

Maternal brooding in the children's python (*Antaresia childreni*) promotes egg water balance

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Abstract Parental care provides considerable benefits to offspring and is widespread among animals, yet it is relatively uncommon among squamate reptiles (e.g., lizards and snakes). However, all pythonine snakes show extended maternal egg brooding with some species being facultative endothermic. While facultative endothermy provides thermal benefits, the presence of brooding in non-endothermic species suggests other potential benefits of brooding. In this study we experimentally tested the functional significance of maternal brooding relative to water balance in the children's python, *Antaresia childreni*, a small species that does not exhibit facultative endothermy. Clutch evaporative water loss (EWL) was positively correlated with clutch mass and was much lower than expected values based on individual eggs. The conglomerate clutch behaved as a single unit with a decreasing surface area to volume ratio as clutch size increased. Maternal brooding had a dramatic impact on evaporation from eggs, reducing and possibly eliminating clutch EWL. In a separate experiment, we found that viability of unattended eggs is highly affected by humidity level, even in the narrow range from 75 to 100% relative humidity at 30.5°C (20–33 mg m⁻³ absolute humidity). However, the presence of the brooding female ameliorated this sensitivity, as viability of brooded clutches at 75% relative humidity was higher

than that of non-brooded eggs at either the same absolute humidity or at near-saturated conditions. Overall, these results demonstrate that brooding behavior strongly promotes egg water balance (and thus egg viability) in children's pythons.

Keywords Evaporative water loss · Egg · Parental care · Snake · Evaporation · Water balance

Introduction

Parental care provides considerable fitness benefits (Clutton-Brock 1991). Most widely studied are the benefits associated with energy provisioning and protection from predators, but other components of parental care may also enhance offspring survival. Understanding the fitness returns that are provided by particular instances of parental care increases our understanding of the costs and benefits of parental care and may provide insight into how such behaviors evolved. While parental care is ubiquitous among mammals and birds, it is much less common and less extensive in ectothermic vertebrates (although numerous fishes, anurans, and crocodylians display some form of parental care). In most squamates (i.e., lizards and snakes) the contribution to offspring from the male is merely genetic, while the female provides offspring with energy in the form of yolk and selects an appropriate nest site. Parental contributions made after oviposition or parturition is rare in squamates, and, where documented, it is mostly restricted to maternal defense of eggs or neonates (Price 1988; Butler et al. 1995). In addition to providing defense of offspring (reviewed in Shine 1988), maternal egg attendance in scincid lizards can also promote water balance (Somma and Fawcett 1989).

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One particularly well recognized form of parental care in squamates is egg brooding by female pythons (Shine 2004). In all species of pythons examined to date, females tightly coil around their clutch and typically remain with the clutch until hatching. While this behavior may provide protection from predators, it likely provides additional benefits to the developing offspring. In some pythons, the brooding female is facultatively endothermic and thus can maintain elevated and stable clutch temperatures during incubation (Hutchison et al. 1966; Vinegar et al. 1970; Van Mierop and Barnard 1976, 1978; Harlow and Grigg 1984; Slip and Shine 1988). Many studies of terrestrial vertebrates have demonstrated the influence of thermal conditions on the offspring's phenotype, performance, viability, and rate of development (Burger and Zappalorti 1988; Deeming and Ferguson 1991; Shine et al. 1996a; Elphick and Shine 1998; Shine 2004). Results from the few studies that have been made using pythons indicate that incubation temperature can affect egg viability, incubation time, offspring morphology, and offspring behavior (Vinegar 1973; Branch and Patterson 1975; Shine et al. 1997b). Thus, the thermal benefits of python egg brooding are apparent.

While facultative endothermy is a well-known component of egg brooding in pythons, only a minority of the python species that have been studied are actually facultatively endothermic (Shine 1988). Many python species lack endothermic capability, despite the fact that all species brood their eggs. Thus, python brooding is not solely linked to facultative endothermy, and the ubiquity of this behavior suggests that alternative benefits exist. While non-endothermic brooding can provide thermal benefits to the developing embryos through increases in both insulation and thermal inertia, it is also feasible that brooding provides developmental benefits that are not linked to thermoregulation (Shine 2004). Importantly, studying the evolutionary origin of python brooding is a rather complex issue, because it is difficult to ascertain whether any currently observed benefit of brooding would have represented an increase in fitness at the time of the evolutionary emergence of the strategy (Shine 1985, 2004). Still, the clarification of the proximal significance of this specialized behavior is a necessary step that should bring significant insight.

