



Energy expenditure for parental care may be trivial for brooding pythons, *Python regius*

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Intensive parental care improves reproductive success. Why, then, do most animal species not provide parental care to their progeny? Life history theory suggests that the costs must be too high unless they are balanced by considerable fitness benefits. The notion that intensive parental care necessarily entails major costs is based both on intuition and on abundant empirical data. We monitored intensive parental care during brooding in 48 female ball pythons in equatorial Africa (south Togo). Female ball pythons remain coiled around their clutch for 2 months and refuse to feed during nest attendance. Surprisingly, brooding females lost less than 6% of their initial body mass over this 2-month period. The magnitude of mass loss was independent of the duration of brooding (experimentally manipulated to 0, 15 or 60 days) or clutch size (normal, enlarged and reduced clutch sizes). Maternal brooding substantially improved hatching success at little energy cost to the female. This paradoxical result reflects the high ambient temperatures in the study area, meaning that only rarely did nest-attending females need to shiver (a costly thermogenic behaviour observed in all python species). The presence of the mother tightly coiled on the clutch reduced water loss and avoided deleterious yolk desiccation. Thus, intensive brooding over a long period does not necessarily entail major energy expenditure for the mother, but none the less can significantly improve reproductive success. Maternal energy costs during brooding (direct expenditure and possible foregone foraging opportunities) were not influenced by clutch size, demonstrating fecundity-independent costs of reproduction.

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Virtually all endothermic vertebrates, and many ectothermic animals (including arthropods, molluscs and fish), display intensive and energy-consuming parental care to protect their offspring, to keep them in optimal hydric and thermal conditions during development, and through various forms of food provisioning during or after embryonic development (Clutton-Brock 1991; Alcock 1993). Without this expenditure of energy and input of resources, embryos and neonates of many taxa could not survive (Webb 1987; Vleck & Hoyt 1991). Parental care is facultative in some species, but it none the less significantly improves offspring survival when provided (Madsen &

Shine 1999). Nevertheless, despite strong advantages for reproductive success, the vast majority of taxa do not provide any form of care to their progeny. This scarcity of parental care is probably due to the strong associated costs experienced by the parents (Calow 1979; Bell & Koufopanou 1986; Clutton-Brock 1991). The relative costs and fitness benefits of intensive parental care have been incorporated into life history theory, within the general context that reproductive effort necessarily entails substantial costs of reproduction (Stearns 1992). The influence of this notion has been so strong that reports of the absence of costs of reproduction have been interpreted as examples of hidden costs, and considerable theoretical and empirical efforts have been devoted to unmask them (Calow 1979; Bell & Koufopanou 1986; Reznick 1992; Weeks 1996; Monaghan & Nager 1997; Reznick et al. 2000; Veasey et al. 2001).

A widespread form of intensive parental care is nest attendance by the parents (usually the mother). Nest

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attendance enhances offspring fitness through various behaviours, such as predator deterrence, oxygenation of the clutch, protection against harsh environmental conditions, or supply of water and heat (Somma 1990; Clutton-Brock 1991; Vleck & Hoyt 1991). Such behaviours obviously improve offspring survival and developmental conditions (Clutton-Brock 1991; Vleck & Hoyt 1991; Shine et al. 1997), but they also expose the parents to significant risk of predation (Houston & MacNamara 1999). In addition, nest attendance is often associated with profound physiological changes such as the loss of feather coverage with the formation of incubation patches in birds (total loss of plumage in hornbills), or even the death of the parent (Wodinsky 1977; Polis 1981; Finch 1990; Clutton-Brock 1991; Alcock 1993; Veasey et al. 2001). As well as major survival costs, nest attendance may entail major energy expenditure. (Wurtman & Miller 1976; Clutton-Brock 1991). For example, birds lose mass rapidly during brooding (up to 33% of postlaying mass, Prince et al. 1981; Vleck 1981), because their metabolic rates increase by 19–50% (Williams 1996; Monaghan & Nager 1997; but note that mass loss during brooding may be a benefit in birds, Moreno 1989). Lactation in small mammals is even more expensive (Epstein 1978; Johnson et al. 2001). One of the most crucial aspects of brooding in birds is the heat transfer from the parents to the clutch. As endothermic animals, birds can readily warm up their developing offspring through brooding and counteract environmental temperature fluctuations (Williams 1996).

Although many nonendothermic animal species attend their eggs, pythons are the only ones known to generate heat metabolically (by shivering thermogenesis) to warm the clutch (Shine 1988; Somma 1990, 2003a, b). Detailed studies in both the field and the laboratory have shown that female pythons use shivering thermogenesis to maintain high and constant temperatures within the clutch when ambient temperatures fall (facultative endothermy; Noble 1935; Cogger & Holmes 1960; Hutchinson et al. 1966; Van Mierop & Barnard 1978; Harlow & Grigg 1984; Shine 1988; Somma 1990, 2003a, b). This ability is widespread among python species, and may be ubiquitous (Shine 1988). Although there are numerous reports of female pythons failing to shiver while brooding, other studies document maternal thermogenesis in many of the same species (Shine 1988). These apparent contradictions reflect the facultative nature of shivering thermogenesis in brooding pythons: the female shivers only when ambient temperatures fall below some critical minimum (Harlow & Grigg 1984; Slip & Shine 1988).

