

Obligate costs of parental care to offspring: egg brooding-induced hypoxia creates smaller, slower and weaker python offspring

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Python egg brooding typifies parental care because it consists of multiple behaviours that provide for multiple developmental needs. For example, tightly coiling around the eggs benefits embryonic water balance, but periodic female postural adjustments improve embryonic gas exchange. Regardless of these postural adjustments, egg brooding creates a hypoxic intra-clutch environment that constrains embryonic metabolism. We further examined this novel and useful parental care model to determine: (1) any fitness-related costs of egg brooding to offspring; (2) whether any long-term costs are alleviated by postural adjustments. We artificially incubated Children's python (*Antaresia childreni*) clutches and modulated oxygen partial pressure (PO_2) to create three treatments: normoxic (NRM, 20.3 kPa O_2), brooding [BRD, PO_2 profile typical of clutch PO_2 ($PO_{2clutch}$) in maternally brooded clutches, 15.8–19.3 kPa O_2] and low (LOW, predicted PO_2 profile of maternally brooded $PO_{2clutch}$ if females did not make postural adjustments, 14.4–18.6 kPa O_2). Using various metrics from ~12 days pre-hatching to 14 days post-hatching, we demonstrated that NRM offspring were larger, faster and stronger than BRD offspring. As only hatchling heart mass differed between BRD and LOW treatments (LOW > BRD), postural adjustments may not alleviate hypoxia-related costs to embryos. Our results demonstrate that parental care may represent a compromise between competing developmental needs and thus entails obligate costs to the offspring. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, 98, 414–421.

ADDITIONAL KEYWORDS: adaptive significance – Children's python (*Antaresia childreni*) – offspring quality – respiration – snake – trade-off.

INTRODUCTION

Parental care is a widespread behaviour adopted by many vertebrates, especially endothermic ones, and has consequently received considerable attention (Clutton-Brock, 1991; Farmer, 2000; Huang, 2006). Parental care meets many critical needs of offspring during development, such as water balance, energy acquisition and temperature regulation (Clutton-Brock, 1991; Deeming, 2004). Given these multiple needs, trade-offs between competing developmental variables often exist. Although likely important to understanding the adaptive significance of parental behavioural suites, such intra-offspring trade-offs are less studied than parent–offspring trade-offs.

One exceptional, yet understudied, case of post-oviposition parental care among squamates (lizards and snakes) is egg-brooding behaviour exhibited by all pythons (family Pythonidae), in which the female coils around her clutch during incubation (Vinegar, Hutchison & Dowling, 1970; Slip & Shine, 1988; Pearson, Shine & Williams, 2003). Python egg brooding is a dynamic process, wherein the egg-brooding female spends most of the time tightly coiled around her eggs, but periodically loosens her coils (Stahlschmidt & DeNardo, 2008; Stahlschmidt, Hoffman & DeNardo, 2008). Tight coiling maintains embryonic water balance at the cost of embryonic respiration, while postural adjustments facilitate embryonic respiration at the cost of embryonic water balance (Stahlschmidt & DeNardo, 2008; Stahlschmidt *et al.*, 2008). Although maternal egg brooding is critically

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important to offspring survival and phenotype in biologically relevant nest environments (Aubret *et al.*, 2005; Lourdaï, Hoffman & DeNardo, 2007), it creates a hypoxic developmental environment that reduces embryonic metabolism (Stahlschmidt & DeNardo, 2008). However, the long-term, fitness-related costs to embryos associated with hypoxia induced by python egg brooding are unknown.