Like the thermal environment during development, egg water balance acts as a major constraint on reproductive success (Deeming and Thompson 1991; Brown and Shine 2005). While some lizards (gekkonids) produce highly calcified eggs, the vast majority of oviparous squamates laid parchment-shelled eggs that are porous and allow considerable water flux (Ackerman et al. 1985; Ackerman 1991; Deeming and Thompson 1991). Thus, significant uptake of water from the environment can occur over the

course of incubation (Ackerman et al. 1985; Packard 1991), resulting in dramatic swelling and increase in mass of the eggs. Size restriction at the pelvic inlet is such that often eggs that are fully hydrated after laying are larger than the maximum size that can be oviposited. However, any hydric benefits of eggshell porosity are dependent on the hygroscopic condition of the nest. When exposed to dry conditions, most squamate eggs undergo severe dehydration (Packard 1991; Du 2004). Alteration of egg water balance can have dramatic effects on egg viability, offspring phenotype, performance, and energy use (Packard et al. 1980; Packard and Packard 1988; Packard 1991; Brown and Shine 2005). In pythons, egg desiccation can extend incubation time and reduce hatching success (Aubret et al. 2003).

As a result of the thermal and hydric sensitivity of squamate eggs, nest site selection is critical to offspring success (Packard 1991; Warner and Andrews 2002; Brown and Shine 2004). However, many environmental factors (e.g., climate variations, site availability) can impede the ability of a female to locate an adequate nest site, which could lead to the death of the eggs or the production of poorer quality offspring. Additionally, nest sites that are thermally or hydrically optimal may be detrimental for other reasons. For example, thermally superior nest sites of water pythons (*Liasis fuscus*) have a higher risk of depredation (Madsen and Shine 1999). Maternal brooding might buffer the microclimatic effects on hydrostasis so that other developmental conditions can be optimized. Female pythons often coil entirely around their eggs, thereby eliminating any egg exposure to the air or the substrate (Walsh 1979; Grace 1997). This contrasts sharply with most squamates that lay parchment-shelled eggs; for those species, important exchanges of water between the eggs and the substrate usually occur (Belinsky et al. 2004). Maternal brooding might provide strong benefits by controlling evaporative water loss (EWL) from eggs and by protecting the clutch from deleterious hydric conditions of the microclimate. Though the brooding Malayan pit viper, *Calloselasma rhodostoma*, does not cover its eggs as thoroughly as brooding pythons, this snake alters body posture (and thus coverage of its eggs) based on humidity (York and Burghardt 1988). Additionally, brooding reduces egg water loss in the ball python, *Python regius*, and thereby increases hatchling success (Aubret et al. 2005). Unfortunately, no quantitative data are available regarding EWL from eggs and the functional impact that brooding has on egg water balance in squamates (Oftedal 2002).

We experimentally tested whether maternal brooding promotes egg water balance in children's pythons, *Antaresia childreni*, a python species that broods its eggs but is not facultatively endothermic. We hypothesized that brooding significantly reduces egg EWL and thus increases

hatching success. We specifically address the magnitude and determinants of EWL from the clutch, the impact of maternal brooding on clutch EWL, and the effect of atmospheric humidity and maternal brooding on hatching success.

Materials and methods

Study species and maintenance

Children's pythons are medium-sized (up to 1,200 mm snout-vent length (SVL), 600 g body mass), non-venomous, constricting snakes that inhabit rocky areas in northern Australia from Kimberley, Western Australia, to eastern Gulf of Carpentaria, Queensland (Wilson and Swan 2003). Children's pythons nest at the end of a long dry season (July–September, Austral winter) when ambient conditions are relatively warm but dry. In the nest cavities, the eggs have little if any contact with the substrate. Thus, python eggs are subjected to greater hydric challenge than what is typical for squamate eggs.

The snakes in this study are part of a long-term captive snake colony maintained at Arizona State University. Snakes were housed individually in $91 \times 71 \times 46$ cm cages located in a room maintained at 25°C with a 12:12 light/dark cycle. Continuous access to supplemental heat was provided using a sub-surface heating element (Flexwatt, Flexwatt Corp., West Wareham, MA) under one side of each cage. Breeding occurred in February 2004 after a 2-month wintering period. Sixteen healthy females commenced vitellogenesis (follicle size >15 mm) and laid viable eggs. Oviposition occurred between early April and mid-June 2004. In each case of oviposition the female rapidly adopted a typical coiled position around her eggs. At oviposition, the female was temporarily removed from her clutch, the masses of the clutch and female were recorded (± 0.1 g) and the number of eggs was counted. As in other pythons, the eggs of *A. childreni* mostly adhere to each other and generally form a compact conglomerate shortly after oviposition (Ross and Marzec 1990). This precluded the weighing of individual eggs, so we calculated mean egg mass of each clutch (clutch mass divided by the number of eggs in that clutch). After processing, the female and her eggs were placed in a polypropylene box housed in a thermally controlled room that maintained ambient temperature (T_a) at $30.5 \pm 0.5^\circ\text{C}$. This temperature was chosen, since it approximates the selected body temperature of gravid female children's pythons prior to oviposition (Lourdais et al. submitted) and brooding by this non-endothermic species would provide no thermal benefits. All females returned to a brooding posture shortly after placement into the polypropylene box.

Experimental design

Measurement of evaporative water loss

Nine snakes and their clutches were used in hygrometric trials. All trials were conducted within 6 h of oviposition. While eggs are moist with oviductal secretions at the time of oviposition, the surface of the egg dries within minutes of oviposition and prior to the female coiling about her eggs. Thus, the eggshells were always dry when the experiment was conducted. The housing cages were equipped with removable perforated aluminum floors that could be transferred to the test chamber, thereby only minimally disturbing the female. Each trial consisted of three immediately consecutive steps in which measurements were made of total EWL, clutch EWL, and female EWL. All snakes were run in this order, rather than in random fashion, so that the coiling posture during brooding was stable. Even with this precaution, one of the snakes refused to stay coiled on her eggs during the trial; thus only clutch EWL data were collected for that individual, and her data were not included in the analyses that compared brooded and non-brooded EWL. Individual eggs that were not adhered to the rest of the clutch (three eggs from three different females) were used in separate measurements of evaporation to assess EWL from individual eggs and thus to determine any benefit from eggs adhering to each other.

Experimental apparatus: Hygrometry trials were conducted within a test chamber housed in an environmental chamber maintained at $T_a = 30.3^\circ\text{C}$, reproducing the selected body temperature of gravid female children's pythons (see above). The test chamber ($40 \times 30 \times 16$ cm, 19.2 l) was constructed almost entirely of glass to minimize hygroscopicity. A Type-T (copper-constantan) thermocouple was used to measure T_a within the test chamber. Each of two opposing walls of the test chamber were equipped with a threaded, borosilicate glass hose connector (#7 Chem-Thread, Chemglass, Vineland, NJ) for connection to minimally hygroscopic influx and efflux tubing (Bev-A-Line, Thermoplastic Processes Inc., Stirling, NJ). Unlike most squamate eggs, brooded python eggs have minimal, and sometimes no, contact with the substrate, because the female often lifts the entire clutch from the surface. Accordingly, evaporative flux of python eggs is dependent on the difference in the partial pressure of water between the egg surface and the atmosphere rather than on the water potential of the substrate. Trials were thus conducted with influent air at an absolute humidity of 15 g m^{-3} (i.e., 50% relative humidity at the chamber temperature of 30.3°C). Anecdotal reports of natural brooding sites suggest children's pythons use varied locations including burrows, termite mounds, and root boles (Bedford pers com). Clearly, environmental conditions among nesting sites vary and

therefore the selection of a single testing condition cannot reflect all nest environments. However, based on limited field data, the chosen humidity reflects the conditions of at least some potential nest sites. To achieve humidity control of the inflow air, we precisely mixed two streams of air—one saturated with water vapor, the other dry—to produce a combined influent stream of the proper humidity. The saturated air stream was created by first sending it through an industrial air purifier (#PCDA11129022, Puregas, Denver, CO) to remove water vapor and carbon dioxide, then through a mass-flow controller (#FMA-A2409, Omega Scientific, Stamford, CT), and finally through three serially connected water columns, each approximately 150 cm in depth. The water columns were contained in copper tubes placed in the environmental chamber. Thus, the water was maintained at ambient temperature, and the dewpoint of the air exiting the columns was equal to ambient temperature. The dry air stream was sent through the purifier and then through a rotameter (Omega Scientific, Stamford, CT) before joining with the saturated stream. We calibrated the mass-flow controller and the rotameter for dry, CO₂-free air using a soap-film flow meter. We determined the proper air-mixing ratio by sending the combined influent to a dewpoint hygrometer (#RH100, Sable Systems, Las Vegas, NV) that we calibrated with nitrogen (zero gas) and experimental air sent through the water columns (span gas). We calculated influx ($l\ h^{-1}$) as the sum of the three constituent fluxes: (1) dry flux through the mass-flow controller; (2) dry flux through the rotameter; and (3) the rate at which water vapor was added to the air stream. Average influx was $124\ l\ h^{-1}$, resulting in a 99% air turnover every 44.6 min (Lasiewski et al. 1966). Effluent air was sent from the test chamber to a borosilicate glass spill-tube, from which a pump delivered a sub-sample of the effluent air to the dewpoint hygrometer. While the hygrometer exhibited little or no drift, we minimized the effects of any drift by calculating evaporative flux based on elevation in dewpoint above an individualized baseline value obtained by flowing air through the sealed, empty test chamber before each trial.

Experimental protocol: Prior to each trial, we moved the cage containing the brooding snake from the housing room to the environmental chamber in which the trials were conducted, and the snake and its clutch were allowed at least 2 h to come to thermal equilibrium. This time was deemed sufficient because of the relatively small size of the brooding female, the minimal difference in thermostatic temperature between the housing room and environmental chamber (0.2°C), and results from pilot trials that verified that cloacal temperature of a non-brooding female moved to an experimental chamber 5°C warmer than its housing temperature reached thermal equilibrium within 1 h. We did not measure body temperature of brooding females prior to trials, thereby avoiding risk of such a disturbance

leading to alter brooding posture and possibly even clutch abandonment.

After the acclimation period, the female, coiled around her eggs, was carefully transferred from the housing cage to the test chamber and allowed at least 45 additional minutes for restabilization of body temperature and ambient dewpoint. Measurements recorded during the ensuing 20 min were used in the analysis of total EWL. The test chamber was then opened long enough for careful removal of the adult female, and the test chamber was resealed with only the eggs therein. After another stabilization period, measurements were recorded for analysis of clutch EWL. Immediately thereafter, the eggs were removed from the test chamber and placed in a container lined with dampened vermiculite (but there was no direct contact between the eggs and vermiculite). The female was then returned to the test chamber for a final period of stabilization after which measurements were recorded for analysis of female EWL. Upon placement into the test chamber, females typically explored the chamber initially, but settled down and remained inactive in an uncoiled posture for extended periods. Data were collected during the quiescent periods. At the completion of the three-part trial, the female and her eggs were promptly returned to the housing cage, whereupon the female readily coiled around her clutch.

Calculations: We used hygrometric dewpoints to calculate vapor pressures using an eighth order polynomial describing saturation vapor pressure as a function of air temperature (Flatau et al. 1992). Vapor pressures were used to calculate vapor densities using the Ideal Gas Law (Campbell and Norman 1998). Finally, evaporative fluxes ($mg\ h^{-1}$) were calculated by multiplying vapor densities ($mg\ ml^{-1}$) by rates of flow of air ($ml\ h^{-1}$). We calculated absolute evaporative fluxes ($mg\ h^{-1}$) as well as fluxes relative to clutch mass or snake mass ($mg\ g^{-1}\ h^{-1}$). Unfortunately, we could not measure clutch surface area because of the complex configuration of the conjoined eggs. However, given that general clutch shape and egg density are relatively consistent, clutch mass correlates well with surface area.

Impact of absolute humidity on clutch viability

In addition to quantifying EWL of brooded and non-brooded eggs, we sought to verify the vulnerability of python eggs to desiccation. More specifically, we sought to address the specific effect of absolute humidity (which is equivalent to atmospheric water vapor density) and notably sub-saturated air on developmental success and hatching success. Since python eggs typically have little or no contact with the ground, we avoided any direct contact of the eggs with a wet substrate that could have permitted direct

water absorption or adsorption. The 16 clutches were allocated to one of the three following treatment groups:

Group 1: Unsaturated air (five clutches without the mother). Absolute humidity = 20–25 mg m⁻³ (i.e., relative humidity of 75–80% at 30.5°C)

Group 2: Saturated air (five clutches without the mother). Absolute humidity = 30–33 mg m⁻³ (i.e., relative humidity of 95–100% at 30.5°C)

Group 3: Unsaturated air (as in treatment 1, but with the female present and brooding, six snakes and their clutches).

We examined the influence of treatment on mean hatching success, defined as the fraction of fertile eggs in a clutch that hatched. We defined fertile eggs as ones that, at the time of oviposition, had chalky white shells as, based on experience; python eggs opened in this state always had an embryo. Since some embryos were fully developed but unable to hatch, we also considered mean developmental success, which we defined as the sum of newborns and fully developed un-hatched snakes divided by the number of fertile eggs in the clutch. While snakes that fully develop but fail to hatch are not ecologically different from infertile eggs or early embryonic deaths (i.e., no offspring result), we calculated developmental success in an effort to determine when the incubation process failed. The nine clutches that were tested in EWL measurements were equally allocated among these groups. The brooding snakes (Group 3) were sensitive to visual disturbance and two individuals abandoned their clutches during the course of incubation.

Experimental apparatus: The absolute humidity (measured using HOBO Pro data loggers, Onset Computer, Bourne, MA) was low in the temperature-controlled room housing the incubation boxes (9 mg m⁻³, 30% relative humidity), but much higher in an empty solid-lid incubation box that was equipped with a water bowl (28 mg m⁻³, 90% relative humidity). Therefore, we created the unsaturated experimental condition (20–25 mg m⁻³) by drilling three small holes (5 mm in diameter) in the lid of the incubation boxes. Air was saturated (30–35 mg m⁻³) by bubbling through a 2 m copper water column, and the emerging stream was split at a manifold to provide separate airlines supplying individual boxes.

Statistics

Statistics were performed using JMP (version 5.1, SAS institute, Cary, NC) and R (version 2.1.1, R development core team 2003). We examined the determinants of clutch EWL using linear regressions. We used paired sample *t*-tests to examine the influence of brooding on EWL and to compare total EWL with EWL of the females alone.

We examined the influence of treatment on hatching and developmental success using the general linear model (GLM) procedure. We specified the number of successfully hatched or developed offspring in a clutch as the response variable and clutch size (i.e., the number of eggs in a clutch) as a binomial denominator, using a logit link and a binomial error distribution. However, quasi likelihood estimations were used to estimate the scale parameter, and the significance of terms in the model was tested using *F*-tests (Wilson and Hardy 2002). Unless otherwise stated, values are reported as mean ± 1 standard deviation and results were considered statistically significant if *P* < 0.05.

Results

Magnitude and determinants of clutch evaporative water loss

The mean absolute clutch EWL for the nine clutches examined was 1,029 mg h⁻¹. Clutch EWL was tightly correlated with clutch mass ($Y = 4.37X + 532.06$, $R^2 = 0.75$, $F(1,7) = 20.95$, $n = 9$, $P < 0.002$, Fig. 1) but not clutch size ($R^2 = 0.16$, $F(1,7) = 1.34$, $n = 9$, $P = 0.28$). The relationship between clutch size and clutch mass was not significant ($R^2 = 0.39$, $F(1,7) = 4.65$, $n = 9$, $P = 0.07$), possibly reflecting the inter-individual variation in mean egg mass (range 8.12–12.65 g). However, this result must be taken with caution, as the relationship might have been significant with a larger sample size.

Evaporative water loss from three eggs (mean mass 9.61 g, range 7.97–11.30 g) placed singly in the chamber resulted in a mean EWL rate of 254.46 ± 15 mg h⁻¹ (range 241.1–270.45 mg h⁻¹) The small 12% variation in isolated egg EWL despite a 42% range in egg mass is a result of

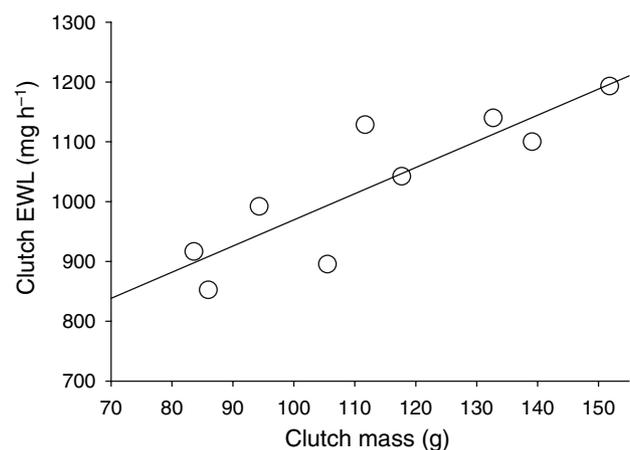


Fig. 1 Relationship between clutch EWL (mg h⁻¹) and clutch mass (g). Each open circle represents one clutch

larger eggs having greater absolute, but smaller mass-specific, rates of evaporation because of reduction of surface area to volume ratio with increasing egg size. In comparison to the relatively small effect that variation in egg size had on isolated egg EWL rate, clutch “average” egg EWL (calculated as clutch EWL / clutch size) was only 40% of isolated egg values (mean = $99.94 \pm 15 \text{ mg h}^{-1}$, range 68.88–130.94 mg h^{-1}). Isolated egg EWL values were used to calculate values for clutch EWL that would be expected if eggs were independent (theoretical EWL: $254.46 \times$ clutch size). Expected clutch EWL was always higher than observed clutch EWL (mean = 2,689 vs. 1,029 mg h^{-1}). More importantly, the deviation between observed and expected clutch EWL decreased with increasing clutch size ($Y = -229.79X + 765.11$, $R^2 = 0.97$, $F(1,7) = 113.40$, $n = 9$, $P < 0.0001$, Fig. 2) but not clutch mass ($R^2 = 0.20$, $F(1,7) = 1.79$, $n = 9$, $P = 0.22$).

Impact of maternal brooding on egg water balance

Maternal brooding strongly affected egg water balance, reducing EWL, on average, 15-fold (mean values 90.47 ± 62 vs. $1,008.51 \pm 111 \text{ mg h}^{-1}$ for total EWL and clutch EWL, respectively, paired t -test: $t = 24.61$, $P < 0.001$, Fig. 3). Furthermore, total EWL tended to be lower than female EWL (paired t -test, $t = 2.99$, $P < 0.06$, Fig. 4), and this difference became significant when excluding one female that was only partially wrapped around her eggs during the trial (mean values 73.10 ± 41 vs. $121.02 \pm 55 \text{ mg h}^{-1}$ for total EWL and female EWL, respectively, paired t -test, $t = 4.98$, $P < 0.002$). Female body size range was narrow (82–110 cm SVL) and female EWL was not significantly related to SVL ($R^2 = 0.13$, $F(1,7) = 0.93$, $P = 0.37$) or post-oviposition body mass ($R^2 = 0.07$, $F(1,7) = 0.51$, $P = 0.50$; mean mass-adjusted female EWL = $0.37 \text{ mg g}^{-1} \text{ h}^{-1}$).

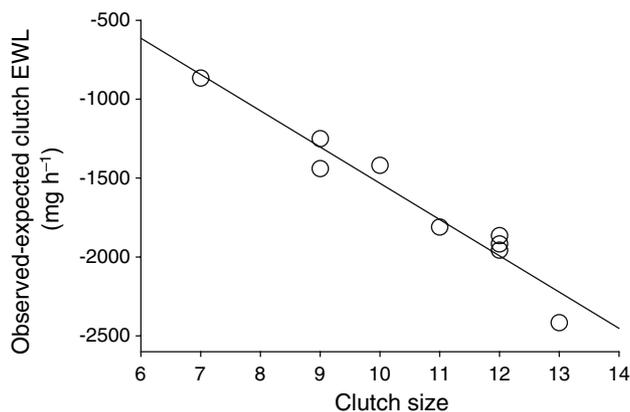


Fig. 2 Relationship between the deviation between observed and expected EWL and clutch size. Each open circle represents one clutch

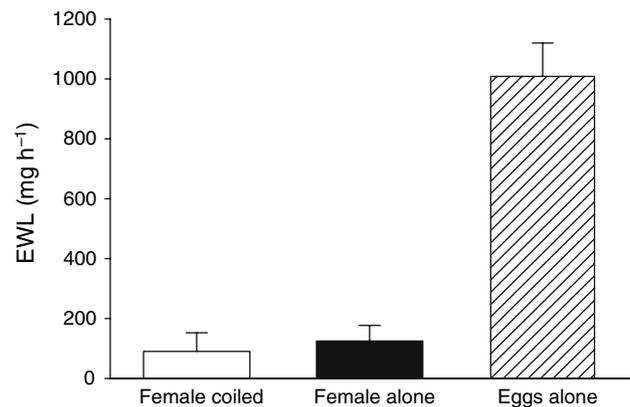


Fig. 3 Effect of maternal attendance on EWL (mg h^{-1}). Error bars indicate one standard deviation. Note the extreme reduction in water loss when the female is coiled around her eggs compared with data from eggs alone

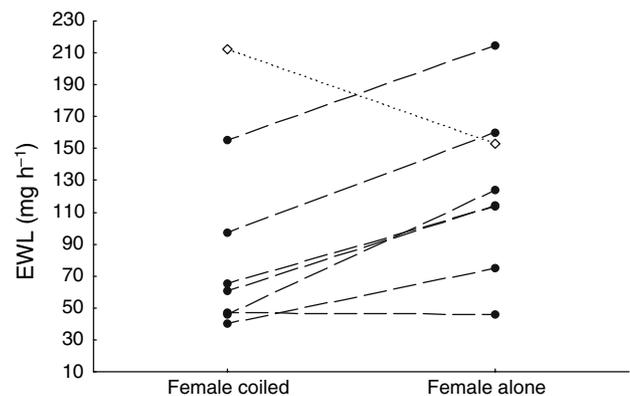


Fig. 4 Paired comparisons of EWL (mg h^{-1}) measured when **a** females were coiled around their eggs and **b** females were alone. EWL was lower when females were coiled around their eggs, despite the presence of the eggs. One individual (open diamond, dotted line) was only partially coiled during the measurements, and thus the measurement of EWL while brooding was relatively high, reflecting the contribution from the exposed egg surface

Impact of absolute humidity on clutch viability

We found a strong influence of treatment on egg viability (GLM procedure, $F(2, 13) = 43.04$, $P < 0.001$ and $F(2, 13) = 29.27$, $P < 0.001$, respectively, for developmental and hatching success; Table 1). In unsaturated, non-brooded conditions (Group 1) rapid egg collapse was observed over the first 2 weeks of incubation, and all of these clutches desiccated within 3 weeks (clutch mass declined to approximately 50% of initial clutch mass). Similarly, eggs of two clutches abandoned by brooding snakes (part of Group 3) rapidly desiccated. Saturated air (Group 2) significantly enhanced success of development and hatching of non-brooded eggs (Table 1). However, there was considerable fungal growth and 21 of the 45 eggs in this group failed to hatch. Among the four clutches successfully brooded by

Table 1 Influence of incubation treatment (eggs alone in unsaturated conditions, alone in saturated conditions, or brooded by female in unsaturated conditions) on proportional developmental success (DS) and hatching success (HS)

Treatment	RH	AH	DS	HS	n clutch	n eggs
Unsaturated	75–80	20–25	0	0	5	43
Saturated	95–100	30–33	0.60	0.51	5	45
Unsat + female	–	–	0.90	0.80	4	31

RH relative humidity, %; AH absolute humidity, mg m^{-3} . See text for details

females in unsaturated conditions (remainder of Group 3), there was significantly higher developmental success and hatching success, and there was no fungal development (Table 1).

Discussion

Clutch evaporative water loss

Our hygrometry experiments quantitatively demonstrate benefits associated with brooding under moderate absolute humidity (i.e., $15 \text{ g m}^{-3} = 50\%$ relative humidity at 30.3°C). We found that clutch EWL was considerably lower than what would be predicted if each egg behaved as an independent entity. That is, clutch EWL was less than 38% of the product of mean isolated egg EWL and clutch size. This result supports the idea that the ability of the eggs to remain cohered in a compact conglomerate is important for egg water balance in that it reduces exposure of individual eggs to the environment, and, as suggested by Ackerman et al. (1985), the clutch can be “viewed as a very large egg showing lower sensitivity to water loss.” A recent study (Marco et al. 2004) on the green lizard, *Lacerta schreiberi*, suggested that egg aggregation possibly poses a cost, in that it may limit water uptake in buried eggs. However, this phenomenon was only detected for “intermediate” values of soil water potential. Similar costs might exist for python eggs. However, because maternal brooding removes direct contact between the eggs and the substrate, water balance will primarily rely on gaseous exchanges. Despite the hydrostatic advantages that conglomeration provides, clutch EWL was still high (Fig. 3), with absolute values averaging slightly more than 1 g h^{-1} (mean clutch mass = 113 g). Clutch EWL was positively correlated with clutch mass, probably reflecting a larger absolute surface area for water exchange (Packard 1991).

The major finding of this study was the functional impact of maternal brooding on clutch EWL. Brooding dramatically reduced clutch EWL and thus significantly promoted egg water balance. Importantly, total EWL was

lower than female EWL (Fig. 4), the latter of which was comparable with previous findings in squamate reptiles (Prange and Schmidt 1969; Dmi’el 1998, 2001; Winne et al. 2001). Thus, EWL in brooding females might only represent female EWL, with cutaneous loss being altered by the change in surface area of exposed skin resulting from the coiled posture (though changes in ventilatory EWL due to changes in metabolic rate could also account for this difference). Regardless of the source of the difference in EWL from brooding and non-brooding females, our results suggest that maternal brooding not only reduces egg water loss but might nearly eliminate it. The proximate functional mechanism of this result is likely based on a decrease of the surface area of the clutch that is available for water exchange. Indeed, females were often coiled entirely around their eggs, eliminating any egg exposure to the air. Similar observations of the ability of female pythons to completely isolate their clutches from the environment have been made in other species, such as the green tree python, *Morelia viridis*, and the black-headed python, *Aspidites melanocephalus* (Walsh 1979; Grace 1997). Because of the tightness in body coiling, the exchange of air between the environment and the clutch is greatly reduced (Van Mierop and Barnard 1978), creating an appropriate hydric microclimate within the “coil nest” (Oftedal 2002). A clutch-size manipulation experiment in the ball python showed a decreased hatching success in experimentally increased clutch sizes, presumably because of the female’s inability to cover all the eggs (Aubret et al. 2003). Our experimental study provides robust empirical support for those observations and underlines the efficacy of python maternal brooding in terms of water conservation. However, the impact of such tight brooding on embryonic gas exchange remains unexplored. On occasion, brooding females loosen their coils and expose some egg surface (one female in this study and additional unpublished data; Ellis and Chappell 1987). This behavior may reflect attempts by the female to promote egg gas exchange despite possible increased water loss, but further studies are needed to explore such a trade-off.

Impact of absolute humidity on clutch viability

In addition to the quantitative hygrometry data demonstrating egg water balance benefits, our study also demonstrates the impact of brooding on egg viability, presumably by providing a gas-exchange buffer between the clutch and the microenvironment. Non-brooded children’s python eggs cannot maintain water balance even in relatively wet atmospheric conditions (i.e., absolute humidity = $20\text{--}25 \text{ g m}^{-3}$; $75\text{--}80\%$ relative humidity at 30.5°C). These results support predictions and previous observations on parchment-shelled eggs (Oftedal 2002; Du 2004), including those on

another python species (Aubret et al. 2003, with *P. regius*). Saturated air significantly enhanced developmental success, yet 40% of eggs incubated in saturated conditions in the absence of the female died, possibly as a result of fungal development. Thus, while eggs are sensitive to water loss in wet but unsaturated air, extremely high (saturated) humidity may also lead to egg death through different mechanisms. Overall, these results suggest that python eggs are extremely sensitive to absolute humidity. Allowing the female to brood the eggs resulted in the highest egg viability, underscoring the importance of maternal attendance.

Relevance of experimental findings

Under our laboratory conditions, brooding clearly provides a superior hydric environment for developing eggs compared to exposed non-brooded eggs. The susceptibility of python eggs to dehydration in their natural environment is verified by the discovery of variably dehydrated eggs of a sympatric python (*L. fuscus*) in an abandoned nest within a varanid burrow system (Madsen and Shine 1999). Females often abandon clutches laid in these burrow systems, since the thermal environment does not require brooding. However, the benefits of nest abandonment (e.g., increased foraging opportunity) come with a cost in terms of increased risks of depredation and dehydration (Madsen and Shine 1999).

In order to record EWL rates, our flow-through hygrometry system required airflows that likely exceed those of natural nest cavities. However, results from our clutch viability experiment demonstrate that even in conditions of very low flow rate (no forced convection, merely free convection through three small holes), eggs are at risk of dehydration even with relatively high humidity.

In sum, while there are natural microenvironments that are suitable for python egg incubation independent of maternal brooding, brooding provides hydric benefits that allow females to exploit nesting sites that would otherwise be inadequate. Such sites, while hydrically inferior, may feature other beneficial nest characteristics (e.g., better temperature profile, reduced risk of depredation). In addition to providing a greater spatial range of nesting opportunities, maternal brooding might also allow for an expanded temporal range as well. That is, brooding might allow females to nest during the tail end of the dry season, so that offspring can utilize the wet season for foraging and resultant growth. While this possibility remains untested, several python species, including children's pythons in northern Australia where rainfall is extremely seasonal, brood their eggs during the latter part of the dry season when hydric conditions are at their least favorable. During such times of year, suitable microhabitats for effective incubation of non-brooded eggs may be highly restricted.

Clearly, the proximal and ultimate benefits of maternal brooding in pythons are complex. However, our results largely support and extend the findings of previous laboratory (Aubret et al. 2005) and field studies (Madsen and Shine 1999). Considered as a whole, these data demonstrate that water balance of python eggs is extremely delicate, and successful production of offspring requires a balance among various nest characteristics, particularly temperature, humidity, and depredation risk. Maternal brooding provides a buffer against environmental conditions and allows for successful incubation of eggs under conditions that would otherwise prohibit hatching. Further studies, both in the laboratory and the field, are clearly required to further explore the driving forces for this behavior that is generic among pythons, but otherwise uncommon among squamate reptile taxa.

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