Especially in cool climates, thermogenesis in pythons may involve a high energy expenditure. Metabolic rates of brooding females can be 22 times greater than those of nonbrooding snakes (Harlow & Grigg 1984), and females can lose more than 15% of their postlaying body mass during incubation (Slip & Shine 1988). However, the corresponding benefits may also be considerable because higher incubation temperatures accelerate embryonic development and may also improve offspring 'quality' (Olsson & Shine 1997; Shine et al. 1997; Madsen & Shine 1999). Thus, available data suggest that maternal thermogenesis in pythons is an energetically costly behaviour,

and is maintained by natural selection because of even higher benefits to offspring viability (e.g. Shine et al. 1997).

However, the conclusion that brooding is necessarily very expensive energetically is based on studies on a small and atypical set of pythonid species: those inhabiting relatively cool climates. The most intensively studied taxon in this respect (*Morelia spilota spilota*) extends to cooler climates than does any other python (Cogger & Holmes 1960). Given the facultative nature of shivering thermogenesis, the costs of this activity might be substantially lower in a python species inhabiting a warm climate where nest-attending females rely on metabolic heat production only infrequently. To test this possibility, we measured the energetic effort associated with maternal nest attendance in a python species from equatorial Africa. We focused on one specific paradigm, that intensive parental care (such as intensive brooding) must entail high energy requirements, and we addressed two simple questions. Does prolonged brooding necessarily entail a high energy expenditure for female pythons and does brooding significantly improve offspring viability?

METHODS

Study Animals and Study Site

Ball pythons, *Python regius*, are medium-sized (to 2 m) nonvenomous snakes widely distributed in western Africa (Luiselli & Angelici 1998; Aubret et al., in press). This species is particularly abundant in equatorial regions ranging from east Ghana to Benin. In the wild, mating occurs from November to January, with oviposition 1–2 months later. Females brood their clutch throughout a long (2-month) incubation period, usually in small burrows with high and stable temperatures (Walls 1998). Shivering thermogenesis during brooding has been reported in captivity (Logan 1973). Gravid females and their clutches are collected in large numbers for the commercial pet trade, and we took advantage of this opportunity to obtain our study animals. Our work was conducted from January to June 2000.

The snakes were collected in the vicinity (<50 km) of Lomé, south Togo, and were brought to TOGANIM, a registered Togolese farm. The laying season regularly occurs from the end of February to late March in south Togo (Aubret et al. 2003), and snake hunters employed by TOGANIM collect gravid females at that time of the year by digging them from burrows. At the end of the experiment, all the females were released in the field, along with 10% of the neonates (the rest of the neonates were legally exported to U.S.A., Japan or Europe) under rules set down by local wildlife authorities. None of the animals involved in our study were mistreated, sick or injured. They all survived and were released in good condition (see below). We did not purchase any animals. Permits were not required by the local wildlife authorities; our study simply took advantage of ongoing legal activity at the farm and was carried out within the commercial premises of TOGANIM. Although *P. regius* is heavily commercially exploited over its distribution range, a recent IUCN survey suggests that in Togo this species adapts well

to human influences. In addition, its status as a totem animal for traditional religious beliefs may protect it from local consumption, especially in the south of the country. Finally, effective ranching methods have been developed for Togo populations, notably by TOGANIM (<http://www.iucn.org/themes/ssc/programs/togoreptiles.html>).

Soon after capture (<48 h), adult females were measured (snout–vent length, SVL, ± 0.5 cm) and weighed on an electronic scale (± 1 g). We also recorded the number of ventral scales, jaw length (from the tip of the snout to the posterior point of the quadratomaxillary junction), head width (above the eyes) and eye diameter.

Experimental Procedure

Forty-eight gravid female ball pythons were captured shortly before oviposition, and maintained individually in small wooden cages (50 × 50 × 30 cm) in a quiet, dark room. Water was provided once every week. Female ball pythons do not feed while incubating their clutches (Ellis & Chappell 1987); although we offered food to the snakes, all reproductive females (gravid or brooding) refused to feed. Nonreproductive females in similar conditions accepted prey. The females laid their eggs 15–45 days after capture, and brooded their clutches for more than 60 days (Aubret et al. 2003).

Because females do not eat while brooding, we expected a marked decrease in their body mass from laying to hatching. The magnitude of that mass loss might be all-or-none (perhaps reflecting some physiological cost independent of reproductive expenditure: Ladyman et al. 2003) or alternatively might be proportional to the duration of brooding or the mass of the brooded clutch or both (Monaghan & Nager 1997). To quantify the energy expenditure of brooding, we manipulated both brooding duration and clutch size. For this purpose, we divided the females into five groups.

(1) Ten females brooded their clutches until hatching (control).

(2) Ten females brooded their clutches for the first 15 days after laying, then the clutches were removed and put into an artificial incubator (brooding interrupted).

(3) Ten females were removed from their clutches immediately after laying, and the clutches put into an artificial incubator (no brooding).

(4) Nine females brooded reduced clutches, with 42% of eggs removed (brooding effort reduced).

(5) Nine females brooded enlarged clutches, with 50% more eggs added to the clutch (brooding effort increased).