Chronic hypoxia often elicits compensatory changes in developmental morphology, such as cardiac hypertrophy (*Alligator mississippiensis*, Daudin 1802, Crossley & Altimiras, 2005). Despite such plasticity, hypoxia can decrease embryonic growth rate (*Alligator mississippiensis*, Warburton, Hastings & Wang, 1995; Crossley & Altimiras, 2005; *Salmo trutta*, Linnaeus 1758, Roussel, 2007), reduce hatchling mass (Crossley & Altimiras, 2005), delay the development of thermogenesis (*Gallus gallus*, Linnaeus 1758, Azzam, Sdzuy & Mortola, 2007), reduce predator avoidance ability of juveniles (Roussel, 2007) and reduce sexual development (*Danio rerio*, Hamilton-Buchanan, 1822, Shang, Yu & Wu, 2006). Although insightful, these studies reared embryos in arbitrary oxygen concentrations, such as 14% (Shang *et al.*, 2006), 26% (Roussel, 2007), 48% (Crossley & Altimiras, 2005), 72% (Crossley & Altimiras, 2005; Azzam *et al.*, 2007) or 81% (Warburton *et al.*, 1995) of normoxia. However, in at least one study, experimenters determined the detrimental effects of developmental hypoxia (e.g. reduced embryonic growth rate and survival) on embryos by creating an artificial oxygen tension (PO_2) environment that models *in vivo* developmental PO_2 (Parker & Andrews, 2006). Similarly, in this study we used previously quantified intra-clutch oxygen tensions ($PO_{2\text{clutch}}$) in Children's pythons (*Antaresia childreni*, Gray 1842, Stahlschmidt & DeNardo, 2008) to determine the hypoxia-related costs of egg brooding to *A. childreni* embryos.

Experimental manipulation of relevant proximate factors (e.g. developmental PO_2) may elucidate the adaptive significance of traits (e.g. parental behaviour). Thus, we incubated *A. childreni* clutches in different PO_2 treatments and hypothesized that python egg brooding represents an adaptive trade-off between embryonic water balance and respiration that is enhanced by modulating behaviours (i.e. tight coiling and postural adjustment). Specifically, we predicted that (1) hypoxia induced by python egg brooding entails fitness-related costs to offspring (e.g. reduced hatchling mass and strength) and (2) these costs are mitigated, at least partially, by hypoxia-alleviating postural adjustments. Support for these predictions would demonstrate the importance of balancing individual parental behaviours to meet the dynamic needs of the developing offspring using a simple, quantifiable parental care model that modu-

lates several critical developmental variables, such as embryonic temperature (Z. Stahlschmidt & D. DeNardo, unpubl. data), respiration (Stahlschmidt & DeNardo, 2008) and water balance (Lourdaï *et al.*, 2007; Stahlschmidt *et al.*, 2008).

MATERIAL AND METHODS

STUDY SPECIES AND CLUTCH INCUBATION

We used a long-term captive colony of *A. childreni* maintained at Arizona State University (ASU) for this study. *Antaresia childreni* are relatively small (< 1 m) pythons from northern Australia (Wilson and Swan, 2003) and they do not employ facultative thermogenesis while egg brooding (Stahlschmidt & DeNardo, in review). Husbandry and breeding of the animals followed that described previously (Lourdaï *et al.*, 2007). In 2007, we allowed seven females to brood their respective clutches for the entire incubation period in 1.9-L egg-brooding chambers supplied with hydrated air and maintained at optimal incubation temperature (31.5 °C, Lourdaï *et al.*, 2007) (herein, MAT clutches). In 2008, 22 females similarly incubated their clutches for the first 6 days as they underwent other non-invasive, behavioural studies. During these 6 days, clutches were not oxygen-limited because early stage *A. childreni* embryos have < 20% of the metabolic requirements for oxygen consumption of late-stage embryos and exhibit a very low critical oxygen tension (i.e. the minimal partial pressure of oxygen that supports normal respiratory gas exchange) (Stahlschmidt & DeNardo, 2008).

