding behavior exhibited by pythons serves as an adjustable diffusive barrier that balances the hydric and respiratory needs of developing embryos. Furthermore, the physiologically and behaviorally quantifiable nature of the python brooding system allowed us to perform a multi-faceted assessment of a simple parental care model. Although parental care provides an overall benefit to offspring, we have demonstrated that individual parental care behaviors entail both costs and benefits for developing offspring. Specifically, the tightly coiled python brooding posture enhances egg water balance at a cost to embryonic metabolism (Stahlschmidt & DeNardo 2008), whereas postural adjustments enhance clutch respiratory gas exchange at a cost to egg water balance. Future studies should elucidate the long-term consequences of tight coiling on offspring (e.g., reduced hatching success or offspring quality) and potential environmental cues used by the female to regulate parental care behavior.

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