The entire clutch was weighed within 12 h of laying. We discarded abnormal eggs (undersized or with incomplete shells, Aubret et al. 2003) and weighed the remaining eggs (individually when possible, but python eggs are strongly adherent, see Aubret et al. 2003 for details on the procedure) at oviposition, and then every 15 days until hatching. The artificial incubators were small wooden boxes (50 × 20 × 20 cm) filled with wood shavings similar to those used by local farmers to incubate snake eggs for the international pet trade. However, the dark room at our disposal was large and relatively open to airflow

whereas local farmers incubate the eggs in small, relatively closed rooms. Each clutch was placed individually in the middle of a box, close to the surface, and covered by a thin layer of shavings.

Incubation time was defined as the time from the day of laying to when the neonatal snakes began to slit their eggshells. Several neonates died within 10 days of hatching (as frequently observed in other snake species, Bonnet et al. 2001). Consequently, we distinguished between hatching success (proportion of eggs that hatched) and offspring viability (proportion of eggs that produced 'healthy' neonates). These two variables are correlated, however, because unhatched eggs were also counted as nonviable offspring. Incubation temperatures were recorded using four automatic recorders (Tinytag Ultra, –40 to 85 °C; total number of records = 7900; delay between each record 16 min 30 s). Two temperature recorders were attached to the clutch in one brooded and one artificially incubated clutch, respectively. We placed another temperature recorder in a potential natural nest site: a tortoise burrow. This allowed us to compare natural incubation temperatures to those we monitored in our experiment. We also recorded the ambient temperature of the room where we placed the five sets of clutches and females.

Energy Expenditure

Because brooding female pythons do not feed, their changes in body mass provide a simple index of the energy expenditure of brooding. Direct measurements of metabolic rates and energy allocation have validated this approach: changes in body mass accurately reflect the overall energy budgets of reproductive female snakes (Bonnet et al. 2001, 2002; Lourdaïs et al. 2002, 2003; Ladyman et al. 2003). More generally, changes in body mass of snakes over time are tightly linked to the use of body reserves (Bonnet et al. 2002). The low-maintenance energy requirements of ectothermy facilitate use of this simple measure, because body mass remains stable through time unless the organism initiates energy-expensive activities. In contrast, the high daily energy requirements of endotherms (with consequent high rates of input, and of maintenance metabolic costs) mean that body mass is sensitive to short-term fluctuations in feeding and metabolic rates. Hence, studies on endotherms require more complex methods to assess the magnitude of energy expenditure of activities such as brooding (Bonnet et al. 1998a, 2002).

The only major assumptions required for our use of body mass decrements to assess metabolic costs of brooding are the following.

(1) Body composition remains similar in females from the different experimental treatments. If snakes of one group lost mass because of breakdown of body fat reserves whereas another group broke down proteins, an equal rate of overall loss in body mass would represent different energy expenditures (because an equivalent mass of fat contains more energy than the same mass of protein: Robin et al. 1998). However, our groups were similar in all

major respects at both the beginning and the end of our experiments (see below), rendering use of different metabolic substrates unlikely.

(2) Body mass also changes through shifts in hydration; thus, we weighed each female at least twice a week, before and after providing her with drinking water.

(3) Ambient temperatures strongly modify metabolic rates and thus energy expenditure; but in our study, all females were kept at identical ambient thermal regimes (in the same room), so that any thermal differences between treatments reflect facultative endothermy by the females.

The females we used were caught in the field by digging them out from burrows before oviposition. They were maintained under thermal conditions likely to occur in natural laying sites (see [Results](#)). In fact, in natural nests such as tortoise burrows, ambient temperatures and humidity may well be more stable than those experienced in our incubation room ([Lagarde et al. 2002](#)). Consequently, the rates of energy expenditure (and hence, variations in body mass) we recorded are likely to be similar to (or greater than) those experienced by brooding female pythons in equatorial Africa.

Analyses

Several variables were not normally distributed. After \ln -transformation, the normality assumption was met ($P > 0.24$ in all Shapiro–Wilk W tests) for all variables except initial clutch size ([Aubret et al. 2003](#)). For clutch size we also tested for differences using a Kruskal–Wallis one-way ANOVA by ranks. Because many of the traits we recorded are correlated with maternal size (e.g. head dimensions, clutch size: [Aubret et al. 2003, in press](#)) we included maternal body size as the covariate in ANCOVAs with experimental treatment group as the factor ([Garcia-Berthou 2001](#)). Slopes were homogeneous in all ANCOVAs, and thus we report only intercept tests.

Snake body condition (mass relative to length: an accurate estimator of body reserves in snakes, [Bonnet et al. 1998b](#); [Aubret et al. 2002](#)) was analysed in two ways.

(1) To compare the five groups of females, we used ANCOVA with \ln maternal mass as the dependent variable and \ln SVL as the covariate (as recommended by [Garcia-Berthou 2001](#)).

(2) To examine shifts during different phases of the reproductive cycle among the groups of females, we used residual scores from a general least-squares linear regression of \ln maternal mass against \ln SVL, based on measurements of a large sample of nonreproductive adult females ($N = 300$) collected from our study area ($r^2 = 0.359$; $F_{1,298} = 167.20$, $P < 0.0001$; \ln body mass = $2.3066 \times \ln$ SVL – 3.5707). The advantage of this latter measure is that it represents the deviation from ‘average’ body condition within the population (reproductive or recently fed animals were not used in this analysis). We can thus easily (and graphically) compare the body condition of each of our experimental groups with that of ‘normal’ conspecifics, rather than relying entirely on comparisons within the experimental animals (which might, for example, all be highly emaciated).