On incubation day 7 (ID7), we separated the 2008 clutches from their respective females, as well as counted the eggs and weighed the clutches. We then assigned each clutch to one of the following treatments: normoxic (NRM, $N = 7$), brooding hypoxic (BRD, $N = 8$) and low hypoxic (LOW, $N = 7$). To reduce desiccation in a viable micro-environment, we partially buried clutches in 60–80 mL of moistened Perlite and suspended them with fine mesh above 200–300 mL of distilled water in 1 L dual-ported plastic containers. We placed all of the clutch-housing containers in a 765-L incubator (no. 3770; Forma Scientific Inc., Marietta, OH, USA) maintained at 31.5 ± 0.2 °C for the remaining incubation duration. Clutches in the NRM treatment received compressed room air. Clutches assigned to the BRD treatment received a mixture of compressed N_2 and air to mimic the PO_2 profile of brooded *A. childreni* eggs (Fig. 1; Stahlschmidt & DeNardo, 2008). During tight coiling, $PO_{2\text{clutch}}$ decreases in a logarithmic fashion until a postural adjustment causes it to rapidly increase up to 1 kPa O_2 (Stahlschmidt & DeNardo, 2008). We used data from our previous study (Stahlschmidt &

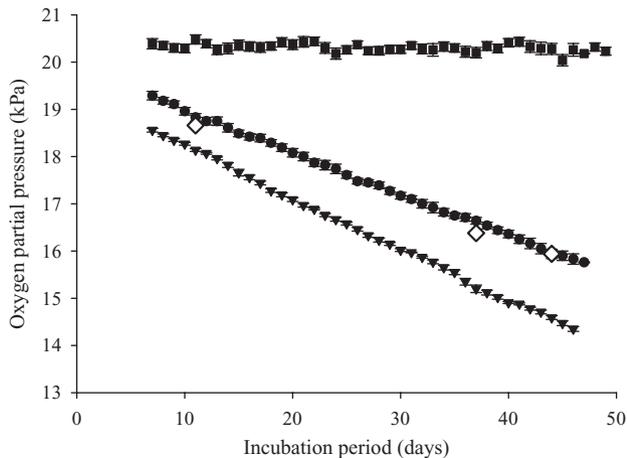


Figure 1. Oxygen regimes for normoxic (NRM, ■), brooded (BRD, ●) and low (LOW, ▼) *Antaresia childreni* clutches during incubation. The BRD oxygen regime was modelled after maternally egg-brooded intra-clutch oxygen partial pressures (◇) from Stahlschmidt & DeNardo (2008). Displayed symbols are $\bar{X} \pm \text{SEM}$ error bars.

DeNardo, 2008) to estimate the points at which $PO_{2\text{clutch}}$ would reach a diffusion equilibrium in the absence of postural adjustments throughout incubation. Thus, we created the LOW treatment using a mixture of N_2 and air to approximate what we predicted a clutch would experience if females did not use postural adjustments during egg brooding (Fig. 1).

We hydrated influent air supplies to near vapour saturation by bubbling them through heated columns of distilled water and maintained the flow rate to each clutch at 50 mL min^{-1} using adjustable flow meters (FL-344; Omega Instruments, Stamford, CT, USA) that were calibrated under experimental conditions. Daily, we determined the % O_2 of each treatment's supply air by running gas samples through an O_2 analyser (S-3A; Applied Electrochemistry Inc., Sunnyvale, CA, USA). We converted % O_2 to PO_2 (% $O_2/100$ barometric pressure) using barometric pressure recorded daily from a gas analyser (FC-1B; Sable Systems, Las Vegas, NV, USA). Oxygen tension for the BRD and LOW treatments supplies was changed daily by adjusting the flow meters for the N_2 and air (Fig. 1). For simplicity, this experimental design did not incorporate the O_2 fluctuations experienced by maternally brooded clutches during postural adjustments. However, as postural adjustments generally entail fluctuations of $< 0.5 \text{ kPa } O_2$ (Stahlschmidt & DeNardo, 2008), we feel that neglecting these periodic fluctuations in our experimental design did not affect our results.

At hatching, we recorded fitness-related measurements of offspring, transferred them into individual

500-mL plastic containers maintained at 31.5°C and provided them drinking water *ad libitum* for the duration of the study.

OFFSPRING FITNESS-RELATED MEASUREMENTS

In 2008, we made the following morphometric and performance measurements during incubation (duration: $\bar{X} \pm \text{SEM} = 47.1 \pm 0.6$ days) and after hatching:

Incubation day 7 (ID7). Clutch mass was measured ($\pm 0.01 \text{ g}$, V-200; Acculab, Edgewood, NY, USA) and mean egg mass was determined (clutch mass/clutch size). At random, we terminally sampled one egg per clutch to determine embryo mass and sampled egg mass ($\pm 0.0001 \text{ g}$, AG245; Mettler-Toledo, Columbus, OH, USA). These measurements validated the similarity of the clutches prior to treatment.

Incubation day 35 (ID35). As on ID7, we randomly sampled one egg from each clutch to determine egg mass and embryo mass. After killing the embryo (via decapitation), we isolated, removed and weighed the heart within 2 min of removal from each sampled embryo to determine heart wet mass ($\pm 0.0001 \text{ g}$).

Day of hatching (H). We determined mean incubation duration, hatching success {[number of hatchlings/(clutch size at ID7-2)] $\times 100$ }, hatching duration (incubation duration of last hatchling – incubation duration of first hatchling) and average hatchling body mass at hatching. We determined snout-vent length (SVL) by temporarily immobilizing each hatchling between $\sim 5 \text{ cm}$ of open-cell foam and a $\sim 3\text{-mm}$ sheet of transparent acrylic, tracing its outline with a dry-erase marker and using a cloth tape ($\pm 0.5 \text{ mm}$) to measure the tracing. We measured head length (i.e. the distance from the snout to the posterior process of the quadrate bone) using digital calipers ($\pm 0.0005 \text{ mm}$, FB-2400; Frei and Borel, Oakland, CA, USA). We also determined body condition for each hatchling in two ways: (1) residuals from a SVL vs. body mass regression line (derived from the best fit of pooled data) and (2) using analysis of covariance (ANCOVA) to determine the effect of treatment on hatchling body mass after controlling for SVL. We used a digital scanner (Scanjet 3670; Hewlett-Packard, Palo Alto, CA, USA) to capture images of the ventral scale patterns of each hatchling that we later analysed for abnormalities (e.g. significant scale asymmetry or extra scales). Lastly, as on ID35, we determined heart wet mass for a single hatchling killed from each clutch.

Also at H and 1 days post-H, we agitated each hatchling with a padded copper tube for 1 min at room temperature ($25.9 \pm 1.0^\circ\text{C}$). We counted the strikes for each 1-min session and used the higher count to determine maximum strike frequency (a metric of defensive behaviour) for each snake. After

each strike session, we transferred hatchlings to an environmental chamber maintained at 31.5 °C. After acclimation, we forced hatchlings to undergo three sequential swimming trials in 3 m × 2 cm troughs maintained at 31.5 °C. We used the fastest of the six trials (i.e. three sequential trials for two consecutive days) to determine maximum swimming speed (an estimate of predation avoidance).

In 2007, we measured incubation duration, hatching success, hatchling body condition and maximum strike frequency data for the MAT clutches. We compared the MAT, BRD and NRM clutch data to validate that the BRD treatment adequately mimicked the naturally brooded developmental environment.

Fourteen days post-hatching (14DPH). As at H, we determined average hatchling body mass, SVL, body condition, heart wet mass, relative wet mass of heart and head length. We compared H and 14DPH measurements to determine SVL growth rate ((14DPH SVL – H SVL)/14 days), body mass loss rate ((H mass – 14DPH body mass)/14 days) and head length growth rate ((14DPH head length – H head length)/14 days) for each hatchling. Daily, we checked all hatchlings' individual 500-mL containers for sheds to determine the duration to first shed for each hatchling.

After morphometric processing and at room temperature (25.9 ± 1.0 °C), we attached one end of a string to the vent of each hatchling using medical tape and the other end of the string to a force transducer (FT-100; iWorx Systems Inc., Dover, NH, USA) that was calibrated daily using an interfacing software (LabScribe; iWorx Systems Inc.). Then, we pulled each hatchling taut, held their heads and allowed them to contract for 1 min. We recorded measurements using the LabScribe software and conducted a second trial immediately after the first. For each hatchling, we determined maximum contraction strength (an estimate of prey acquisition ability and predation avoidance) to be the greatest contraction elicited during either trial. We used the trial with the higher rate of contractions to determine contraction rate for each hatchling.

STATISTICAL ANALYSES

We analysed data using JMP IN (version 5.1.2; SAS Statistical Institute, Inc., Cary, NC, USA) and determined experiment-wide significance at $\alpha < 0.05$ for all tests. To determine any significant differences among treatments, we used single-factor analysis of variance (ANOVA) tests on parametric data. To determine the effect of time within treatment groups (e.g. wet mass of heart), we used repeated measures ANOVA (rmANOVA) tests on parametric data with non-significant sphericity. To determine the treatment

effect between specific predicted comparisons (e.g. NRM vs. BRD and BRD vs. LOW), we used sequential Bonferroni-corrected two-sample Student's *t*-tests on parametric data and similarly corrected Mann–Whitney *U*-tests on non-parametric data (Holm, 1979). As some variables varied significantly among treatment groups (e.g. hatchling body mass) and likely contributed to other offspring variables (e.g. hatchling contractile strength), we used ANCOVA tests on parametric data to further determine the treatment effect between specific predicted comparisons. ANCOVA tests are more appropriate than comparing 'relative variables' (e.g. relative contractile strength = contractile strength/body mass) as most variables exhibit allometric, not isometric, relationships with one another (Packard & Boardman, 1988). We performed power analyses on non-significant parametric data and displayed all results as $\bar{X} \pm \text{SEM}$.

RESULTS

At the onset of the experiment (i.e. ID7), we detected no significant differences among treatments for clutch size ($\bar{X} = 8.5 \pm 0.4$, $P = 0.47$), clutch mass ($\bar{X} = 99.6 \pm 3.7$ g, $P = 0.06$), mean egg mass ($\bar{X} = 11.7 \pm 0.4$ g, $P = 0.19$), sampled egg mass ($\bar{X} = 11.8 \pm 0.6$, $P = 0.18$) and embryo mass ($\bar{X} = 0.669 \pm 0.044$, $P = 0.20$). Thus, treatment-dependent corrections for subsequent dependent variables (e.g. ANCOVA tests with egg mass as a covariate) were not necessary.

At ID35, significant differences in heart mass were detected between NRM and BRD clutches, indicating that hypoxia associated with egg brooding creates cardiac hypertrophy in late-stage python embryos (Table 1). By H (i.e. ~12 days later), NRM and BRD offspring exhibited significantly different body mass, heart mass, head size, locomotor ability and defensive behaviour (Table 1). Significant differences in morphometric (e.g. mass and body condition) and performance (i.e. swimming speed) measurements were again detected at 14DPH (i.e. mass and contractile strength) (Table 1). Throughout the experiment, the only difference between BRD and LOW clutches was in the wet mass of embryo hearts on the day of hatch (Table 1). Upon further comparison of NRM and BRD clutches, we found non-significant effects of treatment on hatchling head length at hatching and body mass 14 days post-hatch after controlling for the effect of body mass at hatching (Table 1). Thus, hypoxia-induced differences in hatchling mass had significant carryover effects on other morphometric variables.

In addition to variables described in Table 1, differences existed among groups for incubation duration ($F_{2,19} = 4.5$, $P = 0.03$, NRM: 48.7 ± 0.6 days, BRD:

Table 1. Summary of all fitness-related offspring metrics in comparisons of *Anataresia chidreni* clutches in treatments of normoxia (NRM; $N = 7$) and hypoxia equivalent to maternal egg-brooding (BRD; $N = 8$), as well as comparisons between clutches of BRD and estimated hypoxia that would result if egg brooding occurred without postural adjustments (LOW; $N = 7$) at incubation day 35, day of hatch and 14 days post-hatching. NRM, BRD and LOW values are displayed as $\bar{X} \pm \text{SEM}$

	NRM vs. BRD			BRD vs. LOW		
	t-test		ANCOVA	t-test		ANCOVA
	P	1- β	P	1- β	P	1- β
Day 35 (ID35)						
Egg mass (g)	13.0 ± 0.9	11.6 ± 0.5	0.23	0.53	0.78	0.86
Embryo mass (g)	4.551 ± 0.410	4.836 ± 0.309	0.6	0.65	0.19	0.92
Heart mass (mg)	22.2 ± 2.0	38.6 ± 0.9	<0.0001	-	0.296*	-
Day of hatch (H)						
Incubation duration (day)	48.7 ± 0.6	46.8 ± 0.8	0.075	0.52	0.32†	> 0.99
Hatching duration (day)	1.3 ± 0.3	1.8 ± 0.3	0.22	0.52	0.077†	0.19†
Hatching success (%)	87.1 ± 3.7	80.0 ± 10.1	0.39	0.56	0.073†	0.092†
Hatchling mass (g)	8.55 ± 0.37	7.43 ± 0.35	0.047	-	0.041‡	0.98
Wet mass of heart (mg)	24.2 ± 2.0	25.7 ± 2.7	0.66	0.81	0.005†	0.02
Snout-vent length (SVL, mm)	221.7 ± 2.9	217.5 ± 3.5	0.38	0.55	0.26†	0.51
Body condition (residual g body)	0.44 ± 0.27†	-0.28 ± 0.15	0.04	-	0.61	0.66
Head length (mm)	14.14 ± 0.15	13.60 ± 0.10	0.01	-	0.063†	0.81
Maximum strike frequency (per min)	15.1 ± 1.8	8.4 ± 2.2	0.038	-	0.034†	0.82
Maximum swimming speed (m/s)	0.14 ± 0.01	0.11 ± 0.01	0.016	-	0.037‡	0.16
14 day post-hatch (14DPH)						
Hatchling mass (g)	7.99 ± 0.33	6.82 ± 0.33	0.027	-	0.18†	0.59
Wet mass of heart (mg)	23.3 ± 1.0	25.6 ± 2.1	0.36	0.54	0.081§	0.42§
SVL (mm)	237.2 ± 3.4	227.2 ± 6.4	0.2	0.52	0.037¶	0.75
Body condition (residual g body)	0.49 ± 0.17	-0.11 ± 0.36	0.18	0.53	0.93¶	0.83*
Hatchling SVL growth rate (mm day ⁻¹)	1.106 ± 0.159	0.689 ± 0.422	0.4	0.56	-	0.56
Hatchling mass loss rate (g day ⁻¹)	0.040 ± 0.006	0.044 ± 0.005	0.61	0.73	0.62¶	0.82*
Head length (mm)	14.68 ± 0.11	13.91 ± 0.15	0.0014	-	0.076¶	0.15
Head length growth rate (mm day ⁻¹)	0.038 ± 0.004	0.022 ± 0.010	0.17*	-	0.15¶	0.49
Maximum contraction strength (N)	1.45 ± 0.10	0.82 ± 0.09	0.00052	-	0.73¶	0.24
Contraction rate (min ⁻¹)	9.3 ± 0.5	9.4 ± 0.4	0.78	0.79	-	0.11
Ventral scale abnormality (% of hatchlings)	19.0 ± 5.1	6.3 ± 6.3	0.14	0.516	0.0030¶	0.27
Duration to first shed (day)	11.2 ± 0.9	9.9 ± 0.6	0.26	0.52	0.86§	0.15
					0.19¶	0.94
					0.25¶	0.90¶
					0.17¶	0.59
					0.5	0.50¶

*Mann-Whitney U-test results.
 †ANCOVA covariate: body mass at H.
 ‡ANCOVA covariate: snout-vent length at H.
 §ANCOVA covariate: snout-vent length at 14DPH.
 ¶ANCOVA covariate: body mass at 14DPH.
 -, Not applicable.

46.8 ± 0.8 day. LOW: 45.9 ± 0.6 day) and 14DPH body condition ($F_{2,19} = 4.5$, $P = 0.03$, NRM: 0.034 ± 0.001 g mm⁻¹, BRD: 0.030 ± 0.001 g mm⁻¹, LOW: 0.029 ± 0.001 g mm⁻¹). See Supplementary Table 1 in Supporting Information for complete results of NRM, BRD and LOW comparisons from ID35 to 14DPH.

Within treatments, wet heart mass of BRD and LOW clutches decreased significantly among ID35, H and 14DPH time points (BRD: $F_{2,4} = 11$, $P = 0.0067$; LOW: $F_{2,4} = 4.1$, $P = 0.04$).

At H, there were no differences between BRD clutches and MAT clutches for incubation duration (BRD: 46.8 ± 0.8 day, MAT: 48.6 ± 0.7 day, $t_{13} = -1.8$, $P = 0.10$), hatching success (BRD: 82 ± 4%, MAT: 87 ± 4% $t_{13} = -0.46$, $P = 0.65$), body condition (BRD: 0.034 ± 0.001 g mm⁻¹, MAT: 0.037 ± 0.001 g mm⁻¹, $t_{13} = -1.6$, $P = 0.13$) and strike frequency (BRD: 8.4 ± 2.2, MAT: 5.9 ± 2.0, $t_{13} = 0.83$, $P = 0.42$), suggesting that our BRD treatment adequately mimicked the maternally brooded developmental environment. Further, variables significantly different between NRM and BRD clutches (Table 1) were similarly different between NRM and MAT clutches (body condition: $t_{12} = 2.2$, $P = 0.049$; strike frequency: $t_{12} = 3.4$, $P = 0.0053$), which suggests that the NRM treatment did not mimic the maternally brooded environment.

DISCUSSION

In support of our first prediction, we demonstrated that hypoxia associated with python egg brooding entails significant negative effects to offspring despite compensatory alterations in developmental morphology (i.e. cardiac hypertrophy). Hatchlings incubated in hypoxic conditions relevant to egg brooding were smaller, slower and weaker than hatchlings incubated under normoxic conditions (Table 1). We further predicted that periodic postural adjustments during egg brooding serve to minimize hypoxia-related costs to embryos. However, our second prediction was generally not supported by our results as only a difference in the wet mass of hatchling hearts existed between BRD and LOW treatment clutches. Thus, our results do not fully support an adaptive behaviour hypothesis regarding the use of multiple behaviours (i.e. tight coiling and postural adjustments) to balance embryonic water balance and respiration in egg brooding *A. childreni*.

The detrimental effects of hypoxia on embryonic development have been widely documented among oviparous taxa (birds: Azzam *et al.*, 2007; crocodilians: Warburton *et al.* 1995; fish: Roussel, 2007; flies: Frazier, Woods & Harrison, 2001). During chronic hypoxia, reptile embryos enhance their access to ambient oxygen by increasing the surface area available for gas exchange (i.e. chorioallantoic vasculature)

(Corona & Warburton, 2000) or, like *A. childreni*, the ability to pump blood to the periphery (Crossley & Altimiras, 2005). Notably, in addition to differences in *A. childreni* heart mass among PO_2 treatments, there was also an effect of time on heart mass within BRD and LOW treatment groups. That is, when reared in biologically relevant levels of PO_2 , embryos had relatively larger hearts when in the egg than after hatching despite significant body growth. These results suggest that the oxygen diffusion capability of an eggshell with very high gas conductance (Thompson & Speake, 2004) forces morphological compensation by the embryos. Bird embryos are similarly oxygen-limited during incubation (Ar, Girard & Rodeau, 1991); thus, amniotic development across many taxa may obligate respiration-related costs or, minimally, morphological compensation that is outweighed by benefits to egg water balance.

Regardless of morphological compensation in *A. childreni* embryos, PO_2 levels typical of brooding reduced embryonic metabolism (Stahlschmidt & DeNardo, 2008) and altered offspring traits. Although many quantifiable metrics are termed 'fitness-related variables', empirically verifying the link between phenotype, particularly hatchling phenotype, and fitness is often logistically difficult (Shine, 2004). While we did not validate this link to *A. childreni* fitness, we demonstrated significant effects on an array of metrics that are known to be fitness-related in other species – hatchling body mass: turtles (Janzen, Tucker & Paukstis, 2000; Paitz *et al.*, 2007), snakes (Brown & Shine, 2005); locomotion: turtles (Delmas *et al.*, 2007) lizards (Husak, Fox & Lovern, 2006); predator defence behaviour: spiders (Person, Walker & Rypstra, 2002); gape size: larval salamanders (Kohmatsu, Nakano & Yamamura, 2001). In water pythons (*Liasis fuscus*, Peters 1873), a short incubation duration has a strongly positive association with yearling survivorship in the field (Madsen & Shine, 1999) and hatchling size in the laboratory (Shine *et al.*, 1997). However, our results indicate that under hypoxic conditions python embryos hatch earlier but are less fit than those incubated in normoxia, which supports a variation of Shine's 'safe harbour' hypothesis (Shine, 1978). Accordingly, incubation duration is dictated by the relative costs of embryonic and juvenile developmental stages where adverse incubation conditions expedite hatching.

Notably, we found no difference in offspring fitness between our BRD and LOW treatment groups, which is counter to our adaptive behaviour hypothesis. This result raises the question: If postural adjustments during egg brooding are detrimental to embryonic water balance and do not significantly reduce the respiration-related costs of egg brooding, why do egg-brooding female pythons periodically adjust their

posture? Explanations may be biological, logistical or both. First, postural adjustments may be hard-wired or solely as a result of female restlessness. However, this possibility is refuted by the fact that female *A. childreni* alter their egg-brooding behaviour to significantly enhance clutch temperature (Z. Stahlschmidt & D. DeNardo, unpubl. data).

A more convincing explanation for the lack of a difference in fitness between BRD and LOW treatment offspring is a logistical one – our modelled PO_2 incubation regime for LOW clutches may have been too conservative. Our PO_2 profile for the BRD treatment was based on an extrapolation of empirical data (Fig. 1) and the effectiveness of this approach was validated by the lack of differences between MAT and BRD clutches for fitness-related variables. However, for the LOW treatment, we had to estimate the PO_2 profile for a fictitious situation (constant tight coiling throughout incubation). As it is difficult or impossible to quantitatively estimate the cumulative effect of postural adjustments, it is highly possible that continuous coiling would lead to far greater oxygen suppression than that which we provided in our LOW treatment. Conversely, our modelled LOW treatment may have been realistic, whereby python egg-brooding behavioural adjustments balance thermal and hydric, but likely not respiratory, developmental needs.

Our experimental design focused on the impact of hypoxia, but, in doing so, the impact of developmental hypercapnia remains unknown. Brooding-induced elevations in P_{CO_2} (i.e. partial pressure of CO_2) may play a role in the adaptive significance of egg-brooding postural adjustments. Studies examining the effect(s) of hypercapnia on amniotic development are scarce. However, in early stage chicks, developmental hypercapnia often entails minimal costs or confers benefits, such as increased hatching success (Willemsen *et al.*, 2008) or increased embryonic growth rate (Bruggeman *et al.*, 2007).

Because of its physiologically and behaviourally quantifiable nature, python egg brooding continues to serve as a useful model for the study of intra-offspring and parent-offspring trade-offs associated with parental care. Current and previous studies have evaluated proximate factors, such as developmental temperature (Z. Stahlschmidt & D. DeNardo, unpubl. data), water balance (Lourdais *et al.*, 2007; Stahlschmidt *et al.*, 2008) and hypoxia (Stahlschmidt & DeNardo, 2008; Table 1), to help elucidate the adaptive significance of python egg brooding. Results demonstrate that the hydric and thermal benefits of egg brooding to developing offspring are provided at a respiratory cost that has long-term consequences to the offspring. Future studies to better understand the significance of python parental care should examine

the extent to which environmental conditions and behavioural responses can diminish costs.

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