

## RESEARCH ARTICLE

# Maternal influences on early development: preferred temperature prior to oviposition hastens embryogenesis and enhances offspring traits in the Children's python, *Antaresia childreni*

Sophie Lориoux<sup>1,2,\*</sup>, Dale F. DeNardo<sup>3</sup>, Root Gorelick<sup>4</sup> and Olivier Lourdais<sup>1,3</sup>

<sup>1</sup>Centre d'Etudes Biologiques de Chizé, CNRS, 79360, Villiers en Bois, France, <sup>2</sup>Université de Poitiers, 40 avenue du recteur Pineau, 86022 Poitiers, France, <sup>3</sup>School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA and

<sup>4</sup>Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6

\*Author for correspondence (sophie.lorioux@gmail.com)

Accepted 13 December 2011

### SUMMARY

Embryonic life is particularly sensitive to its surroundings, and the developmental environment can have long-lasting effects on offspring. In oviparous species, the impacts of the developmental environment on offspring traits are mostly examined during development within the egg. However, as more than 25% of the development of squamate reptiles can occur prior to oviposition, we explored the effect of thermal conditions on development prior to oviposition in an oviparous snake species, the Children's python (*Antaresia childreni*). We housed gravid female pythons under three thermal cycles: an optimal regime that reflected maternal preference in a non-constrained environment (constant preferred body temperature of gravid females,  $T_{set}=31.5^{\circ}\text{C}$ ) and two mildly suboptimal regimes that shared the same mean temperature of  $27.7^{\circ}\text{C}$ , but differed in the duration at  $T_{set}$ . In one of the constraining regimes, females had access to  $T_{set}$  for 4 h daily whereas in the other regime, females never reached  $T_{set}$  (maximal temperature of  $29.0^{\circ}\text{C}$ ). Thermal treatments were maintained throughout gravidity in all three groups, but, after oviposition, all eggs were incubated at  $T_{set}$  until hatching. Compared with the optimal regime, the two suboptimal regimes had a longer duration of gravidity, which resulted in delayed hatching. Between the two suboptimal regimes, gravidity was significantly shorter in the treatment that included time at  $T_{set}$ . Furthermore, suboptimal regimes influenced offspring traits at hatching, including body morphology, antipredator behavior, strength and metabolism. However, partial access to maternal  $T_{set}$  significantly enhanced several offspring traits, including performance. Our results demonstrate the importance of time at  $T_{set}$  on early development and suggest an adaptive significance of maternal thermoregulation prior to oviposition.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/215/8/1346/DC1>

Key words: prenatal parental care, thermoregulation, early development, oviparity.

### INTRODUCTION

Embryonic development is sensitive to many factors, including hormones, toxins, energy, water availability and temperature. Minor variation in the conditions experienced by an embryo can translate into major phenotypic modifications (Deeming, 2004) and influence key life history traits such as fecundity or survival (Lindström, 1999; Lumma and Clutton-Brock, 2002). Epidemiological studies in humans and experimental studies in animals have demonstrated that suboptimal developmental conditions have immediate effects that can alter morphology, behavior and survival (Burger, 1998; Webb et al., 2001; Meylan and Clobert, 2005; Denver, 2009). These conditions can also have long-lasting consequences on adult life (Clark and Galef, 1995; Wells, 2003; Gorman and Nager, 2004; Dolinoy et al., 2007; Gardner et al., 2009).

Embryonic thermal sensitivity is a universal vertebrate feature (Farmer, 2000) and eggs are particularly vulnerable to environmental conditions. Incubation temperature affects offspring phenotype (Shine et al., 1997; Elphick and Shine, 1998; Downes and Shine, 1999; Qualls and Andrews, 1999; Mullins and Janzen, 2006; Eiby and Booth, 2009), influencing such traits as locomotor performance (Elphick and Shine, 1998; Braña and Ji, 2000; Ashmore and Janzen,

2003), viability (Shine and Harlow, 1993; Eiby et al., 2008; DuRant et al., 2010), behavior (Sakata and Crews, 2003) and even sex determination (Ewert et al., 1994; Lang and Andrews, 1994; Pieau et al., 1999; Valenzuela and Lance, 2004). Embryonic development is temporally variable (Andrews, 2004) with differentiation (e.g. the establishment of the neuroendocrine axis and body plan) being predominant during initial stages, whereas later development consists of more continuous aspects of embryonic growth and fetal life. Environmental perturbations at early stages can lead to dramatic and often irreversible effects on embryonic organization (Wells, 2003; Kunes and Zicha, 2006; Mueller and Bale, 2006; Oxendine et al., 2006; Zambrano et al., 2005).

In many oviparous amniotes, early embryonic development occurs within the mother prior to oviposition. Thus, oviparous females can exert profound influences on their developing offspring (Mousseau and Fox, 1998; Shine, 2004b). Maternal thermoregulatory shifts during embryonic life are widespread in invertebrates (Tallamy and Wood, 1986; Machado and Raimundo, 2001) and vertebrates (see Farmer, 2000; Shine, 2004a). Through behavioral thermoregulation, mothers can attempt to maintain their developing embryos at an optimal temperature, and thus

buffer early developmental conditions from environmental perturbation. Because of the specific nature of thermal effects [i.e. non-depreciable care (Bernardo, 1996)], a small increase in time at or near the preferred temperature may considerably enhance offspring quality.

Thermoregulatory shifts associated with intra-oviductal development have been reported in most viviparous squamates with pregnant females showing modified (either higher or lower) thermal preferences and, perhaps more importantly, more precise thermoregulation (Robert et al., 2006; Shine, 2006; Gardner-Santana and Beaupre, 2009). Recent experimental work in oviparous squamates suggests that maternal influences on early developmental environment may also provide offspring benefits similar to those typically attributed to viviparous species (Shine, 2006). However, the duration of embryonic retention is much shorter, and therefore there remains a need to better understand embryonic sensitivity during early development (Lourdais et al., 2008).

Female pythons provide extended parental care to their developing embryos by tightly coiling around their eggs, often for the entire incubation period (Shine, 2004a; Aubret et al., 2005b; Lourdais et al., 2007). Female pythons also retain their eggs longer than most other squamates (approximately 30% of development occurs within the female python). Behaviorally, gravid female pythons show a modified thermoregulatory behavior, leading to an elevated and more precise body temperature compared with when they are non-reproductive (Lourdais et al., 2008). These developmental and behavioral aspects of python gravidity suggest that early development in pythons is highly sensitive to temperature, and that females actively regulate early developmental temperature. However, how the thermal environment during early embryonic development affects offspring quality is unknown, and thus the implications of maternal thermoregulation can only be speculated.

Our general hypothesis is that maternal preferred body temperature [ $T_{set}$ , 31.5°C (Lourdais et al., 2008)] during pre-oviposition developmental stages is critical to optimize offspring quality. We experimentally manipulated thermal conditions during gravidity (between ovulation and oviposition) in a small species, the Children's python [*Antaresia childreni* (Gray 1842)]. We tested the following predictions: (1) development is faster when embryos are continuously exposed to  $T_{set}$  early in their life (pre-oviposition development) compared with exposure to temperatures below  $T_{set}$ ; (2) thermal conditions during pre-oviposition development influences offspring traits, with higher quality when early embryos are continuously exposed to  $T_{set}$ ; and (3) when thermoregulation is constrained (i.e. suboptimal conditions), maternal exposure to  $T_{set}$ , independent of differences in mean incubation temperature, will increase the phenotypic quality of offspring.

## MATERIALS AND METHODS

### Study species and maintenance

Snakes used in this study were part of a captive colony of Children's pythons maintained at the Centre d'Etudes Biologiques de Chizé, France. Children's pythons 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 and Swan, 2003). Snakes were housed individually in plastic cages (35.5×63×15 cm) in a room maintained on a 12h:12h light:dark cycle. Prior to breeding, the snakes were provided a thermal gradient (25–35°C) by maintaining room temperature at 20°C and providing continuous supplemental heat using a sub-surface heating cable under one end of each cage. Water was available *ad libitum* in bowls, and snakes were offered one mouse every 1–2 weeks.

### Breeding protocol

To induce reproductive activity, both males and females were moved for 6 weeks to a 6h:18h temperature cycle of 31°C:16°C. Breeding occurred after returning the snakes to their normal thermal regime. Each day a new male was introduced to each female. Courtship and mating generally occurred within a few hours of the male being introduced, and mating typically lasted 4–12 h. Males were transferred to a new female each day unless copulation was occurring at the time of the intended transfer.

Ultrasonography (Micromaxx, SonoSite, Inc., Bothell, WA, USA) was used to assess reproductive status. Pre-laying ecdysis occurs in many oviparous snakes, providing an additional indicator of reproductive status. Skin shedding occurs 1 week before oviposition in colubrid snakes (Markel, 1990), but somewhat earlier in pythons (3–4 weeks pre-oviposition in the Children's python). In the Children's python, ecdysis occurs, on average, 23.1±2.6 days before oviposition and this delay seems to be temperature dependent (Lourdais et al., 2008). When a female was found to be gravid (as determined by ultrasonic imaging of ovoid ova aligned linearly down the coelomic cavity), she was randomly assigned to one of the three thermal treatments (see below), removed from her cage and placed in a smaller opaque plastic container (10.5×30×16.5 cm) within a climatic chamber (Vötsch Industrietechnik, VP 600, Balingen, Germany). A few days before oviposition, the female was transferred to a 1.9l opaque Teflon-coated chamber covered with wire netting to allow gas exchange. At oviposition, the female and her eggs were transferred to a 31.5°C [ $T_{set}$  in the Children's python (Stahlschmidt et al., 2011)] constant incubation regime until hatching. Eggs were inspected daily, and hatchlings were removed after all eggs in a clutch had hatched. Hatchlings from the same clutch were maintained in a single plastic cage (10.5×30×16.5 cm) at a constant temperature of 31.5°C. Females were not fed during the entire reproductive period as they typically are aphagic during this time, but water was available *ad libitum* except when the females were brooding their eggs. In snakes, neonates rely primarily on their body reserves [fat stores and remaining vitellus (Charland, 1989; Lance and Morafka, 2001; Pandav et al., 2006)] for their first weeks of life. Thus, they were not fed during the study period, but were provided water *ad libitum*.

### Experimental thermal treatments during gravidity

In this species, gravidity induces a significant shift in thermal preferences and basking intensity. Gravid females maintain a higher, more precise body temperature (31.5°C) when compared with non-reproductive females (28.7°C) (Lourdais et al., 2008). When given the opportunity, they will maintain this higher body temperature throughout the day and night. Our goal was to examine the significance of maternal thermoregulation by examining the impact of temperature during early embryonic life on development and offspring quality. Three thermal treatments were designed using existing knowledge of female *A. childreni* thermal preference and behavior (Lourdais et al., 2008): 24 h  $T_{set}$ , 4 h  $T_{set}$  and NR.

In the 24 h  $T_{set}$  treatment, gravid females ( $N=11$ ) were constantly exposed to maternal preferred body temperature (31.5°C; Fig. 1). This treatment reflects female behavioral choice when given permanent access to supplemental heat (Lourdais et al., 2008). In natural conditions, the combination of basking activities (during the day) and appropriate burrow selection (at night) can provide such constant temperatures (Shine and Madsen, 1996; Webb et al., 2001).

In the 4 h  $T_{set}$  treatment, gravid females ( $N=10$ ) were exposed to  $T_{set}$  for a short period (4 h daily) and then to 26.0°C for 10 h (mean temperature=27.7°C; Fig. 1). The transition between the two

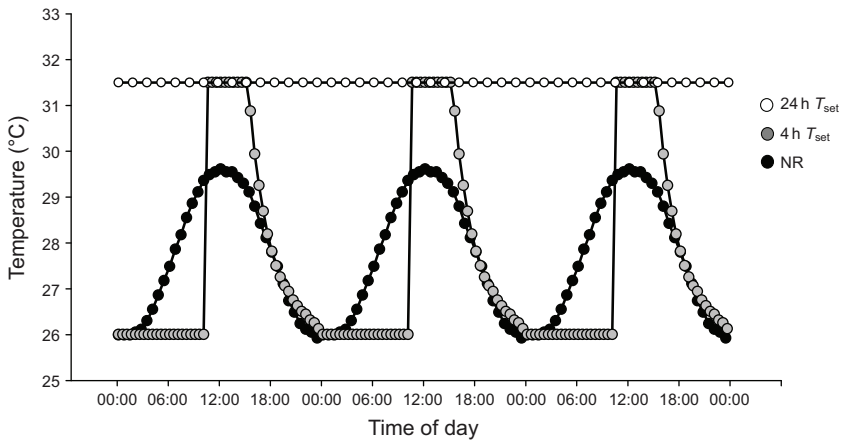


Fig. 1. Thermal treatments applied during gravidity in female Children's python (*Antaresia childreni*). Temperature patterns were obtained from temperature loggers placed in climatic chambers housing the animals. In the 24 h  $T_{set}$  treatment (open circles), gravid females were continuously exposed to their preferred temperature (31.5°C). In the cyclic 4 h  $T_{set}$  treatment (gray circles), gravid females could reach optimal temperature for 4 h per day, whereas in the cyclic NR treatment (black circles), gravid females were exposed to the typical thermal cycle of non-reproductive females and thus had no access to their preferred temperature. The mean temperatures of the 4 h  $T_{set}$  and NR treatments were the same (27.7°C).

temperatures was rapid during temperature increase (1 h) and longer during the temperature decrease (9 h), mimicking natural asymmetrical fluctuations in body temperature. A rapid increase in body temperature is associated with basking efforts, while the slow decrease is a result of insulation provided by the nighttime retreat. This cycle reproduces constrained conditions such as what occurs when weather limits basking opportunities.

In the NR treatment, gravid females ( $N=9$ ) had no access to their preferred temperature ( $T_{set}$ ). Instead, this treatment broadly corresponds to the thermal regime maintained by non-reproductive females when provided a thermal gradient (Lourdais et al., 2008). Although thermal regimes are highly variable among non-reproductive females, for experimental purposes we provided a consistent regime of a 24 h sinusoid with 29.6 and 26.0°C as the maximal and minimal temperatures, respectively. Mean temperature (27.7°C) was the same as for the 4 h  $T_{set}$  group (Fig. 1).

This design provided ecologically relevant thermal cycles to assess offspring benefits provided by the typical shift in female preferred temperature with gravidity (comparing 24 h  $T_{set}$  with NR) and to evaluate the influence of exposure to  $T_{set}$  independent of mean developmental temperature (comparing 4 h  $T_{set}$  with NR). This design does not allow us to fully disentangle the complexity of thermal influences [e.g. the two suboptimal regimes differ in time at  $T_{set}$  and variance (Shine and Elphick, 2001; Shine, 2004c)]; however, these three regimes were relevant considering previously collected information on female behavior. Additionally, we avoided any exposure of embryos to temperatures above  $T_{set}$  as females given a thermal choice rarely elevate body temperature above  $T_{set}$ , likely because of the potential for major developmental alterations.

Experimental thermal treatments were applied throughout gravidity using climatic chambers. The impact of chamber treatment on snake body temperature was first validated using physical models made from rubber tubes filled with water (Lelièvre et al., 2010), reproducing the thermal characteristics of the study species (correlation coefficient=0.95).

#### Variables measured

##### Reproduction phenology

For each female, we recorded the dates of pre-oviposition ecdysis, oviposition and hatching of the eggs. Time period between ecdysis and oviposition provided an estimate of gravidity duration (Lourdais et al., 2008).

##### Offspring traits

To quantify the impact of thermal manipulations during gravidity on offspring quality, we considered a set of traits related to fitness.

#### Morphology

Morphological data were collected at hatching and 2 weeks after hatching to evaluate growth rates. Because hatching snake size (both mass and length) is well documented to have a positive influence on neonatal survival (Sinervo et al., 1992; Brown and Shine, 2004), we measured snout–vent length ( $\pm 0.5$  mm), total length ( $\pm 0.5$  mm) and body mass ( $\pm 0.01$  g). We also measured tail length ( $\pm 0.5$  mm) and jaw length ( $\pm 0.01$  mm). Tail length is an important parameter in snakes as a longer tail positively influences locomotor performance (Aubret et al., 2005a). Snakes are gape-limited predators and jaw size impacts the range of potential prey items and thereby individual performance and fitness (Forsman and Lindell, 1993). We estimated body condition, which reflects the amount of energy stores, as the residual of the regression of body mass on snout–vent length (Jayne and Benett, 1990; Madsen and Shine, 1999; Lourdais et al., 2002), both variables log-transformed.

#### Swimming and contraction performance

We measured swimming and contraction force for each neonate. All trials were performed at a room temperature of  $20.0 \pm 1.0$ °C. We conducted swimming trials in a PVC swimming track ( $3.5 \times 0.3 \times 0.16$  m, length  $\times$  height  $\times$  width) with water maintained at 31.5°C. We encouraged snakes to swim at their maximum speed by gently tapping their tails throughout the trial. Using a stopwatch, we recorded the time ( $\pm 0.01$  s) for the head to traverse the raceway (trial length=2.5 m). Between each trial, snakes were handled out of the water for 10 s and then replaced at the swimming starting point. We conducted three successive trials on two consecutive days within 3 days of hatching. We used maximum and mean swimming speed over all trials in the analysis.

Snakes react to handling, especially attempts to stretch out their bodies, by vigorous and extended body contraction. Contraction force was assessed between 2 and 3 weeks after hatching. We quantified this behavior using a dynamometer (Force Gauge 200N, Fisher Scientific Bioblock, Illkirch, France). The tail of the snake was attached to the dynamometer using a string. We then immobilized the body, maintaining the snake in a stretched position on a flat surface. To avoid residual traction, the dynamometer was reset just before the beginning of each test. Body contraction was stimulated by gentle palpation of the back. The highest value ( $\pm 0.05$  N) obtained during 1 min of stimulation was recorded. During the same time, we also collected the number of contractions as an estimate of defensive motivation. Three consecutive trials (1 min each) were performed for each individual. We used maximum and mean contraction force and maximum and mean contraction number in the analyses.

### Antipredator behavior

Defensive behavior was quantified within 3 days of hatching. Two trials were conducted over two successive days. Snakes were placed in an arena and stimulated with a stick to mimic a predator attack (Stahlschmidt and DeNardo, 2009). The number of strikes elicited over 1 min was recorded.

### Metabolic rate

We used closed-system respirometry to measure standard metabolic rate 2 weeks after hatching. Trials were conducted at  $31.5 \pm 1.0^\circ\text{C}$  in opaque test chambers (average internal volume 1900 ml; the exact volume was incorporated into the analyses) covered with a glass lid made air-tight using closed-cell foam. Each test chamber contained a small piece of paper towel to allow the snake to hide, and thus remain inactive. To assure that the temperature had stabilized, the animals were placed in their test chambers inside the environmental chamber for 2 h prior to conducting the standard metabolic rate trial. Trials were conducted midday between 11:00 and 16:00 h. Outside air was drawn into each chamber using a Bioblock Scientific 5 l air pump for 3 min assuring 99% air turnover (Lasiewski et al., 1966). Baseline samples of outside air were collected at the onset of each trial. The test chambers were sealed, and the time of closure was recorded. Trial duration ( $180.1 \pm 0.4$  min) ensured sufficient  $\text{O}_2$  suppression (0.1%). At the end of each trial, time was recorded and an end air sample was collected using two 140 ml syringes connected to the chamber *via* a three-way stopcock. The  $\text{O}_2$  concentration of the sample was then determined using an oxygen analyzer (resolution 1 p.p.m.; FC10A, Sable Systems, Las Vegas, NV, USA). Using an infusion pump (KDS 210, KD Scientific, Inc., Holliston, MA, USA) set at a controlled rate ( $18 \text{ ml min}^{-1}$ ), each air sample was stripped of moisture by first passing through Drierite and then sent to the analyzer. The analyzer was calibrated at the infusion pump flow rate prior to each session. Oxygen consumption ( $\pm 0.01 \text{ ml h}^{-1}$ ) was calculated as: (baseline oxygen concentration – final oxygen concentration)  $\times$  volume of the trial chamber / duration of the trial.

### Statistical analyses

All statistical comparisons were performed with R version 2.12.1 (R Development Core Team 2010) using linear models (lm models, stats package), linear mixed models (lme models, nlme package) and generalized linear mixed models for binomial distribution (glmer models, lme4 package). For each test, the distribution was checked and variables were transformed when necessary. Significance was determined at  $\alpha=0.05$  for all tests. Experiments were conducted in 2008 and 2010, with treatments evenly distributed across years. We found no year effect, so this parameter was not included in the analyses. Some females ( $N=3$ ) reproduced in both years, and their contribution was accounted for in the analyses (random effect on female identity).

We analyzed data of reproduction phenology using a one-factor ANOVA to test for the effect of thermal treatment on gravidity duration and incubation duration. Because siblings are not independent statistical units (Massot et al., 1994), in all other statistical tests on offspring traits, we used linear mixed model analyses with female identity as a random factor. Statistical significance of the random term was estimated using likelihood ratio tests (LRTs) between the full and reduced models.

Although energy allocation into the eggs (i.e. clutch size and clutch mass) occurs during vitellogenesis, parameters such as maternal size and clutch size are known to influence offspring traits (Olsson and Shine, 1997; Bonnet et al., 2001; Andrews, 2004). To control for

maternal investment and allocation trade-offs, we included clutch size adjusted for mother snout–vent length (residuals of the regression of clutch size on mother snout–vent length) in the models for snout–vent length and body mass at birth. Body condition was calculated as the residual values from the regression of body mass against snout–vent length. Body proportions (i.e. tail length and jaw length relative to body length) were analyzed using snout–vent length as a covariate. Growth patterns were analyzed using initial measurement at hatching as a covariate. For snout–vent length growth analyses we also included body condition as a covariate. Proportion of strikers and strike number were analyzed using female identity as a random factor and treatment as a fixed factor. Strike number data were log-transformed to verify normality assumption. Swimming speed was analyzed using female identity as a random factor, treatment as a fixed factor, and snout–vent length at birth as a co-factor. We analyzed data of traction force using linear mixed models with female identity as a random factor, treatment as a fixed factor, and body mass at day 14 as a covariate. Metabolic rate was measured using female identity as a random factor, treatment as a fixed factor, and body mass at day 14 as a covariate. To test for effects of thermal treatment and other co-factors (described above), we used the model with the smallest Akaike's information criterion (AIC). When two models differed by less than two AIC, we chose the more parsimonious (supplementary material Tables S1–S3). When the effect of thermal treatment was statistically significant, Tukey's *post hoc* tests were performed to allow for  $2 \times 2$  comparisons in the selected model (multcomp package). Values are presented as means  $\pm$  s.e.m.

## RESULTS

### Influence of treatment on reproduction phenology

Thermal treatment influenced gravidity duration (one-way ANOVA,  $F_{2,27}=56.431$ ,  $P<0.001$ ; Fig. 2A) and the effect of female identity was significant (LRT $<0.001$ ). Gravidity duration was shortest in snakes in the 24 h  $T_{\text{set}}$  treatment ( $22.5 \pm 1.0$  days), intermediate in snakes in the 4 h  $T_{\text{set}}$  treatment ( $32.7 \pm 1.1$  days) and longest in snakes in the NR treatment ( $38.0 \pm 1.1$  days; Tukey's *post hoc* tests: 4 h  $T_{\text{set}}$  vs 24 h  $T_{\text{set}}$ ,  $t=-6.655$ ,  $P<0.001$ ; 24 h  $T_{\text{set}}$  vs NR,  $t=-10.421$ ,  $P<0.001$ ; 4 h  $T_{\text{set}}$  vs NR,  $t=3.400$ ,  $P=0.006$ ). Egg incubation duration was also significantly different among treatments (one-way ANOVA,  $F_{2,26}=7.197$ ,  $P=0.003$ ; Fig. 2B). *Post hoc* tests revealed no differences in incubation duration between the two constraining thermal treatments ( $47.8 \pm 0.6$  and  $47.8 \pm 0.5$  days for 4 h  $T_{\text{set}}$  and NR, respectively;  $t=0.066$ ,  $P=0.998$ ). However, incubation was significantly longer in snakes in the 24 h  $T_{\text{set}}$  treatment ( $50.1 \pm 0.5$  days; Tukey's *post hoc* test, 24 h  $T_{\text{set}}$  vs 4 h  $T_{\text{set}}$ ,  $t=3.162$ ,  $P=0.011$ ; 24 h  $T_{\text{set}}$  vs NR,  $t=3.291$ ,  $P=0.007$ ).

### Influence of treatment on hatchling traits

One hundred and ninety-one snakes hatched during this study: 62 in the 24 h  $T_{\text{set}}$  treatment, 61 in the 4 h  $T_{\text{set}}$  treatment and 68 in the NR treatment. All values and the results of the Tukey's *post hoc* tests are presented in Tables 1 and 2.

### Offspring morphology and growth

Thermal treatment did not influence offspring snout–vent length (Table 1). We found a significant effect of mother and clutch size (adjusted by snout–vent length) on hatchling size ( $F_{1,85}=13.433$ ,  $P<0.001$ ). Considering body proportions, jaw length was not influenced by thermal treatment, but was influenced by snout–vent length at hatching ( $F_{1,186}=35.840$ ,  $P<0.001$ ; see Table 1). Tail length was significantly influenced by thermal treatment (Table 1) and also by snout–vent length ( $F_{1,183}=50.673$ ,  $P<0.001$ ). Hatchlings from the

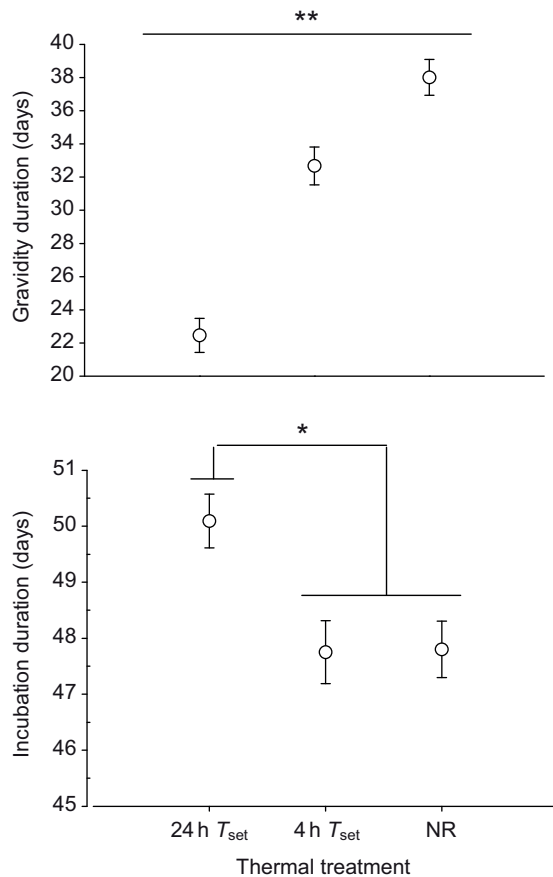


Fig. 2. Influence of thermal treatment during gravidity on (A) gravidity and (B) incubation durations. Error bars represent  $\pm$ s.e.m. \* $P<0.05$ ; \*\* $P<0.01$ .

24 h  $T_{set}$  treatment had a longer tail than hatchlings from both of the thermally constrained treatments (Table 1). Body mass was influenced by thermal treatment (Table 1) and adjusted clutch size ( $F_{1,183}=29.337$ ,  $P<0.001$ ). Body condition was influenced by thermal treatment (Table 1). Hatchlings from the NR treatment were heavier and had a higher body condition than hatchlings from the two other treatments (Table 1).

Thermal treatment did not influence growth in body size (Table 1), which, instead, was mainly determined by snout–vent length at hatching ( $F_{1,183}=28.964$ ,  $P<0.001$ ) and initial body condition

( $F_{1,183}=19.465$ ,  $P<0.001$ ). Smaller individuals at birth exhibited higher growth rates over the study period ( $r^2=-0.24$ ,  $P<0.001$ ). Tail growth was not influenced by thermal treatment (Table 1). Hatchlings from the 24 h  $T_{set}$  treatment had greater jaw growth than hatchlings from the two other treatments (Table 1).

#### Offspring behavior

Thermal treatment did not influence the proportion of individuals eliciting strikes (glmer model for binomial distribution,  $P>0.05$ ). Among striking individuals, total strike number (log-transformed data) was not different among treatments.

Maximal swimming speed recorded was significantly influenced by thermal treatment. Variation between successive trials was minor (CV: 2.01, 1.48 and 3.47% for 24 h  $T_{set}$ , 4 h  $T_{set}$  and NR, respectively). Hatchlings from the NR treatment were significantly slower than individuals from the 24 h  $T_{set}$  treatment, whereas hatchlings from the 4 h  $T_{set}$  treatment were not significantly different from either of the other two groups (Table 2). Average swimming speed was also influenced by thermal treatment (Table 2). NR treatment hatchlings had a slower mean speed compared with the other two treatments (Table 2).

Thermal conditions during gravidity influenced maximal contraction force (Table 2). Variation between trials was highest in snakes in the 24 h  $T_{set}$  treatment (CV: 30.0, 18.9 and 9.1% for 24 h  $T_{set}$ , 4 h  $T_{set}$  and NR, respectively). Still, maximal contraction force was significantly lower in the NR treatment compared with the other two treatments (Table 2). Average contraction force over the three trials was also different among treatments, suggesting a lower endurance for hatchlings from the NR treatment (Table 2). The maximum number of contractions, as well as the average number of contractions, was also influenced by thermal treatment (Table 2). Both of these values were lower for hatchlings from the NR treatment (Table 2).

#### Metabolism

The standard metabolic rate of hatchlings at 14 days of age was significantly influenced by thermal treatment (Table 2) and also by body mass ( $F_{1,203}=13.661$ ,  $P<0.001$ ). Hatchlings from the NR treatment had a significantly higher standard metabolic rate than hatchlings from either of the other two treatments (Table 2).

#### DISCUSSION

We addressed the influence of pre-oviposition, embryonic temperature on reproductive phenology and offspring traits in an

Table 1. Influence of thermal treatment and female identity during gravidity on morphological traits and growth patterns of hatchling pythons (*Antaresia childreni*)

Morphological trait	Gravidity treatment			Thermal treatment	Female identity (LRT)
	24 h $T_{set}$	4 h $T_{set}$	NR		
Snout–vent length (mm)	237.3 $\pm$ 1.6	231.7 $\pm$ 1.6	235.6 $\pm$ 1.4	n.s.	<0.001
Body mass (g)	8.16 $\pm$ 0.14 <sup>a</sup>	8.14 $\pm$ 0.14 <sup>a</sup>	9.19 $\pm$ 0.13 <sup>b</sup>	$F_{2,183}=6.651$ , $P=0.002$	0
Tail length (mm)	28.4 $\pm$ 0.2 <sup>a</sup>	27.5 $\pm$ 0.2 <sup>b</sup>	27.4 $\pm$ 0.19 <sup>b</sup>	$F_{2,183}=4.112$ , $P=0.018$	<0.001
Jaw length (mm)	13.93 $\pm$ 0.05	13.94 $\pm$ 0.05	13.86 $\pm$ 0.04	n.s.	<0.001
Body condition	-0.004 $\pm$ 0.004 <sup>a</sup>	-0.002 $\pm$ 0.004 <sup>b</sup>	0.029 $\pm$ 0.006 <sup>c</sup>	$F_{1,184}=15.769$ , $P<0.001$	<0.001
Snout–vent length growth (mm day <sup>-1</sup> )	1.17 $\pm$ 0.07	1.15 $\pm$ 0.07	1.25 $\pm$ 0.07	n.s.	0.007
Jaw growth (mm day <sup>-1</sup> )	0.032 $\pm$ 0.002 <sup>a</sup>	0.020 $\pm$ 0.002 <sup>b</sup>	0.017 $\pm$ 0.001 <sup>b</sup>	$F_{2,184}=13.428$ , $P<0.001$	0.310
Tail growth (mm day <sup>-1</sup> )	0.091 $\pm$ 0.009	0.053 $\pm$ 0.009	0.067 $\pm$ 0.008	n.s.	0.323

Fitted values are presented as means  $\pm$  s.e.m.

LRT, likelihood ratio test; n.s., not significant.

Results of Tukey's *post hoc* tests are indicated with superscripted letters next to fitted mean values when the effect of thermal treatment was significant.

Different letters represent significant differences ( $P<0.05$ ) between groups.

Table 2. Influence of thermal treatment and female identity during gravidity on performance traits and physiology of hatchling pythons (*Antaresia childreni*)

Performance trait	Gravidity treatment			Thermal treatment	Female identity (LRT)
	24 h $T_{set}$	4 h $T_{set}$	NR		
Proportion of strikers (%)	67.7±6.0	48.4±6.4	59.0±5.6	n.s.	<0.001
Total strike number	20.09±2.67	17.83±2.84	14.52±2.20	n.s.	1
Maximal swimming speed (cm s <sup>-1</sup> )	22.28±0.73 <sup>a</sup>	20.69±0.51 <sup>a,b</sup>	20.70±0.56 <sup>b</sup>	$F_{2,184}=4.020$ , $P=0.020$	0.029
Mean swimming speed (cm s <sup>-1</sup> )	17.65±0.51 <sup>a</sup>	17.12±0.37 <sup>a</sup>	16.14±0.41 <sup>b</sup>	$F_{2,184}=6.459$ , $P=0.020$	0.04
Maximal strength force (N)	1.16±0.07 <sup>a</sup>	0.99±0.06 <sup>a</sup>	0.86±0.05 <sup>b</sup>	$F_{2,170}=12.905$ , $P<0.001$	0.024
Mean strength force (N)	0.83±0.05 <sup>a</sup>	0.78±0.05 <sup>a</sup>	0.64±0.04 <sup>b</sup>	$F_{2,170}=5.234$ , $P=0.006$	0.962
Maximum number of contractions	8.09±0.52 <sup>a</sup>	8.26±0.57 <sup>a</sup>	6.44±0.37 <sup>b</sup>	$F_{2,170}=24.009$ , $P<0.001$	<0.001
Mean number of contractions	5.78±0.36 <sup>a</sup>	5.81±0.34 <sup>a</sup>	4.77±0.28 <sup>b</sup>	$F_{2,170}=11.877$ , $P<0.001$	<0.001
O <sub>2</sub> consumption (ml h <sup>-1</sup> )	0.87±0.05 <sup>a</sup>	0.75±0.10 <sup>a</sup>	1.13±0.10 <sup>b</sup>	$F_{2,203}=5.944$ , $P=0.003$	<0.001

Values are presented as means ± s.e.m.

LRT, likelihood ratio test; n.s., not significant.

Results of Tukey's *post hoc* tests are indicated with superscripted letters next to fitted mean values when the effect of thermal treatment was significant.

Different letters represent significant differences ( $P<0.05$ ) between groups.

oviparous squamate, the Children's python. By utilizing treatments that mimic ecologically relevant conditions, we show that altered female thermal profiles associated with gravidity alter the timing of development as well as fitness-relevant morphology and performance of offspring. Additionally, we demonstrated that exposure to maternal preferred temperature, even if of limited duration, significantly shortened gravidity and enhanced fitness-relevant offspring traits independent of any effects of mean incubation temperature.

#### Early embryonic temperature influences gravidity duration and date of hatching

Thermal treatment strongly impacted gravidity duration. As predicted, suboptimal conditions significantly slowed development. Gravidity was, respectively, 45.5 and 69.2% longer with the 4 h  $T_{set}$  and NR treatments. Although the two suboptimal treatments had the same mean daily temperature, gravidity duration was significantly shorter when embryos had limited exposure to  $T_{set}$ . A slight but significant decrease in egg incubation duration was observed in the two suboptimal regimes. These results could be interpreted as a positive compensatory response of the embryos. For instance, a recent study demonstrated compensatory cardiac response during embryonic life in turtles with accelerated developmental rate (Du and Shine, 2010). A non-exclusive hypothesis is that there is a prolonged retention of eggs when conditions are suboptimal, enabling more of the developmental phase to occur within the female (Warner and Andrews, 2003). Additional studies on embryonic stage at oviposition are required to address these hypotheses. Regardless, total developmental time from ecdysis to hatching was significantly longer in the constrained groups (72.5 days for 24 h  $T_{set}$ , 80.3 days for 4 h  $T_{set}$  and 85.8 days for NR). Based on these results, we can conclude that maternal thermoregulation during gravidity hastens developmental rate, resulting in an earlier oviposition date and, thus, hatching date. This result is consistent with previous finding (Shine, 2006; Lourdais et al., 2008). Date of birth is a crucial parameter in squamates, as it impacts the time that the neonates have in a favorable environment and thus survival (Olsson and Shine, 1998a; Olsson and Shine, 1998b; Warner and Shine, 2007). It is noteworthy that a shorter duration of gravidity may also be beneficial to the mother by reducing the period of physical burden (Shine, 1980; Shine, 2004a) and associated ecological risks.

#### Early embryonic temperature affects offspring quality

Thermal treatment during gravidity affected morphology, performance and behavioral traits of hatchlings despite incubation of all eggs at the same temperature. Offspring body mass and body proportions were significantly affected by thermal treatment. Neonates from the two suboptimal treatments had reduced jaw growth at 2 weeks of age and significantly shorter tails at birth, the latter reflecting previous results in lizards incubated at cooler temperatures (Elphick and Shine, 1998; Qualls and Andrews, 1999). Our results suggest a deleterious impact of suboptimal conditions on head plasticity. Adaptive plasticity (enhanced head growth) has been previously studied in response to a trophic environment (Forsman, 1996; Forsman and Shine, 1997; Aubret et al., 2004). In our study, hatchlings from the NR regime were heavier and had a higher body condition, yet had reduced jaw growth. Similar effects were found in lizards (Shine and Harlow, 1996) and may be interpreted as altered efficiency of yolk conversion to body tissues.

Offspring from the NR treatment had the highest metabolic rates, which may represent a positive compensation for suboptimal temperatures during gravidity. Yet in our context, high standard metabolic rate may also be disadvantageous (high maintenance costs) because this parameter was measured in fasting animals 15 days after hatching. Metabolic responses to incubation temperature have been reported in other species (Deeming and Ferguson, 1991; Birchard and Reiber, 1995; O'Steen and Janzen, 1999). However, metabolic rate may change dramatically after hatching (Schultz et al., 2008). As only a single measurement was made in our study, it is impossible to conclude whether the response is irreversible (developmental acclimation) or developmentally plastic (reversible acclimation) (Angilletta, 2009). Still, the difference in standard metabolic rate was detected after standardized incubation conditions (45 days), and thereby reflects long-term effects of early embryonic temperature.

Most importantly, behavior at hatching was affected by thermal treatment. Snakes are independent at birth, and altered defensive behavior may affect survival (Burger, 1998). Swimming speed is a relevant estimator of locomotor and predator evasion abilities (Webb et al., 2001; Stahlschmidt and DeNardo, 2009), whereas contraction force provides an indicator of neonate defensive response to handling (Stahlschmidt and DeNardo, 2009), as well as an estimate of strength (Lourdais et al., 2005). Hatchlings from the NR treatment consistently demonstrated reduced anti-predator behavior,

with reduced swimming speed endurance and reduced contraction force (both maximal and mean) compared with the 4h  $T_{set}$  and 24h  $T_{set}$  treatments. Reduced strength can be especially problematic in constricting snakes, which rely on force to subdue their prey. Our data consistently identified reduced offspring quality in the suboptimal treatments. Although morphology was affected for some traits, major differences were detected in physiology and behavior. As early development is highly sensitive to temperature, thermoregulatory shifts displayed by gravid females (e.g. the 24h  $T_{set}$  vs the NR treatments) are likely adaptive. Maternal behavioral shifts and thermal precision likely provide great benefit by hastening development rate and enhancing offspring quality and current reproductive value.

#### Exposure to preferred body temperature enhances offspring phenotype

Thermal sensitivity of development has attracted considerable scientific interest. Thermal variance, as well as mean temperature, can modify hatchlings' phenotypic traits (Webb et al., 2001; Shine, 2004b; Du and Shine, 2010). Such effects are essential to consider when studying maternal thermoregulation, which is likely affected by basking opportunities and diel cycle. We found that although continued access to preferred body temperature was optimal for offspring development, even a short period at preferred temperature was sufficient to significantly shorten gravidity duration and enhance fitness-relevant offspring traits, even when the mean developmental temperature was kept constant (i.e. the 4H- $T_{set}$  vs the NR treatments). For most behavioral traits, offspring from the 4h  $T_{set}$  treatment were not different from those from the 24h  $T_{set}$  treatment, whereas neonates from the NR treatment displayed lower quality. Our design did not allow us to fully disentangle thermal influences because the three groups also differed in thermal variance. However, the fact that offspring from the 4h  $T_{set}$  treatment were either equivalent or closer to those from 24h  $T_{set}$  treatment in terms of phenological and offspring traits indicates that access to preferred body temperature may be more relevant than mean temperature and thermal variance during development (Georges, 1989; Du and Shine, 2010). Active maternal thermoregulation likely provides a robust means to extend exposure to optimal developmental temperature and thereby enhance offspring quality (Wapstra, 2000). These results suggest non-linear thermal benefits on development that need to be addressed more thoroughly.

#### CONCLUSIONS

Our study clearly supports the importance of maternal regulation at early stages of development on both phenological and offspring traits. Overall, our results highlight the importance of exposure to thermal preference during early embryonic life. Viviparity may have evolved *via* extension of pre-existing adaptations in oviparous squamates, as suggested by Shine (Shine, 2006). We provide the first experimental support for this 'pre-adaptation' hypothesis in snakes. Pythons are quite unique among squamates because all species are oviparous and show maternal egg care (Vinegar, 1970; Harlow and Grigg, 1984; Slip and Shine, 1988; Lourdaï et al., 2007; Stahlschmidt and DeNardo, 2008; Stahlschmidt et al., 2008). Although brooding has attracted considerable interest (Aubret et al., 2005b; Lourdaï et al., 2007; Stahlschmidt and DeNardo, 2008), many python species (possibly all) display specific thermoregulatory behavior (inverted basking) during gravidity. Hence, regulation of early developmental conditions may be a general, possibly ancestral trait within pythons that favored further maternal investment after oviposition (egg brooding).

#### ACKNOWLEDGEMENTS

We thank David Pinaud, Mathieu Authier and Michaël Guillon for their help with statistical analyses. Julie Volette, Gersende Dangoisse and Hélène Lisse participated in data collection. We thank Christian Thibource and Xavier Duchemin for snake care.

#### FUNDING

Financial support was provided by the Fyssen Foundation, the Natural Sciences Engineering Research Council of Canada, and the Centre National de la Recherche Scientifique (Institut Ecologie et Environnement).

#### REFERENCES

- Andrews, R. M. (2004). Embryonic development. In *Reptilian Incubation: Environment, Evolution and Behaviour* (ed. D. C. Deeming), pp. 75-102. Nottingham: Nottingham University Press.
- Angilletta, M. J. (2009). *Thermal Adaptation: a Theoretical and Empirical Synthesis*. Oxford: Oxford University Press.
- Ashmore, G. M. and Janzen, F. J. (2003). Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia* **134**, 182-188.
- Aubret, F., Shine, R. and Bonnet, X. (2004). Adaptive developmental plasticity in snakes. *Nature* **431**, 261-262.
- Aubret, F., Bonnet, X. and Maumelat, S. (2005a). Tail loss, body condition and swimming performances in tiger snakes, *Notechis ater occidentalis*. *J. Exp. Zool.* **303**, 894-903.
- Aubret, F., Bonnet, X., Shine, R. and Maumelat, S. (2005b). Why do female ball python (*Python regius*) coil so tightly around their eggs? *Evol. Ecol. Res.* **7**, 743-758.
- Bernardo, J. (1996). Maternal effects in animal ecology. *Am. Zool.* **36**, 83-105.
- Birchard, G. F. and Reiber, C. L. (1995). Growth, metabolism and chorioallantoic vascular density of developing snapping turtles (*Chelydra serpentina*): Influence of temperature. *Physiol. Zool.* **68**, 799-811.
- Bonnet, X., Naulleau, G., Shine, R. and Lourdaï, O. (2001). Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. *Oikos* **92**, 297-308.
- Braña, F. and Ji, X. (2000). Influence of incubation temperature on morphology, locomotor performance and early growth of hatchling wall lizards (*Podarcis muralis*). *J. Exp. Zool.* **286**, 422-433.
- Brown, G. P. and Shine, R. (2004). Maternal nest-site selection and its consequences for offspring fitness in a tropical snake (*Tropidonophis mairii*, Colubridae). *Ecology* **85**, 1627-1634.
- Burger, J. (1998). Effects of incubation temperature on hatchling pine snakes: implications for survival. *Behav. Ecol. Sociobiol.* **43**, 11-18.
- Charland, M. B. (1989). Size and winter survivorship in neonatal western rattlesnakes (*Crotalus viridis*). *Can. J. Zool.* **67**, 1620-1625.
- Clark, M. M. and Galef, B. G. (1995). Prenatal influences on reproductive life history strategies. *Trends Ecol. Evol.* **10**, 151-153.
- Deeming, D. C. (2004). *Reptilian Incubation: Environment, Evolution and Behaviour*. Nottingham: Nottingham University Press.
- Deeming, D. C. and Fergusson, M. W. J. (1991). *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge: Cambridge University Press.
- Denver, R. J. (2009). Stress hormones mediate environment-genotype interactions during amphibian development. *Gen. Comp. Endocrinol.* **164**, 20-31.
- Dolinoy, D. C., Weidman, J. R. and Jirtle, R. L. (2007). Epigenetic gene regulation: linking early developmental environment to adult disease. *Reprod. Toxicol.* **23**, 297-307.
- Downes, S. J. and Shine, R. (1999). Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? *Oecologia* **120**, 9-18.
- Du, W. G. and Shine, R. (2010). Why do the eggs of lizards (*Bassinia duperreyi*: Scincidae) hatch sooner if incubated at fluctuating rather than constant temperatures? *Biol. J. Linn. Soc.* **101**, 642-650.
- DuRant, S. E., Hepp, G. R., Moore, I. T., Hopkins, B. C. and Hopkins, W. A. (2010). Slight differences in incubation temperature affect early growth and stress endocrinology of wood duck (*Aix sponsa*) ducklings. *J. Exp. Biol.* **213**, 45-51.
- Eiby, Y. A. and Booth, D. T. (2009). The effects of incubation temperature on the morphology and composition of Australian brush-turkey (*Alectura lathamii*) chicks. *J. Comp. Physiol. B* **179**, 875-882.
- Eiby, Y. A., Worthington Wilmer, J. and Booth, D. T. (2008). Temperature-dependent sex-biased embryo mortality in a bird. *Proc. R. Soc. Lond. B* **275**, 2703-2706.
- Elphick, M. J. and Shine, R. (1998). Long-term effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biol. J. Linn. Soc.* **63**, 429-447.
- Ewert, M. A., Jackson, D. R. and Nelson, C. E. (1994). Patterns of temperature-dependent sex determination in turtles. *J. Exp. Zool.* **270**, 3-15.
- Farmer, C. G. (2000). Parental care: The key to understanding endothermy and other convergent features in birds and mammals. *Am. Nat.* **55**, 326-334.
- Forsman, A. (1996). An experimental test for food effects on head size allometry in juvenile snakes. *Evolution* **50**, 2536-2542.
- Forsman, A. and Lindell, L. E. (1993). The advantage of a big head: swallowing performance in adders, *Vipera berus*. *Funct. Ecol.* **7**, 183-189.
- Forsman, A. and Shine, R. (1997). Rejection of non-adaptive hypotheses for intraspecific variation in trophic morphology in gape-limited predators. *Biol. J. Linn. Soc.* **62**, 209-223.
- Gardner, D. S., Ozanne, S. E. and Sinclair, K. D. (2009). Effect of the early-life nutritional environment on fecundity and fertility of mammals. *Philos. Trans. R. Soc. Lond. B* **364**, 3419-3427.

- Gardner-Santana, L. C. and Beupre, S. J.** (2009). Timber rattlesnakes (*Crotalus horridus*) exhibit elevated and less variable body temperatures during pregnancy. *Copeia* **2009**, 363-368.
- Georges, A.** (1989). Female turtle from hot nests: is it duration of incubation or proportion of development at high temperatures that matters? *Oecologia* **81**, 323-328.
- Gorman, H. E. and Nager, R. G.** (2004). Prenatal developmental conditions have long-term effects on offspring fecundity. *Proc. R. Soc. Lond. B* **271**, 1923-1928.
- Harlow, P. and Grigg, G.** (1984). Shivering thermogenesis in a brooding diamond python, *Python spilotes spilotes*. *Copeia* **1984**, 959-965.
- Jayne, B. C. and Bennett, A. F.** (1990). Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**, 1204-1229.
- Kunes, J. and Zicha, J.** (2006). Developmental window and environment as important factors in the expression of genetic information: a cardiovascular physiologist's view. *Clin. Sci.* **111**, 295-305.
- Lance, V. A. and Morafka, D. J.** (2001). Post natal lecithotroph: a new age class in the ontogeny of reptiles. *Herpetol. Monogr.* **15**, 124-134.
- Lang, J. W. and Andrews, H. V.** (1994). Temperature-dependent sex determination in crocodylians. *J. Exp. Zool.* **270**, 28-44.
- Lasiewski, R. C., Acosta, A. L. and Bernstein, M. L.** (1966). Evaporative water loss in birds. I. Characteristics of the open flow method of determination and their relation to estimates of the thermoregulatory ability. *Comp. Biochem. Physiol.* **19**, 445-457.
- Lelièvre, H., Le Hénauff, M., Blouin-Demers, G., Naulleau, G. and Lourdaïs, O.** (2010). Thermal strategies in two sympatric colubrid snakes with contrasted exposure. *J. Comp. Physiol. B* **180**, 415-425.
- Lindström, J.** (1999). Early development and fitness in birds and mammals. *Trends Ecol. Evol.* **14**, 343-348.
- Lourdaïs, O., Bonnet, X., Shine, R., DeNardo, D., Naulleau, G. and Guillon, M.** (2002). Capital-breeding and reproductive effort in a variable environment: a longitudinal study of viviparous snake. *J. Anim. Ecol.* **71**, 470-479.
- Lourdaïs, O., Brischoux, F. and Barantin, L.** (2005). How to assess musculature and performance in a constricting snake? A case study in the Colombian rainbow boa (*Epicratis cenchria maurus*). *J. Zool.* **265**, 43-51.
- Lourdaïs, O., Hoffman, T. and DeNardo, D.** (2007). Maternal brooding in the Children's python (*Antaresia childreni*) promotes egg water balance. *J. Comp. Physiol. B* **177**, 569-577.
- Lourdaïs, O., Heulin, B. and DeNardo, D.** (2008). Thermoregulation during gravidity in the Children's python (*Antaresia childreni*): a test of the pre-adaptation hypothesis for maternal thermophily in snakes. *Biol. J. Linn. Soc.* **93**, 499-508.
- Lumma, V. and Clutton-Brock, T.** (2002). Early development, survival and reproduction in humans. *Trends Ecol. Evol.* **17**, 141-147.
- Machado, G. and Raimundo, R. L. G.** (2001). Parental investment and the evolution of subsocial behaviour in harvestmen (Arachnida, Opiliones). *Ethol. Ecol. Evol.* **13**, 133-150.
- Madsen, T. and Shine, R.** (1999). Life history consequences of nest-site variation in tropical pythons (*Liasis fuscus*). *Ecology* **80**, 89-997.
- Markel, R. G.** (1990). *Kingsnakes and Milk Snakes*. Neptune City, NJ: TFH Publication.
- Massot, M., Clobert, J., Chambon, A. and Michalakakis, Y.** (1994). Vertebrate natal dispersal: the problem of non-independence of siblings. *Oikos* **70**, 172-176.
- Meylan, S. and Clobert, J.** (2005). Is corticosterone-mediated phenotype development adaptive? Maternal corticosterone treatment enhances survival in male lizards. *Horm. Behav.* **48**, 44-52.
- Mousseau, T. A. and Fox, C. W.** (1998). The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**, 403-407.
- Mueller, B. R. and Bale, T. L.** (2006). Impact of prenatal stress on long-term body weight is dependent on timing and maternal sensitivity. *Physiol. Behav.* **88**, 605-614.
- Mullins, M. A. and Janzen, F. J.** (2006). Phenotypic effects of thermal means and variances on smooth softshell turtle (*Apalone mutica*) embryos and hatchlings. *Herpetologica* **62**, 27-36.
- Olsson, M. and Shine, R.** (1997). The limits to reproductive outputs: Offspring size versus number in the sand lizard (*Lacerta agilis*). *Am. Nat.* **149**, 179-188.
- Olsson, M. and Shine, R.** (1998a). Timing of parturition as a maternal care tactic in an alpine lizard species. *Evolution* **52**, 1861-1864.
- Olsson, M. and Shine, R.** (1998b). The seasonal timing of oviposition in sand lizards (*Lacerta agilis*): why early clutches are better. *J. Evol. Biol.* **10**, 369-381.
- O'Steen, S. and Janzen, F. J.** (1999). Embryonic temperature affects metabolic compensation and thyroid hormones in hatchling snapping turtles. *Physiol. Biochem. Zool.* **72**, 520-533.
- Oxendine, S. L., Cowden, J., Hinton, D. and Padilla, S.** (2006). Vulnerable windows for developmental ethanol toxicity in the Japanese medaka fish (*Oryzias latipes*). *Aquat. Toxicol.* **80**, 396-404.
- Pandav, B. N., Shanbhag, B. A. and Saidapur, S. K.** (2006). Functional significance of posthatching residual yolk in the lizard, *Calotes versicolor*. *J. Herpetol.* **40**, 385-387.
- Pieau, C., Dorizzi, M. and Richard-Mercier, N.** (1999). Temperature-dependent sex determination and gonadal differentiation in reptiles. *Cell. Mol. Life Sci.* **55**, 887-900.
- Qualls, C. P. and Andrews, R. M.** (1999). Cold climates and the evolution of viviparity in reptiles: cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus*. *Biol. J. Linn. Soc.* **67**, 353-376.
- R Development Core Team** (2010). *R: a Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Robert, K. A., Thompson, M. B. and Seebacher, F.** (2006). Thermal biology of a viviparous lizard with temperature-dependant sex determination. *J. Therm. Biol.* **31**, 292-301.
- Sakata, J. T. and Crews, D.** (2003). Embryonic temperature shapes behavioural change following social experience in male leopard geckos, *Eublepharis macularis*. *Anim. Behav.* **66**, 839-846.
- Schultz, T. J., Webb, J. K. and Christian, K. A.** (2008). The physiological cost of pregnancy in a tropical viviparous snake. *Copeia* **2008**, 637-642.
- Shine, R.** (1980). Costs of reproduction in reptiles. *Oecologia* **46**, 92-100.
- Shine, R.** (2004a). Adaptive consequences of developmental plasticity. In *Reptilian Incubation: Environment, Evolution and Behaviour* (ed. D. C. Deeming), pp. 187-210. Nottingham: Nottingham University Press.
- Shine, R.** (2004b). Incubation regimes of cold-climate reptiles: the thermal consequences of nest-site choice, viviparity and maternal basking. *Biol. J. Linn. Soc.* **83**, 145-155.
- Shine, R.** (2004c). Seasonal shifts in nest temperature can modify the phenotypes of hatching lizards, regardless of overall mean incubation temperature. *Funct. Ecol.* **18**, 43-49.
- Shine, R.** (2006). Is increased maternal basking an adaptation or a pre-adaptation to viviparity in lizards? *J. Exp. Zool.* **305**, 524-535.
- Shine, R. and Elphick, M. J.** (2001). The effect of short-term weather fluctuations on temperatures inside lizard nests, and on the phenotypic trait of hatchling lizards. *Biol. J. Linn. Soc.* **72**, 555-565.
- Shine, R. and Harlow, P.** (1993). Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* **96**, 122-127.
- Shine, R. and Harlow, P.** (1996). Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. *Ecology* **76**, 1808-1817.
- Shine, R. and Madsen, T.** (1996). Is thermoregulation unimportant for most reptiles? An example using water pythons (*Liasis fuscus*) in tropical Australia. *Physiol. Zool.* **69**, 252-269.
- Shine, R., Elphick, M. and Harlow, P. S.** (1997). The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology* **78**, 2559-2568.
- Sinervo, B., Doughty, P., Huey, R. B. and Zmudo, K.** (1992). Allometric engineering: a causal analysis of natural selection on offspring size. *Science* **258**, 1927-1930.
- Slip, D. J. and Shine, R.** (1988). Reptilian endothermy: a field study of thermoregulation by brooding diamond pythons. *J. Zool.* **216**, 367-378.
- Stahlschmidt, Z. R. and DeNardo, D. F.** (2008). Alternating egg-brooding behaviors create and modulate a hypoxic developmental micro-environment in Children's pythons (*Antaresia childreni*). *J. Exp. Biol.* **211**, 1535-1540.
- Stahlschmidt, Z. R. and DeNardo, D. F.** (2009). Obligate costs of parental care to offspring: egg brooding-induced hypoxia creates smaller, slower and weaker python offspring. *Biol. J. Linn. Soc.* **98**, 414-421.
- Stahlschmidt, Z. R., Hoffman, T. and DeNardo, D. F.** (2008). Postural shifts during egg-brooding and their impact on egg water balance in Children's pythons (*Antaresia childreni*). *Ethology* **114**, 1113-1121.
- Stahlschmidt, Z. R., Brashears, J. and DeNardo, D. F.** (2011). The role of temperature and humidity in python nest site selection. *Anim. Behav.* **81**, 1077-1081.
- Tallamy, D. W. and Wood, T. K.** (1986). Convergence patterns in subsocial insects. *Ann. Rev. Entomol.* **31**, 369-390.
- Valenzuela, N. and Lance, V. A.** (2004). *Temperature-Dependent Sex Determination in Vertebrates*. Washington, DC: Smithsonian Books.
- Vinegar, A., Hutchinson, V. H. and Dowling, H. G.** (1970). Metabolism, energetics and thermoregulation during brooding of snakes of the genus *Python* (Reptilia, Boidae). *Zoologica* **55**, 19-45.
- Wapstra, E.** (2000). Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Funct. Ecol.* **14**, 345-352.
- Warner, D. A. and Andrews, R. M.** (2003). Consequences of extended egg retention in the eastern fence lizard (*Sceloporus undulatus*). *J. Herpetol.* **37**, 309-314.
- Warner, D. A. and Shine, R.** (2007). Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia* **154**, 65-73.
- Webb, J., Brown, G. P. and Shine, R.** (2001). Body size, locomotor speed and antipredator behaviour in a tropical snake (*Tropidonophis mairii*, Colubridae): the influence of incubation environments and genetic factors. *Funct. Ecol.* **15**, 561-568.
- Wells, J. C. K.** (2003). The thrifty phenotype hypothesis: thrifty offspring or thrifty mother? *J. Theor. Biol.* **221**, 143-161.
- Wilson, S. and Swan, G.** (2003). *A Complete Guide to Reptiles of Australia*. Frenchs Forest, NSW: New Holland Publishers.
- Zambrano, E., Martinez-Samaoya, P. M., Bautisca, C. J., Deás, M., Guillén, L., Rodríguez-González, G. L., Guzmán, C., Larrea, F. and Nathanielsz, P. W.** (2005). Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. *J. Physiol.* **566**, 225-236.