To compare temporal changes in maternal mass during incubation, we used multivariate ANOVA with repeated measures over time ([O’Brien & Kaiser 1985](#)). Relative clutch masses were compared using ANCOVAs with clutch mass (\ln) as the dependent variable, postlaying maternal mass (\ln) as the covariate and experimental treatment as the factor. Bonferroni corrections (not reported) did not modify any of our conclusions. Means in the text (except f or adjusted means) are given \pm SD. Data were analysed using Statistica 6.1 ([StatSoft 2003](#)).

RESULTS

Maternal and Clutch Characteristics

Females from the five groups were very similar in terms of their phenotypic and reproductive characteristics ([Table 1](#)). No statistically significant differences were detected between the groups, regardless of whether or not maternal SVL was included as a covariate in the analyses. Our experimental manipulations of clutch size generated highly significant between-group differences in mean values for clutch size and clutch mass ([Table 1](#)).

Incubation Temperatures

All our data loggers ($N = 1929$ data for each recorder) recorded temperatures in a narrow range and with similar means. Ambient temperature averaged $30.5 \pm 1.3^\circ\text{C}$ during the study (range 27.4 – 33.0°C). All the clutches thus experienced similarly high and relatively constant temperatures. Mean temperatures of a maternally brooded clutch ($30.5 \pm 0.8^\circ\text{C}$, range 28.1 – 32.2°C) were slightly higher than those of an artificially incubated clutch ($29.5 \pm 1.2^\circ\text{C}$, range 26.3 – 31.8°C ; difference of 1°C , $P < 0.00001$). This thermal difference between nonbrooded and brooded clutches was probably caused by greater evaporative water loss in nonbrooded clutches (see below). Thermal fluctuations were also slightly buffered by the presence of the mother (mean diurnal ranges of 4.1°C versus 5.5°C for brooded and artificially incubated clutches, respectively). Importantly, temperature records of the potential natural laying site ($28.8 \pm 0.9^\circ\text{C}$, range 25.9 – 31.1°C) suggest that the temperature regimes experienced by unattended clutches in our experiments fell within the range of conditions encountered in natural nests.

Changes in Maternal Mass and Body Condition

Maternal body mass

Body masses remained remarkably consistent over time, regardless of the experimental treatments to which the snakes were exposed. Females that brooded their clutches throughout the 2-month incubation period lost on average only 70.7 ± 66.4 g, representing $5.9 \pm 6.0\%$ of their postlaying body masses (1260.5 ± 283.7 g). As a comparison, the mean clutch mass was 640.3 ± 128.3 g ($N = 48$,

Table 1. Phenotypic traits and characteristics of the clutches of 48 female ball pythons involved in the experiment

Maternal traits and clutch characteristics	Full brooding	Partial brooding	No brooding	Reduced clutches	Enlarged clutches	df	F/H	P
Snout-vent length (cm)	112.5±6.8	114.5±5.0	115.3±8.2	114.6±11.1	115.3±11.3	4, 43	0.20	0.954
Maternal mass at capture (g)	1844.2±311.6	1933.3±288.4	2032.3±440.0	2082.3±648.6	1992.0±648.7	4, 43	0.23	0.843
Body condition at capture (g)*	1939.4±77.2	1931.7±76.7	1989.6±76.8	2075.7±80.9	1947.7±81.0	4, 42	0.36	0.839
Number of ventral scales*	206.4±1.0	206.3±1.0	203.2±1.0	206.4±1.1	204.9±1.1	4, 42	0.49	0.740
Jaw length (mm)*	47.4±0.5	46.1±0.5	46.6±0.5	47.2±0.5	46.9±0.5	4, 42	1.12	0.361
Head width (mm)†	17.0±0.2	16.9±0.2	16.7±0.2	17.1±0.2	17.2±0.2	4, 42	0.80	0.532
Eye diameter (mm)†	5.8±0.1	5.8±0.1	5.8±0.1	5.7±0.1	5.6±0.1	4, 42	0.63	0.641
Laying date±days	9 March±10	11 March±9	7 March±10	12 March±10	6 March±9	4, 43	1.00	0.624
Prelying maternal mass (g)	1782.5±270.3	1886.3±266.6	1974.2±455.5	1946.7±627.4	1917.7±599.9	4, 43	0.18	0.950
Prelying body condition (g)*	1875.4±68.3	1884.8±67.9	1932.6±68.0	1940.2±71.6	1874.5±71.7	4, 42	0.15	0.960
Postlaying body condition (g)*	1236.0±41.9	1198.2±41.7	1301.0±41.7	1304.9±44.0	1271.1±44.0	4, 42	0.78	0.542
Body condition at hatching (g)*	1188.6±40.5	1133.1±40.2	1199.4±40.3	1253.7±42.4	1183.0±42.5	4, 42	0.79	0.539
Initial clutch size	7.3±1.3	7.4±1.1	8.0±1.9	7.0±1.9	7.0±1.3	4, 43	0.71	0.591
Initial clutch size*	7.5±0.4	7.4±0.4	7.9±0.4	7.0±0.4	7.0±0.4	4, 42	0.93	0.454
						4, N=48	2.50	0.645
Initial clutch mass (g)	640.3±128.3	677.8±122.7	679.3±214.9	637.9±283.3	629.8±206.3	4, 43	0.36	0.835
Initial clutch mass (g)*	675.8±38.7	677.2±38.5	663.4±38.5	635.4±40.5	613.3±40.6	4, 42	0.96	0.438
Relative clutch mass (%)‡	54.1±4.5	56.7±7.4	50.7±7.8	47.6±14.0	48.4±6.1	4, 42	2.13	0.094
Manipulated clutch size	7.3±1.3	7.4±1.1	8.0±1.9	3.4±1.5	9.7±2.0	4, 43	17.67	<0.001
						4, N=48	28.37	<0.001
Manipulated clutch mass (g)	640.3±128.3	677.8±122.7	623.4±236.3	316.2±136.1	943.3±217.2	4, 43	15.75	<0.001

$N = 10$ for the first three groups (brooding time modified) and nine for the last two (clutch size modified). Comparisons among the five groups were performed using ANOVAS and ANCOVAS (F ; means: * adjusted by maternal snout-vent length; † adjusted by jaw length). Results from Kruskal-Wallis ANOVA by ranks (H) are also reported for clutch size. Relative clutch mass was calculated as the ratio of clutch mass divided by postlaying maternal mass (%); however, statistical analysis of this variable used ANCOVA with ln-transformed data (‡ adjusted by postlaying maternal mass). Means are given \pm SD; adjusted means are given \pm SE. Mean values are presented without transformation for simplicity, ANOVAS and ANCOVAS were performed on ln-transformed data, however, to meet the normality assumption (see **Methods**). The last two rows provide information on clutch size and clutch mass after manipulation.

54% of the postlaying maternal mass on average); the mean mass of the eggs was 90.0 ± 10.7 g ($N = 48$ clutches). This latter mass represents on average 7.1% of the postlaying body mass of females. In terms of body reserves, the cost of maternal attendance was thus broadly equivalent to the production of an additional egg.

Maternal body condition

Changes in maternal body condition over the period from 15 days before laying to the end of incubation (hatching) differed significantly between the five experimental groups (repeated measures ANOVA with treatment as the factor and subsequent measures of maternal body condition as the dependent variable; Wilk's $\lambda = 0.228$, $F_{7,28} = 134.83$, $P < 0.001$; $P > 0.162$ in all Levene's tests for variance homogeneity; **Fig. 1**). Maternal body condition changed significantly through time ($F_{6,258} = 1164.88$, $P < 0.001$) reflecting considerable weight loss at oviposition (**Fig. 1**), but females in the five treatment groups lost mass at similar rates ($F_{4,43} = 0.44$, $P = 0.78$). A significant interaction between time and treatment ($F_{24,228} = 1.756$, $P = 0.018$) was detected, owing to the rate of mass loss

decreasing through time in the control group, but increasing in the other treatments (**Fig. 1**).

These analyses include the massive changes in maternal mass at oviposition, before the experimental treatments were imposed. A more direct analysis is thus to restrict attention to changes in maternal body condition from the time of oviposition until hatching. The results were largely unaltered (Wilk's $\lambda = 0.331$, $F_{5,20} = 130.30$, $P < 0.001$; effect of time: $F_{4,172} = 156.22$, $P < 0.001$; effect of treatment: $F_{4,43} = 0.76$, $P = 0.55$; interaction: $F_{16,172} = 3.54$, $P < 0.001$). The interaction between time and treatment, detected by the repeated measures analysis, did not translate into any significant differences between the five groups at any time of the experiment (i.e. performing analyses separately at each time period), nor at the completion of the study (after hatching). In each of the latter analyses, female mass loss to that point was unaffected by treatment (ANCOVA with ln maternal mass at the end of the study as the dependent variable, ln SVL as the covariate and treatment as the factor: $F_{4,42} = 0.79$, $P = 0.55$; **Table 1**). Overall, maternal mass loss was modest during the 2 months of incubation, and affected to only a minor (nonsignificant) degree by our experimental

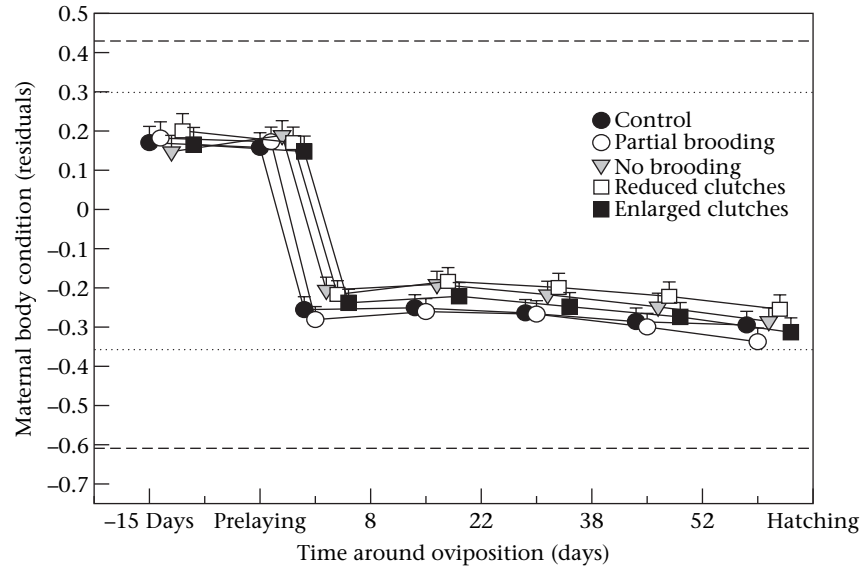


Figure 1. Influence of brooding duration and clutch size manipulation on changes in body condition of female ball pythons during incubation. Mean values \pm SE for maternal body mass are plotted from 15 days before laying to the end of incubation. $N = 10$ for the control, partial and no brooding conditions and nine for reduced and enlarged clutches. The drop in body condition at oviposition corresponds to the expulsion of the eggs. Dashed lines correspond to the extreme values observed in 300 wild nongravid females. Dotted lines correspond to the percentile boundaries (10% and 90%). The lower dashed line indicates a lower critical body condition before which survival is jeopardized. Data points are plotted slightly out of step for clarity.

manipulations (Fig. 1). The results were unchanged when we repeated the above analysis with \ln maternal body mass instead of maternal body condition.

Our body condition index calculations (based on 300 nonreproductive adult females) allowed us to compare the above fluctuations in body conditions during reproduction with the range observed in natural conditions (see dashed lines in Fig. 1). Even after oviposition and brooding, the females were not particularly emaciated; average values for all treatment groups remained well within the range seen in field-captured nonreproductive animals (Fig. 1).

Effect of Treatment on Reproductive Success

Hatching success

Our experimental treatments generated strong differences in egg viability (Contingency table: $\chi^2_4 = 51.3$, $P < 0.001$; Fig. 2), indicating that the mother's presence during incubation strongly enhanced hatching success.

Offspring viability

When we ranked our experimental treatments in terms of the intensity of maternal brooding (from no brooding through interrupted brooding, increased clutch size, control and reduced clutch size), we saw a strong association between this ranking and the viability of the offspring (Kendall correlation: tau = 1.0, $N = 5$, $P = 0.014$). This result suggests that intensive brooding enhances the probability of obtaining healthy offspring.

The eggs lost mass from laying to hatching in all three groups, owing to a net loss of water (Rahn & Ar 1974; Packard & Packard 1988; Ackerman 1991; Packard 1991),

but the amount of mass lost differed significantly between the five treatments (repeated measures of mass over time; Wilk's $\lambda = 0.129$, $F_{3,12} = 106.12$, $P < 0.001$; $P > 0.18$ in all Levene's tests; Fig. 3). All the clutches lost water; the change in mass was least when brooding intensity was high (ranking groups as above; Kendall correlation: tau = 1.0, $N = 5$, $P = 0.014$; Figs 3, 4).

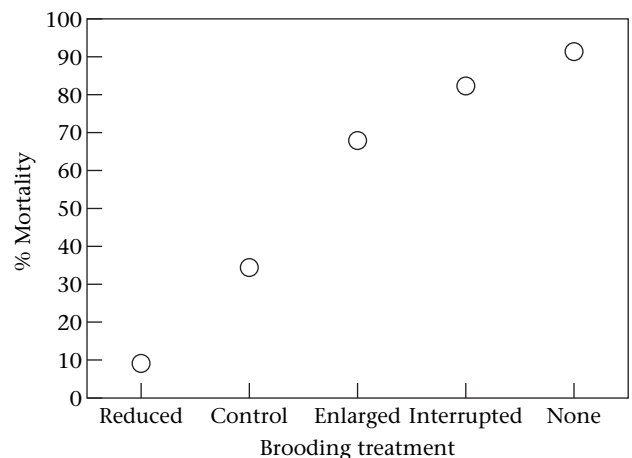


Figure 2. Influence of the intensity of maternal brooding (brooding treatment) on the proportion of eggs that led to viable offspring (neonates that were still healthy 10 days after hatching). 'Reduced' and 'enlarged' refer to reduced and enlarged clutch sizes, respectively. Reduction of clutch size facilitates brooding for the mother, whereas enlarging clutch size impedes brooding because the female cannot cover the entire clutch. Hence, the intensity of brooding per egg decreases from the left to the right of the X axis (see text for statistics).

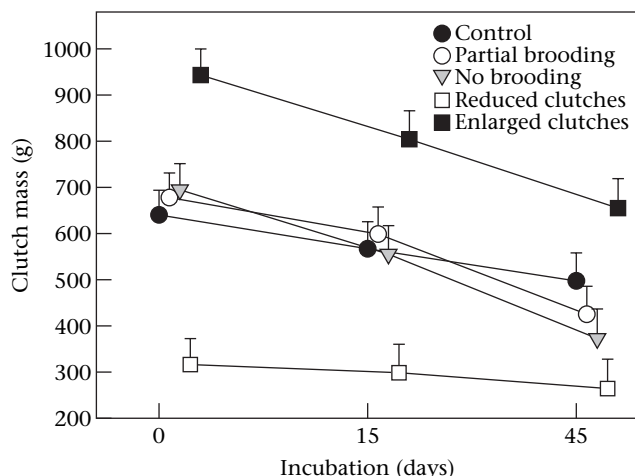


Figure 3. Clutch mass in relation to the day of incubation. Measurements were not taken at the end of incubation (60 days) because many neonates had already hatched by this time.

DISCUSSION

Is Brooding Energetically Expensive?

Our data suggest that female pythons can derive considerable fitness benefits from a modest energy input during prolonged parental care. The major empirical result from our study is strongly counterintuitive: female ball pythons that brooded their clutches for 2 months lost an average of <6% of their postoviposition body mass over that period. In addition, experimental manipulations showed that the magnitude of this minor decrement in maternal body mass was not influenced by either brooding duration or the number of eggs brooded. To our knowledge, our study provides the first report of such a low energy expenditure, unrelated to clutch size and associated with a prolonged intensive brooding in vertebrates. The virtual constancy in maternal mass cannot be attributed to feeding because the females did not eat. Importantly, all the females involved in the experiment

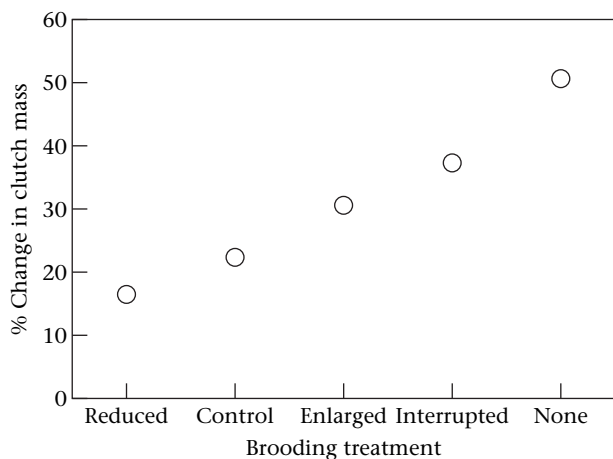


Figure 4. The loss in mass of the eggs in relation to the relative intensity of maternal brooding. See Fig. 2 for definition of the categories on the X axis.

survived and appeared healthy at the conclusion of the study. Furthermore, their body condition remained within the usual range observed in wild-caught nonreproductive animals, well above a critical body condition threshold below which the snakes may not survive (hence, the absence of field records below this level, indicated by the lower dashed line in Fig. 1). This result clearly indicates that the females maintained substantial body reserves over the entire reproductive period, probably in the form of fat bodies and musculature, and consequently did not experience any significant survival cost during nest attendance (Bonnet et al. 1998b, 1999b; Lourdaï et al. 2004). Indeed, snakes are mostly at risk when they leave their shelter to forage, to mate and to reach laying sites (Bonnet et al. 1999a).

Why was maternal mass loss so slow, despite the substantial elevation in maternal metabolic rates demonstrated for reproductive female ball pythons in captivity (Ellis & Chappell 1987)? In fact, Ellis & Chappell documented an increase in metabolic rate in their animals before oviposition (during vitellogenesis) rather than during nest attendance. The high relative clutch mass (54% of the postlaying maternal mass) we observed is consistent with the fact that, in snakes, vitellogenesis is the main period of energy expenditure (Bonnet et al. 1994; Beaupré & Duvall 1998); the postovulation (gestation, brooding) period is usually less demanding, at least under favourable ambient temperatures (Ladyman et al. 2003). The distinction between these two very different physiological stages has often been ignored, perhaps explaining the discrepancy between previous studies (reporting high energy costs of reproduction in brooding reptiles) and our results.

Using Ellis & Chappell's (1987) equation linking body mass to metabolic rate in nonbrooding ball pythons, we can crudely estimate the amount of fat reserves necessary to sustain a snake's metabolic needs during 2 months at an ambient temperature of 30°C. A nonbrooding ball python with a body mass of 1260 g would use roughly 240 g of her fat reserves during 2 months without feeding, about 18% of her initial body mass. The much lower value (6%) obtained in our study suggests that nest-attending females experience more favourable conditions in south Togo, or somehow reduce their energy expenditure, perhaps by prolonged periods of quiescence combined with selective organ down-regulation (Secor & Diamond 1995). The absence of effect of our various brooding/clutch size manipulations further reinforces the notion that brooding is not necessarily expensive per se. Overall, both direct and indirect evidence suggests that the energy requirements for maternal egg attendance were low at the mean ambient temperatures of 30°C representative of natural conditions in south Togo (especially in buffered burrows).

Our results stand in strong contrast to previous studies that have reported higher estimated rates of mass (i.e. energy) loss in brooding females of about 15% of their postlaying body mass (Slip & Shine 1988; Madsen & Shine 1999, 2000). These calculations were imprecise, however, because (1) the free-ranging snakes in these studies were weighed only before the eggs were laid and after they hatched, (2) egg masses for these females were estimated,

not measured directly, and (3) brooding occurred during the dry season, so that part of the mass loss may reflect dehydration (Madsen & Shine 1999). Hence, the exact mass loss after oviposition, a critical measurement to tease apart the respective energy expenditures of vitellogenesis and brooding, was not known. Because clutch mass will be much higher than maternal mass loss during the incubation period (in our study, means of 54% versus <6% of postlaying maternal body mass), even a small percentage error in the estimate of clutch mass might substantially influence the calculated mass loss from brooding.

An additional potential cost to maternal care in pythons may be represented by the missed feeding opportunities while brooding. In the absence of information on the foraging success of nonreproductive females during the brooding period we cannot assess the magnitude or the significance of such a potential cost. However, whatever their magnitude, the existence of such a cost will not alter our main result: brooding per se required very little energy. Even if 'opportunity costs' of nest attendance are substantial, they are likely to be independent of fecundity, as the number of prey foregone by a female will not be related to the size of her clutch. In accord with the fact that maternal energy expenditure was unrelated to clutch size, the overall costs of brooding in ball pythons may be largely independent of maternal fecundity. The relative magnitude of fecundity-independent costs of reproduction may be crucial for the evolution of reproductive strategies such as breeding frequency (Bull & Shine 1979), capital breeding and semelparity (Bonnet et al. 1998a); field studies are required to appreciate their importance better.

Did Brooding Improve Offspring Viability?

For facultative parental care to enhance maternal fitness, the (potentially modest) energy expenditure needs to be balanced by strong benefits to offspring fitness. Ball pythons fulfil this condition also: hatching success and offspring viability were strongly dependent on maternal attendance. Females brood their eggs by coiling around so tightly that the clutch is hidden (Ellis & Chappell 1987; personal observation). Our results show that, at a mean ambient temperature of 30°C, maternal attendance protected the eggs against desiccation (variations in egg mass in the course of incubation are closely related to water exchange: Rahn & Ar 1974; Packard & Packard 1988; Packard 1991) and that this control of water balance might have been the primary cause for enhanced hatching success of maternally brooded clutches (Aubret et al. 2003; O. Lourdais, personal communication). Maternally brooded clutches also averaged 1°C warmer than unattended clutches, possibly reflecting differential evaporative water loss, occasional shivering thermogenesis, or enhanced thermal inertia from maternal covering of the clutch. Preventing desiccation of eggs might have been the initial selection pressure for the evolution of lactation, another form of intensive parental care (Ofstedal 2002a, b). Several authors have reported cases of female reptiles keeping eggs moist by brooding (Fitch 1954; Bels & Van den Dande 1986; York & Burghardt 1988; Somma &

Fawcett 1989) or wetting their bodies with water and then lying on the eggs, thereby reducing desiccation (Packard et al. 1982; Packard & Packard 1988; Somma 1990, 2003a, b). If the main benefit from maternal brooding involves an energetically 'cheap' posture with prolonged immobility rather than energetically expensive behaviours such as shivering, then the balance between costs and benefits may indeed favour intensive parental care such as prolonged brooding.

Why Did Brooding Females Rarely Shiver?

Ambient temperatures are so favourable for incubation in our study area, especially in burrows, that female ball pythons did not shiver, or perhaps did so only very occasionally. This suggestion is supported by the similarity of temperature records among treatment groups, and with thermal regimes in natural laying sites. Indeed, although there are detailed reports of typical shivering thermogenesis within this species (Logan 1973; Pitman 1974), we never observed this behaviour during our (admittedly infrequent) observation periods. Thus, shivering may occur so rarely under favourable conditions that it has little overall effect on energy expenditure. Shivering thermogenesis is probably more frequent at higher latitudes where temperatures are more variable than in equatorial areas such as south Togo.

The clear implication of these results is that shivering may be rare in species with access to incubation environments that provide high, stable temperatures. Most python species worldwide live in relatively warm climates, and, hence, it is plausible that the initial origin of shivering thermogenesis occurred in such an environment. In keeping with this inference, detailed studies on a population of water pythons, *Liasis fuscus*, in tropical Australia showed that females that laid their eggs in unusually warm nest sites not only did not shiver, but also usually abandoned their clutch entirely and allowed it to incubate under ambient temperatures (Madsen & Shine 1999). Females that selected slightly cooler nests remained with the eggs, shivering only sporadically (Shine et al. 1997; Madsen & Shine 1999). Even the minor thermal shift caused by maternal attendance in these cooler nests significantly enhanced offspring viability (Shine et al. 1997).

A plausible scenario is that maternal nest attendance evolved for benefits related to hydration or protection of eggs against predators (as seen in many other reptile taxa; Somma 1990). The water-conserving postures of brooding females inevitably provide a thermal buffer as well; and females also heat their clutches by periods of basking in the sunshine followed by retreat to the cooler nest (e.g. Slip & Shine 1988). Once these behaviours have evolved, it is only a small step to additional means of maternal thermal regulation of nest temperatures. Sporadic muscular shivering to generate metabolic heat would have involved little cost if it occurred only on the infrequent occasions when local ambient temperatures fell briefly during storms (as occurred several times during our own study; at these times, brooded clutches remained at

constant high temperatures but unattended eggs cooled by 2–3°). In keeping with this scenario, metabolic rates of brooding female (but not nonbrooding) *P. regius* show a rapid but transitory elevation in response to a slight drop in air temperature (figure 3 in Ellis & Chappell 1987). We do not know if such brief periods of lower temperatures would affect offspring fitness, but a recent study on incubation of lizard eggs revealed that hatchling phenotypes were strongly affected by even brief exposure to slightly different thermal regimes (Shine & Elphick 2001).

In conclusion, our study shows that intensive parental care such as brooding over a prolonged period does not necessarily entail major energy expenditure to the mother. