The consequences of alternative parental care tactics in free-ranging pythons in tropical Australia

Zachary R. Stahlschmidt[*,1,3], **Richard Shine**[2] **and Dale F. DeNardo**[1]

[1]*School of Life Sciences, Arizona State University, Tempe, Arizona, 85287 USA;* [2]*School of Biological Sciences A08, University of Sydney, Sydney, New South Wales, 2006 Australia; and* [3] *Present address: Department of Psychology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1 Canada*

Summary

1. Life-history theory attributes the evolution of parental care to the benefits to offspring viability outweighing any costs to parental viability. However, the consequences of parental care tactics to parent(s) and the developmental environment have seldom been measured under field conditions.

2. Laboratory research on pythons shows that maternal nest-site selection and egg brooding benefit embryos, but prolonged nest attendance may impose fitness costs to free-ranging females. A population of water pythons (*Liasis fuscus*) in tropical Australia provides an excellent opportunity to examine this parent–offspring trade-off because females exhibit parental care polymorphism wherein some individuals brood their eggs only briefly (< 10 days) post-oviposition ('short brooders') while others remain with their eggs throughout the incubation period (> 50 days; 'long brooders').

3. We used radiotelemetry, temperature and humidity data loggers, ultrasonography, haematological techniques, and habitat analyses to examine the correlates and consequences of maternal nesting decisions in 14 free-ranging female pythons over the 4-month reproductive season.

4. Nest-site selection and maternal attendance enhanced thermal and hydric regimes within the nest. Egg production by reproducing female pythons resulted in high energetic costs (loss of 60% of maternal body mass) and increased parasite load. However, the estimated mass loss because of brooding was (i) low (< 5%), (ii) inversely related to fecundity (females that produced relatively large clutches tended to select lower temperatures and thus lost less mass during brooding) and (iii) surprisingly unrelated to brooding duration. Phenotypic traits of short and long brooders were similar, but long brooders had higher haemoparasite burdens prior to oviposition. Clutches of long brooders were laid in more open sites (less canopy cover) and experienced warmer and more humid conditions than did those of short brooders.

5. Together with previous research, we suggest several explanations for the maintenance of maternal care polymorphism within this population, such as a trade-off between offspring number and quality (long brooders may produce fewer clutches during their lifespan but enhance offspring quality). Our study provides detailed measurements of the correlates and consequences of parental care in a free-ranging reptile, and it clarifies the trade-offs mediated by taxonomically widespread maternal decisions (e.g. nest- or oviposition-site selection and nest attendance).

Key-words: Egg brooding, *Liasis fuscus*, life-history trade-off, maternal care, nest attendance, nest-site selection, parent–offspring trade-off, polymorphism, pythons

Introduction

Parental care can be broadly defined as non-gametic contributions that directly or indirectly contribute to the survival and reproductive success of the offspring (Blumer 1979), and it has evolved convergently in a broad range of taxa (Clutton-Brock 1991). In addition to influencing the fitness of parents and offspring, parental care may be inextricably involved in other evolutionary processes. For example, the degree of parental investment often dictates the degree and direction of sexual selection (e.g. large, brightly coloured male birds and female fish compete for mates that provide high parental investment: Trivers 1972). The form and duration of parental care also

*Correspondence author. E-mail: zrs@dal.ca

influence patterns of social aggregation within populations (Clutton-Brock 1991). Further, parental control of the developmental environment may play a role in the evolution of endothermy (Farmer 2000) and viviparity (Webb, Christian & Shine 2006).

Across animal taxa, there is substantial variation in the degree to which one or both parents provide(s) care to their offspring (Clutton-Brock 1991). Even within a species, parental care polymorphism occurs when variation in parental care traits (e.g. the quality of nest-site selection or the duration of nest attendance) persists within a single population (e.g. birds: Tuttle 2003; Pryke & Griffith 2009; fishes: Henson & Warner 1997; Oliveira *et al.* 2001). Such intraspecific variation may exist when the trade-off between parental costs or offspring benefits is influenced by the parent's physiological state or the environmental conditions of the nest. That is, the appropriate parental strategy to use may depend on conditions specific to the particular parental effort. To better understand the factors involved in parental decisions and the maintenance of parental care polymorphism within a population, we need to examine the correlates and consequences of parental tactics on parents and their offspring.

The scientific literature on parental care has been dominated by studies on mammals and birds (Clutton-Brock 1991; Stahlschmidt 2011), but reptiles also offer excellent model systems for analysis of this topic (Shine 1988). In particular, pythons have emerged as a useful taxon for studying the benefits, costs and trade-offs of parental care (reviewed in Stahlschmidt & DeNardo 2011). The family Pythonidae exhibits female-only parental care, the predominant mode of care when considering all animal taxa (Gross & Shine 1981; Zeh & Smith 1985; Clutton-Brock 1991). In addition to reducing egg predation (Madsen & Shine 1999), parental behaviour by female pythons provides benefits to offspring (improving embryonic viability) by enhancing the developmental environment (e.g. promoting egg water balance: Aubret *et al.* 2003; Lourdais, Hoffman & DeNardo 2007). During egg retention (gravidity), at oviposition (nest-site selection) and throughout incubation (egg brooding), female pythons also improve the thermal developmental environment, which strongly influences fitness-related variables in offspring (e.g. developmental rate, body size, locomotion and behaviour) (Vinegar, Hutchison & Dowling 1970; Shine *et al.* 1997; Madsen & Shine 1999; Lourdais, Heulin & DeNardo 2008; Stahlschmidt, Brashears & DeNardo 2011a; Stahlschmidt & DeNardo 2011). However, parental care in pythons is costly to females in terms of survival and reproductive output (Madsen & Shine 1999) and in reduced body condition (Madsen & Shine 1999; but see Aubret *et al.* 2005).

A compelling model system in which to explore benefits and costs of parental care would be a single population in which: (i) females use alternate parental care strategies and (ii) widely accepted fitness-related variables can be measured. Free-ranging pythons exhibit maternal care poly-

morphism and thus provide exactly this situation (Madsen & Shine 1999). Female water pythons (*Liasis fuscus* Peters 1873) that chose thermally suboptimal nest sites brooded their eggs for the duration of incubation (a tactic we henceforth refer to as 'long brooders', mean ± SD: 58 ± 14 days), whereas conspecific females that chose thermally optimal nest sites abandoned their eggs *c.* 1 week (mean ± SD: 7 ± 5 days) after oviposition ('short brooders', Madsen & Shine 1999). Prolonged brooding imposed multiple fitness-related costs: long brooders were in poorer body condition after hatchlings emerged and were less likely to survive (Madsen & Shine 1999). Long brooders that did survive were less likely to reproduce again in the following year (Madsen & Shine 1999). Interestingly, a small proportion of females (<10%) switched between these dichotomous strategies in subsequent reproductive efforts (Madsen & Shine 1999). The presence of two apparently discrete but not completely fixed maternal care tactics within a single population provides a robust opportunity to assess the effects of variation in parental care tactics and to clarify the factors underlying this dichotomy in nesting decisions (nest-site selection and brooding duration). Thus, we used radiotelemetry, miniature temperature and humidity data loggers, ultrasonography, haematological techniques and habitat analyses to test two hypotheses: (i) maternal nesting decisions impact the developmental environment (fitness-related benefits to offspring; e.g. warmer, more humid micro-climate) and (ii) reproductive effort (e.g. clutch size and prolonged egg brooding) affects care-giving females (fitness-related costs to females; e.g. reduced body condition or increased parasite burden). To further evaluate the correlates of maternal care, we also examined factors associated with maternal tactics, such as the characteristics of nest sites and decisions made prior to oviposition (e.g. behavioural thermoregulation during gravidity).

Materials and methods

STUDY SPECIES AND AREA

Liasis fuscus are large (≥ 2 m total length), semi-aquatic, non-venomous snakes found throughout northern Australia (Wilson & Swan 2008). Their ecology has been intensively studied for the past three decades at Fogg Dam (60 km southeast of the city of Darwin) on the Adelaide River floodplain in the wet-dry tropics (12°34′S, 131°18′E). This area is characterized by high temperatures year-round (monthly mean maximum: 31–34 °C) and highly seasonal rainfall [>75% of annual rain fall (1300 mm) occurs during the wet season from December to March: Madsen & Shine 1996; Shine & Brown 2008]. Our study area was *c.* 10 km south-southeast of Fogg Dam on Beatrice Hill Farm, a government-operated cattle farm situated on the Adelaide River floodplain. Some of the snakes we caught at Beatrice Hill Farm were animals that had been marked at Fogg Dam, suggesting some degree of interchange of individuals between the two areas. However, such interchange likely involves only a small proportion of the population (Ujvari, Shine & Madsen 2011). Mating in this population occurs in the dry season (July–August) with oviposition occurring

1–2 months later (Madsen & Shine 1996). All procedures were approved by the Arizona State University Institutional Animal Care and Use Committee (protocol #08-968R) and the Northern Territory (Australia) Parks and Wildlife Commission (permit #37045).

RADIOTELEMETRY

We surgically implanted radiotransmitters and temperature data loggers to determine each snake's location and hourly body temperature (T_{body}), respectively. In August 2010, we captured adult water pythons and gently palpated each snake to determine its sex and reproductive status. We transported 14 gravid (egg-bearing) snakes to the laboratory at the University of Sydney's Tropical Ecology Research Facility (TERF, <10 km from the study area). We then intra-coelomically implanted each snake with a radiotransmitter (13 g model SI-2; Holohil Systems Ltd., Carp, Ontario, Canada) and a miniature temperature data logger (Thermochron iButton, model DS1921G; Maxim Corp., Dallas, TX, USA) using methods similar to those described previously for rattlesnakes (Taylor, DeNardo & Malawy 2004). Prior to each snake's recovery from anaesthesia, we measured morphological characteristics and collected blood for haematological analyses (see below and Appendices S1 and S2 in the Supporting Information for details). We returned snakes to their locations of capture within 24 h of surgery. From the time of the snakes' release through reproduction (gravidity and egg brooding), we determined the location of each snake using radiotelemetry every morning (07:00–10:00 h) and 0–2 times per evening (18:00–21:00 h). After a snake ceased brooding (see Appendix S3, Supporting information for criteria used to determine the onset and cessation of brooding), we captured it and surgically removed the radiotransmitter and temperature data logger. We again returned snakes to their locations of capture within 24 h of surgery. We were unable to recapture one of the females; thus, we report data on all 14 nests (five short-brooded nests and nine long-brooded nests: Fig. S1, Supporting information), but only 13 females (five short brooders and eight long brooders).

MORPHOLOGICAL AND REPRODUCTIVE CHARACTERISTICS

At gravidity and post-brooding, we measured several morphological variables of each snake to determine the effects of late (post-ovulation) reproductive effort on aspects of morphology plausibly related to fitness, such as body condition and musculature (see Appendix S1, Supporting information for details). We also examined each snake with a portable ultrasound system (MicroMaxx; SonoSite Inc., Bothell, WA, USA) after the implantation surgery, but prior to recovery from anaesthesia, to determine its clutch size (number of viable eggs, Stahlschmidt, Brashears & DeNardo 2011b) and to estimate its clutch mass (see Appendix S1, Supporting information for details).

HAEMATOLOGICAL ANALYSES

During the gravidity and post-brooding phases, we used blood smears to determine the effects of reproduction on leucocyte profile and parasite load (see Appendix S2, Supporting information for details). We identified heterophils and lymphocytes using the criteria of Campbell (2005) because they are the two most prevalent types of leucocytes in pythons, typically comprising >80% of leucocytes (reviewed in Mader 2005). We examined innate immunity in two ways: the sum of heterophils and lymphocytes per 10^3 erythrocytes (H + L), and the ratio of heterophils to lymphocytes

(H : L). The latter measure is often used as a reliable indicator of chronically elevated plasma glucocorticoid concentrations resulting from stress (reviewed in Davis, Maney & Maerz 2008). We also identified the type of haemoparasite in each parasitized erythrocyte (Telford 2008), specifically *Plasmodium*, *Serpentoplasma* and Haemogregarines (*Hepatzoon* and *Haemogregarina*). We determined parasite load as the total number of parasitized erythrocytes per 10^3 erythrocytes.

CHARACTERISTICS OF THE DEVELOPMENTAL, NEST AND SURFACE ENVIRONMENTS

We used miniature data loggers to measure the temperature and humidity of the clutch, nest and surface environments on an hourly basis (see Appendix S4, Supporting information for details). We used dew point (the temperature to which air must be cooled for water condensation to occur) as our humidity metric because, unlike relative humidity, dew point is directly proportional to vapour pressure independent of temperature. From the temperature and humidity data, we determined the mean and standard deviation of the surface temperature ($T_{surface}$), as well as body and nest temperature (T_{body} and T_{nest}, respectively) for all 13 females and 14 nest sites to compare the means and variances in temperature and humidity among sites. We also used daily rainfall data compiled from a weather station located <6 km from our study area (Middle Point AWS, station 014041) to determine the effects of precipitation on temperature and dew point throughout our study. During early incubation, we measured habitat features of each nest site to compare ecological factors [e.g. nest dew point (DP_{nest}) and percentage of canopy closure] with brooding decisions (see Appendix S5, Supporting information for details).

STATISTICAL ANALYSES

Data met the appropriate assumptions of parametric statistics or were transformed as necessary and were analysed using SPSS version 19 (IBM, Armonk, NY, USA). We used a linear mixed-model approach to examine differences in temperature and dew point as a function of incubation stage (early, middle or late incubation; fixed factor), site of measurement (surface, nest or body; fixed factor) and brooding type (short or long brooder; fixed factor). We also examined interactions among these fixed factors and included individual snake identification number as a within-subjects (random) effect. In mixed-model analyses for each dependent variable, we used the model with the lowest Akaike's Information Criterion, which invariably included a scaled identity covariance structure for our random effect and a restricted maximum likelihood estimation. We initially ran the complete model (all effects and interactions) and parsimoniously removed non-significant variables. We then re-analysed our data using the remaining variables to create the most robust model possible, which we report.

We were specifically interested in differences in morphological, haematological and nest traits between short and long brooders. Because the number of variables of interest (>20) exceeded our sample size (13–14), we tested for differences using two-sample *t* tests on data taken during gravidity. We also tested for differences between females of the two brooding types in the ways in which morphological and haematological traits changed over the reproductive cycle using analyses of covariance (post-reproductive trait: dependent variable; brooding type: main effect; gravid trait: covariate). Although this method likely inflated Type I error rate (increased false-positive results), we found few significant differences between brooding types (see *Comparison of brooding phenotypes* below). To test relationships within and among individuals, we used simple linear regression analysis. All values are displayed

as mean ± SEM, and significance was determined at α < 0·05 for all tests.

Results

EFFECTS OF MATERNAL NESTING DECISIONS AND RAINFALL ON THE DEVELOPMENTAL ENVIRONMENT

Mean temperature was independently affected by brooding type (long brooders > short brooders), site of measurement (body > nest > ground surface) and reproductive stage (reproduction > after reproduction) (Fig. 2a, Table 1). However, each of these variables had an interactive effect on mean temperature (Fig. 2a, Table 1). Standard deviation in temperature was influenced by the site of measurement (ground surface > nest > body), reproductive stage (after reproduction > reproduction) and a site*stage interaction (Fig. 2c, Table 1). Mean dew point was independently affected by the site of measurement (body > nest > ground surface) and reproductive stage (reproduction > after reproduction) (Fig. 2a, Table 1). However, several interactions (site*stage, brooding type*site and brooding type*stage) also influenced mean dew point (Fig. 2b, Table 1). Standard deviation in dew point was influenced by site of measurement (ground surface > nest > body), reproductive stage (after reproduction > reproduction) and a brooding type*stage interaction (Fig. 2c, Table 1). No brooding type*site*stage interaction significantly influenced any of the temperature or humidity variables.

In September, our study area was relatively warm and dry (Fig. 1). Warmer $T_{surface}$ during early incubation coincided with warmer T_{nest} for both brooding types during early incubation relative to the middle period of incubation (Fig. 2a). The nests of short brooders had relatively low dew points during early incubation (Fig. 2b) because of a lack of rainfall (Fig. 1) and the low nest temperatures of short brooders relative to long brooders (dew point temperature cannot exceed ambient temperature; Fig. 2a). Rain fell throughout October (Fig. 1), and this corresponded with cooler (Fig. 2a) and less variable (Fig. 2c,d) T_{nest} during the middle period of incubation. Rainfall during mid- and late incubation coincided with a reduction in the dew point disparity between the nests of the two brooding types (Fig. 2b,d).

EFFECTS OF REPRODUCTION ON CARE-GIVING FEMALES

After reproduction had concluded, females weighed an average of 903 ± 93 g (60 ± 2%) less than they had prior to oviposition. Much of the loss of mass was explained by reproductive output (clutch size vs. maternal mass loss, $F_{1,11} = 47$, $R^2 = 0.81$, $P < 0.001$). In contrast, the estimated additional loss of body mass because of brooding was low (100 ± 26 g, 4 ± 1%). Although body mass may have been lost in the form of water or fat stores, at least

Table 1. Linear mixed-model results for the significant effects of brooding type (short or long brooders), site of measurement (ground surface, nest or body) and stage of reproduction (gravidity or one of the three stages of incubation) on the mean and standard deviation of temperature and dew point

	d.f.	*F*	*P*
Temperature			
Mean			
Intercept	1, 14	40418·2	<0·001
Brooding type	1, 14	6·2	0·027
Site	2, 107	273·1	<0·001
Stage	3, 106	25·7	<0·001
Site*Stage	5, 106	5·6	<0·001
Brooding type*Site	2, 106	4·6	0·012
Brooding type*Stage	3, 106	3·1	0·029
Standard deviation			
Intercept	1, 14	810·1	<0·001
Site	1, 114	584	<0·001
Stage	3, 113	7·8	<0·001
Site*Stage	5, 113	12·1	<0·001
Dew point			
Mean			
Intercept	1, 21	8857·9	<0·001
Site	2, 83	172·3	<0·001
Stage	2, 82	3·7	0·029
Site*Stage	4, 82	7·2	<0·001
Brooding type*Site	2, 82	4·9	0·009
Brooding type*Stage	2, 81	5·3	0·007
Standard deviation			
Intercept	1, 13	111·6	<0·001
Site	2, 89	42·6	<0·001
Stage	2, 88	7·8	0·001
Brooding type*Stage	3, 27	3·4	0·033

See text for further details, and see Fig. 2 for graphic representation.
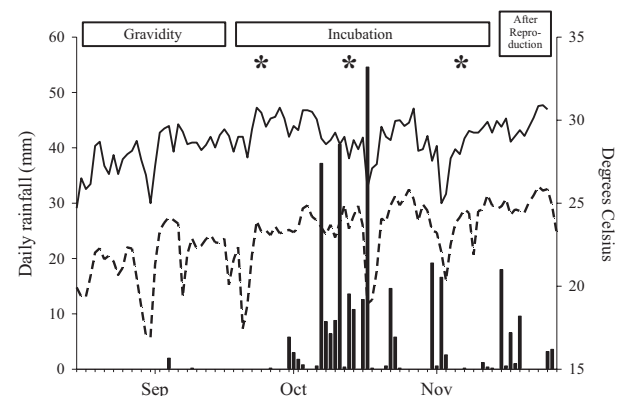
Fig. 1. Daily climatic data (bar: rainfall; solid line: mean temperature at the ground surface; dashed line: mean dew point at the ground surface) at our study area during gravidity, incubation and after reproduction. The name of each month is centred below the first day of the month. Asterisks denote periods at which we measured the thermal and hydric characteristics of each female's nest (see text for details).

some body mass lost was because of muscle catabolism because females exhibited reduced epaxial muscle width after reproduction (gravidity: 17·15 ± 0·33 mm; post-reproduction: 13·81 ± 0·43 mm; $t_{12} = 10$, $P < 0.001$).
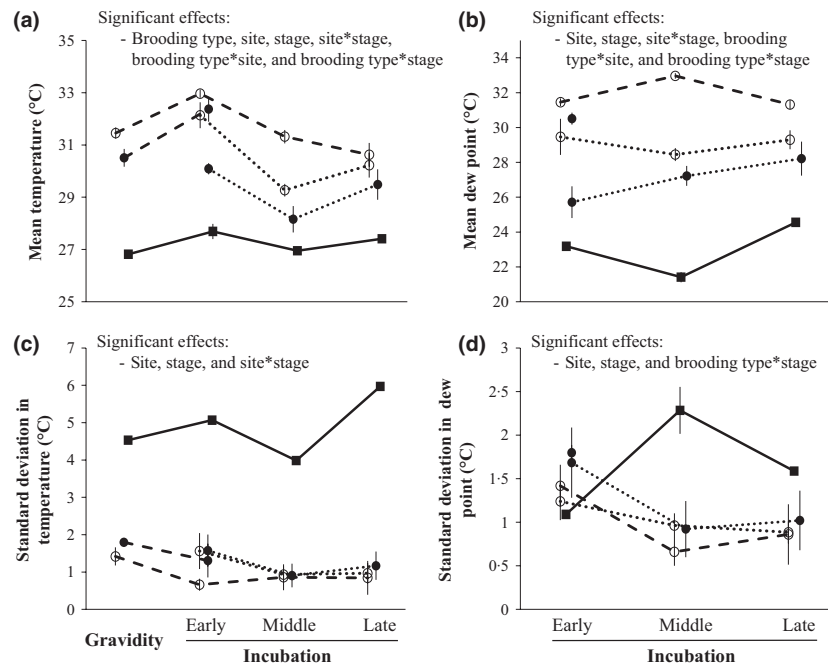
Fig. 2. Thermal regimes of snake body temperatures, nest temperatures and ground surface conditions during the reproductive cycle of water pythons in tropical Australia. The graphs show mean (a) temperature and (b) dew point, and standard deviation in (c) temperature and (d) dew point as measured at three sites: the female python's body (a proxy for embryonic conditions during gravidity and brooding; dashed line), her nest (dotted line) and the ground surface (solid line). Data are shown for both brooding types (short brooders: closed circles; long brooders: open circles) and for multiple reproductive stages: gravidity (14 days pre-oviposition, temperature only) and three stages of incubation (early, middle and late; mean: 6, 26, and 52 days post-oviposition, respectively). Data are from 48 h sampling periods matched at each time point for each female, are displayed as mean ± SEM and are positioned offset to improve visual clarity.

However, a female's clutch size was not significantly related to her loss of epaxial muscle width (in terms of the number of millimetres lost, $F_{1,11} = 0.010$, $R^2 = 0.001$, $P = 0.92$; or the percentage lost, $F_{1,11} = 0.053$, $R^2 = 0.005$, $P = 0.82$). We were unable to regress brooding duration against other variables of interest (e.g. the loss of body mass or increase in parasite load, see below) because of the highly bi-modal (and thus, non-normal) distribution of brooding durations among females within the study population (Fig. S1, Supporting information).

During reproduction, female T_{body} was higher and more stable relative to T_{body} after reproduction (Fig. 2a,c; Table 1). Mean T_{body} during brooding was negatively correlated with clutch size residuals from a snout-vent length (SVL) vs. clutch size regression as well as a body mass vs. clutch size regression; that is, female pythons with relatively larger clutch sizes were cooler while brooding (Fig. 3a; SVL: $R^2 = 0.35$, $F_{1,11} = 5.9$, $P = 0.034$; body mass: $R^2 = 0.60$, $F_{1,11} = 17$, $P = 0.0018$). Gravid T_{body} and brooding T_{body} were positively correlated; that is, females that maintained higher-than-average body temperatures prior to oviposition also maintained higher-than-average body temperatures during incubation (Fig. 3b; mean T_{body}: $R^2 = 0.47$, $F_{1,11} = 9.8$, $P = 0.0095$; standard deviation in T_{body}: $R^2 = 0.45$, $F_{1,11} = 9.1$, $P = 0.012$). A female's mean T_{body} during brooding was positively correlated with the percentage of mass that she lost during brooding ($R^2 = 0.35$, $F_{1,11} = 5.9$, $P = 0.033$), suggesting that mass loss was mostly a result of standard metabolic rate rather than activity.

The mean number of lymphocytes in female pythons was higher after reproduction than during gravidity ($19 ± 2$ and $12 ± 1$ per 10^3 erythrocytes, respectively; $t_{12} = 2.4$, $P = 0.036$). Similarly, H + L increased from $19 ± 2$ to $27 ± 3$ per 10^3 erythrocytes over the course of the reproductive cycle ($t_{12} = 2.5$, $P = 0.026$). After reproduction, parasite load was positively related to H : L ($F_{1,11} = 5.9$, $R^2 = 0.35$, $P = 0.034$) and the number of heterophils ($F_{1,11} = 5.8$, $R^2 = 0.35$, $P = 0.034$). The change in parasite load between gravidity and after reproduction was positively correlated with clutch size residuals; that is, females with relatively larger clutch sizes exhibited greater increases in parasite load over the course of reproduction ($F_{1,11} = 5.0$, $R^2 = 0.31$, $P = 0.047$). Clutch size residuals were also positively correlated with the number of lymphocytes at gravidity ($F_{1,11} = 7.9$, $R^2 = 0.42$, $P = 0.017$). Both clutch size and clutch size residuals were positively correlated with H + L during gravidity (clutch size: $F_{1,11} = 15$, $R^2 = 0.58$, $P = 0.0030$; residuals: $F_{1,11} = 14$, $R^2 = 0.55$, $P = 0.0040$).

COMPARISON OF BROODING PHENOTYPES

Brooding duration was bi-modally distributed; long brooders attended their nests *c.* 50 days longer than short brooders (Fig. S1, Supporting information). Apart from

Table 2. Morphological and haematological traits of short-brooding and long-brooding water pythons (mean ± SEM = 5 ± 1 and 54 ± 2 days of nest attendance, respectively) during gravidity and after reproduction (9 ± 3 days after nest attendance)

	Short brooders	Long brooders
Gravidity		
Snout-vent length (SVL, cm)	154 ± 7	149 ± 3
Body mass (g)	2297 ± 311	2202 ± 193
Body condition (g, residuals of log$_{10}$ transformed SVL vs. body mass regression)	−0·014 ± 0·013	0·018 ± 0·022
Epaxial muscle width (mm)	17·21 ± 0·90	17·24 ± 0·18
Clutch size (number of eggs)	15 ± 2	14 ± 1
Mass-specific clutch size (eggs, residuals of body mass vs. clutch size regression)	1 ± 1	0 ± 1
Length-specific clutch size (eggs, residuals of SVL vs. clutch size regression)	0 ± 1	1 ± 1
Heterophils (per 10^3 erythrocytes)	8 ± 1	7 ± 1
Lymphocytes (per 10^3 erythrocytes)	12 ± 2	13 ± 2
Heterophils and lymphocytes (per 10^3 erythrocytes)	20 ± 2	20 ± 2
Heterophils : lymphocytes (H : L)	0·66 ± 0·08	0·54 ± 0·08
Hemogregarine load (per 10^3 erythrocytes)	2 ± 1 *	7 ± 2 *
Total parasite load (per 10^3 erythrocytes)	3 ± 1 *	8 ± 3 *
Post-reproduction		
Body condition (g)	0·003 ± 0·006	−0·002 ± 0·015
Change in SVL (cm)	0 ± 1	1 ± 1
Change in body mass (g)	−902 ± 173	−915 ± 115
Change in epaxial muscle width (mm)	−3·08 ± 0·38	−3·51 ± 0·48
Change in heterophils (per 10^3 erythrocytes)	−1 ± 1	3 ± 2
Change in lymphocytes (per 10^3 erythrocytes)	10 ± 7	5 ± 1
Change in heterophils and lymphocytes (per 10^3 erythrocytes)	10 ± 8	8 ± 2
Change in H : L	−0·23 ± 0·04	0·00 ± 0·12
Change in hemogregarine load (per 10^3 erythrocytes)	1 ± 0	0 ± 2
Change in total parasite load (per 10^3 erythrocytes)	1 ± 1	3 ± 3

Significant differences between brooding types were determined using two-sample *t* tests or *U* tests. Brooding type-specific changes over reproduction were determined using analyses of covariance, in which brooding type was the main effect and the gravid trait was the covariate. See *Effects of reproduction on care-giving females* for results related to the effects of reproductive state on these traits. Significant differences ($P < 0.05$) are denoted by asterisks, and all values are displayed as mean ± SEM.

haemoparasite burdens prior to oviposition, we found no significant differences in morphology or haematology between the two brooding phenotypes (Table 2). Haemoparasite burdens were no longer significantly different between the brooding types after reproduction ($P > 0.10$).

During gravidity, long brooders adopted higher T_{body} (31·5 ± 0·2 °C) than short brooders (30·5 ± 0·3 °C; $t_{11} = 2.5$, $P = 0.032$; Fig 2a; Table 1). However, during brooding, we detected no significant differences in T_{body} between the two brooding types (32·0 ± 0·2 °C; $t_{11} = 0.50$, $P = 0.63$; Fig 2a; Table 1). Long brooders chose warmer ($F_{1,12} = 5.5$, $P = 0.037$) and more humid ($F_{1,12} = 4.8$, $P = 0.049$) nests, although the differential in thermal and hydric variables between the two brooding types decreased during the incubation period (Fig. 2a,b; Table 1). Because the two brooding types differed in the quality of their nest sites (Fig. 2; Table 1) and the duration of their nest attendance (Fig. S1, Supporting information), clutches from short brooders experienced cooler, less humid conditions (29·9 ± 0·5 °C temperature and 29·2 ± 0·6 °C dew point), whereas clutches from long brooders experienced warmer (31·7 ± 0·2 °C), more humid (31·4 ± 0·3 °C dew point) conditions.

If females were facultatively thermogenic during brooding, they may have been capable of influencing the surrounding nest environment (e.g. maintaining a higher, more stable T_{nest}). Yet, mean $T_{nest} - T_{surface}$ and $DP_{nest} - DP_{surface}$ differences were similar between attended nests during late incubation and unattended nests after incubation (70 days post-oviposition) (temperature: 2·3 ± 0·6 °C; $t_4 = 0.63$, $P = 0.56$; dew point: 4·7 ± 0·7 °C; $t_4 = 0.018$, $P = 0.99$). Similarly, the standard deviation in $T_{nest} - T_{surface}$ and $DP_{nest} - DP_{surface}$ differences was similar between these two nest conditions (temperature: −5·1 ± 0·3 °C; $t_4 = 0.43$, $P = 0.86$; dew point: −1·1 ± 0·2 °C; $t_4 = 0.27$, $P = 0.80$).

The distances between brooding females and their respective nest openings (i.e. the effective lengths of burrows) were similar for short and long brooders (2·07 ± 0·29 m; $t_{12} = 0.26$, $P = 0.80$). The diameter of each nest's opening was also similar between brooding types (13 ± 1 cm; $t_{12} = 0.15$, $P = 0.88$) and was positively correlated with mean T_{nest} during early incubation (6 ± 1 days post-oviposition) (nests with wider entrance holes were warmer: $F_{1,12} = 7.6$, $R^2 = 0.39$, $P = 0.017$). The percentage of canopy closure was negatively correlated with mean T_{nest} during early incubation (shaded nests were cooler: $F_{1,12} = 6.9$, $R^2 = 0.37$, $P = 0.022$), and canopy closure was greater over the nests of short brooders (51 ± 16%) than over those of long brooders (16 ± 6%; $t_{12} = 2.4$, $P = 0.035$). The percentage of ground covered with grass was positively correlated with mean T_{nest} and DP_{nest} during early incubation (T_{nest}: $F_{1,12} = 32$, $R^2 = 0.73$, $P < 0.001$; DP_{nest}: $F_{1,12} = 8.3$, $R^2 = 0.41$, $P = 0.014$), and it was greater outside the nest openings of long brooders (36 ± 5) than short brooders (9 ± 7; $t_{12} = 3.1$, $P = 0.0092$). The two brooding types did not differ in the mean depth of the measurements of T_{nest} and DP_{nest} (49 ± 4 cm into nest opening; $t_5 = 1.1$, $P = 0.34$).

Discussion

In support of our first hypothesis (maternal nesting decisions impact the developmental environment), nest-site
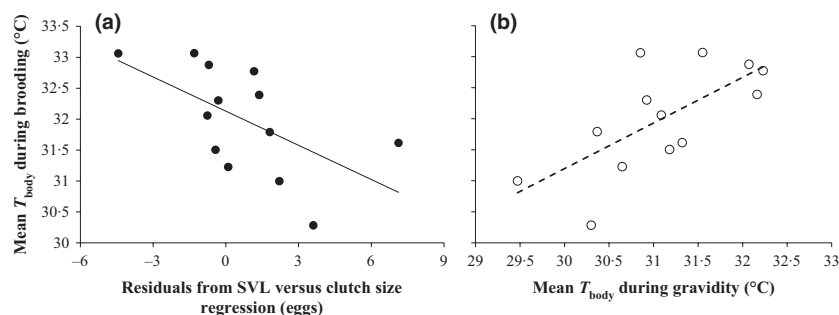
Fig. 3. Significant relationships between female water python body temperature (T_{body}) during brooding and (a) relative clutch size [calculated as the residuals of a snout–vent length (SVL) vs. clutch size regression; $R^2 = 0.35$, $F_{1,11} = 5.9$, $P = 0.034$] and (b) T_{body} during gravidity ($R^2 = 0.47$, $F_{1,11} = 9.8$, $P = 0.0095$).

selection and egg brooding influenced the thermal and hydric conditions under which offspring developed. Although logistical constraints prevented the measurement of hatching success or hatchling phenotypes (e.g. hard rocky terrain and multiple nest openings per burrow), experimental work has shown that developmental temperature and humidity strongly influence the hatching success, body size, behaviour, locomotor performance and/or growth rates of python offspring (temperature: Shine *et al.* 1997; humidity: Lourdais, Hoffman & DeNardo 2007). As in other animals, nest-site selection in *L. fuscus* enhanced developmental temperature and humidity because nests were warmer, more humid and more stable than the surface environment (Fig. 2). Non-random nest-site selection, whereby reproducing females select sites that provide unusually favourable abiotic conditions, occurs in taxa ranging from fruit flies (Dillon *et al.* 2009) to snakes (Brown & Shine 2004).

Although egg brooding by *L. fuscus* provided additional thermal and hydric benefits to the developmental environment, some females exhibited this behaviour only briefly. The ubiquity of egg brooding during early development supports the 'harm to offspring' hypothesis, which predicts that parents should take greater risks for younger offspring (or offspring in poorer condition) because these cases confer the greatest marginal benefit of parental care (Dale, Gustavsen & Slagsvold 1996). Alternatively or additionally, ubiquitous early brooding may be a function of physical constraint, such as a seasonal shift in abiotic conditions: the nests that we monitored exhibited lower and more variable dew points during early incubation (27.5 ± 1.0 °C) likely due to less rainfall (Figs 1 and 2b,d). These lower humidity levels may imperil embryonic survival because laboratory studies on a sympatric python species (*Antaresia childreni* Gray 1842) reported 100% egg mortality because of desiccation when the eggs were incubated in 23–27 °C dew point conditions (Lourdais, Hoffman & DeNardo 2007). Thermal benefits of egg brooding also may diminish as the incubation period progresses (Fig. 2a,c; Table 1), further reducing the benefits of maternal attendance. Also, egg brooding early in incubation may reduce egg predation rates during the period when

chemical cues produced at oviposition might attract potential predators (Shine 1988). Nonetheless, early desertion of eggs by female pythons reduced offspring viability in a previous study (Madsen & Shine 1999: eggs of short-brooding *L. fuscus* exhibited desiccation and increased rates of predation). In support of this putative cost of early termination of parental care, we found freshly predated eggs adjacent to one nest entrance <1 week after the departure of a short-brooding female. In summary, maternal parental care in this system improves embryonic thermal regimes and water balance, and it likely reduces predation risk.

In support of our second hypothesis (reproductive effort affects care-giving females), reproduction also imposed significant costs on female pythons. From gravidity through to egg brooding, females lost *c.* 60% of their body mass and *c.* 19% of their epaxial musculature. Losses in body reserves were expected because *L. fuscus* typically do not feed during reproduction (Madsen & Shine 1999). Some of the costs we measured were fecundity-dependent because clutch size explained a significant amount of the among-female variation (31–81%) in the amount of mass lost and the change in parasite load over reproduction. Undoubtedly, egg production comprised the largest energetic investment by females in our study, but increased T_{body} during reproduction may have also contributed to the energetic costs. Because *L. fuscus* surface activity is minimal during reproduction (particularly during brooding), female *L. fuscus* are aphagic during reproduction (Madsen & Shine 1999), and *L. fuscus* T_{body} conforms to ambient temperature (females are non-thermogenic: Stahlschmidt *et al.* 2012), actual metabolic rate likely approximated standard metabolic rate of females in our study. On the basis of Bedford & Christian's (1998) data, the shift that we documented in T_{body} would have increased maternal energy expenditure (standard metabolic rate) by 14% and 27% during gravidity and egg brooding, respectively.

Despite the elevated maternal temperatures during brooding, the loss in maternal body mass over this phase was trivial compared to that because of egg production (an estimated 4% loss in body mass was attributed to brooding). This result agrees with laboratory research on other pythons (ball python, *Python regius* Shaw 1802: Aubret

et al. 2005; *A. childreni*: O. Lourdais & D. F. DeNardo, unpublished). In our study, the estimated amount of body mass lost to brooding was related to T_{body} during brooding (warmer females lost more mass). Females with relatively large clutches adopted lower T_{body} (Fig. 3a). Although future investigation is required, this result suggests a trade-off between investment early vs. late in the reproductive cycle (clutch size vs. thermoregulation, respectively) given the relationships between these two variables and other variables of interest (e.g. losses in body mass and shifts in parasite load). While early investment (egg production) was likely more energetically expensive than late investment (temperature-dependent increase in metabolic rate) in our study, research in other systems suggests a trade-off between early and late investment. For example, clutch size enlargement in another python resulted in reduced hatching success (*P. regius*: Aubret *et al.* 2003). A recent comparative study of 287 insect species reported that female body size scales positively with clutch size in taxa exhibiting little or no parental care (i.e. early investment is favoured over later investment), whereas it scales negatively in taxa exhibiting intensive parental care (i.e. late investment is favoured over early investment: Gilbert & Manica 2010).

Because of their higher developmental temperature (32 °C vs. 30 °C), the offspring of long brooders had a shorter developmental period that likely resulted in at least an extra week of foraging based on data in *A. childreni* (Z.R. Stahlschmidt & D.F. DeNardo, unpublished data). The long-brooding tactic may also offer other benefits to offspring because of incubation temperature (e.g. larger size or greater locomotor ability: Shine *et al.* 1997). Yet, in spite of these benefits, long-brooding pythons in our study appeared to incur similar energetic costs as did short brooders (Table 2). Apart from parasite burdens prior to oviposition, female pythons adopting these two tactics were phenotypically similar at the onset of the study (Table 2). However, long brooders selected higher T_{body} during gravidity and chose warmer, more humid nests (Fig. 2). Thus, even with a relatively small sample size, we demonstrate that long brooders were better mothers before, at and after oviposition. In this respect, our results conflict with those of a previous investigation of short- and long-brooding pythons at nearby Fogg Dam, which found that brooding tactics were typically fixed from reproductive bout to bout (i.e. maternal care polymorphism may have a substantial genetic component in *L. fuscus*) and that short brooders chose warmer and more thermo-stable nests (Madsen & Shine 1999). These contradictory results may be the result of variation in sampling methods (because of technological advances, our study more rigorously sampled T_{body} and T_{nest}), landscape-level differences between study areas that may influence microclimatic variables (our study area was characterized by pastures, hills and concrete rubble, whereas the Fogg Dam area was characterized by marshes and tropical forest), and/or population-level differences in maternal tactics

(very little interchange of individuals occurs between the two areas: Ujvari, Shine & Madsen 2011).

Polymorphism in reproductive strategies is widespread, and much of the attention has been paid to the link between conspicuous aspects of morphology (e.g. size or colouration) and reproductive behaviours, predominately mating strategies but also parental care behaviour (e.g. Henson & Warner 1997; Tuttle 2003; Mank & Avise 2006; Hurtado-Gonzalez & Uy 2009; Pryke & Griffith 2009). The mechanisms by which chromo-etho morphs are maintained have been extensively reviewed for birds (Roulin 2004) and fishes (Mank & Avise 2006). Yet, the factors involved in maternal care polymorphism without colour or body size variation are less understood (but see Henson & Warner 1997). On the basis of the available information about the implications of parental care in our study system, we propose several explanations that could be investigated in future to better understand the observed polymorphism in maternal care in *L. fuscus*:

1. Long brooders may enter reproduction in better condition than short brooders (all of our snakes were caught during gravidity, which is well after yolk production). Instead of investing in more eggs, long brooders invested in parental care (e.g. higher T_{body} during egg retention and longer attendance of eggs). As a result of this added investment, they were morphologically similar to short brooders and had higher parasite loads at gravidity. Future research should examine the characteristics of short and long brooders prior to the initiation of reproduction.

2. Poor nest-site selection leads to early nest abandonment. In support, short brooders maintained similar T_{body} during brooding as did long brooders, despite choosing cooler nests. Thus, short brooders may have abandoned early because of the high energetic costs of behaviourally thermoregulating their eggs. Yet, behavioural differences between short and long brooders were evident well in advance of nest-site selection (long brooders were warmer during gravidity), and females of other pythons are adept at choosing warm, humid nests (Stahlschmidt, Brashears & DeNardo 2011a). In sum, this scenario is unlikely.

3. Long brooders were able to reduce the costs of brooding. In support, losses in maternal body mass, condition and musculature were similar between short and long brooders. Also, long brooders may have been more tolerant of parasites, reducing the costs of maintaining a higher level of resistance. However, to test this scenario, future research should measure metabolic rate and parasite tolerance in short and long brooders.

4. Egg brooding is energetically inexpensive for both brooding types. Pythons can reduce their energetic requirements by down-regulating the size and function of major organ systems (Secor 2008). Although thermogenesis (intrinsic heat production) during egg brooding increases energetic expenditure by >10-fold in some python species (Burmese python, *Python*

molurus Linnaeus 1758: Vinegar, Hutchison & Dowling 1970; diamond python, *Morelia spilotes spilotes* Lacépède 1804: Harlow & Grigg 1984), *L. fuscus* do not exhibit thermogenesis during egg brooding (Stahlschmidt *et al.* accepted). Thus, most (9 of 14) of the females in our study may have brooded their eggs for the entire incubation period because brooding is not energetically expensive. If this was true, however, we would expect all females to attend their eggs throughout incubation. This scenario is also unlikely, because it underestimates real costs of egg attendance by disregarding 'opportunity costs' (i.e. the foraging opportunities that females forego while brooding: see below).

5. Egg brooding is expensive. Although brooding causes a relatively small reduction in maternal body mass, it precludes foraging that would otherwise allow females to recover the body reserves lost to egg production. Specifically, short brooders can begin to feed *c.* 50 days sooner after oviposition than can long brooders. That accelerated opportunity to recoup reserves may be especially important if prey densities are high during this period or are low after this period. In support of this hypothesis, short-brooding *L. fuscus* at Fogg Dam were in better body condition by the time their eggs hatched than were long brooders (Madsen & Shine 1999).

   a. Increased foraging opportunities may allow short brooders to grow larger (because of their increased energy intake) and thus produce more eggs in subsequent clutches. Our data do not show any such differences: short brooders were not larger at gravidity and did not produce larger clutches (Table 2). However, short brooders may have been younger (because of higher growth rates).

   b. In terms of reproduction, long brooders choose quality, whereas short brooders choose quantity. Short brooders can reproduce more frequently, because they have more time to recover body reserves lost to egg production. Thus, short brooders may invest in more clutches throughout their lifetimes but care for them poorly (they choose quantity over quality), while long brooders invest in fewer clutches but care for them well (they choose quality over quantity). Future research could address these scenarios by monitoring the growth rates and reproductive frequencies of individual snakes throughout ontogeny and after adulthood, which is feasible in *L. fuscus* (Madsen & Shine 1999; Pizzatto *et al.* 2009; this study).

By examining the consequences of maternal nesting decisions, we hypothesize that python maternal care may provide an adaptive alternate strategy to viviparity. Viviparous taxa are expected to exert stronger control over embryonic temperature throughout development relative to oviparous taxa (Shine 2005). Yet, although one-third of embryonic development in pythons occurs *in utero* (Lourdais, Heulin & DeNardo 2008), female *L. fuscus* in our study did not control developmental temperature better during gravidity than during brooding (Fig. 2a,c). This, of course, assumes that developmental temperature equalled T_{body} when females were coiled around the eggs. However, clutch temperature was likely even more stable than T_{body} during brooding because each clutch was insulated by a brooding female. Viviparity maintains embryonic water balance and reduces embryonic predation throughout incubation, and maternal nesting decisions by female *L. fuscus* can similarly meet these needs of offspring (Madsen & Shine 1999; Fig. 2b,d and anecdotal evidence from our study). Further, python maternal care may gain the advantage over viviparity under certain conditions because it offers increased flexibility in maternal commitment. For example, the decision to terminate maternal care early in the developmental period without fully compromising offspring viability (e.g. short brooding in *L. fuscus*) is not an option for viviparous taxa.

In summary, our study of free-ranging pythons provides new insights into the benefits and costs of parental care, and the trade-offs mediated by maternal decision-making at multiple stages (at ovulation, gravidity, oviposition and throughout incubation) in a natural setting. The persistence of two successful and discrete parental tactics within this single population provides an exciting opportunity for further investigation into fundamental aspects of parental care and life-history evolution, such as selective pressures (does maternal care polymorphism persist because of a quality-quantity trade-off?), adaptive plasticity (can females switch from one tactic to the other under certain conditions?) and developmental effects (are parental tactics influenced by proximate factors experienced during ontogeny, such as prey availability or the ambient regimes of temperature and humidity?).

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Histogram of egg-brooding duration in study animals.
Appendix S1. Morphological and reproductive measurements.
Appendix S2. Haematological methods.
Appendix S3. Reproductive states.
Appendix S4. Temperature and humidity recording.
Appendix S5. Habitat analyses.

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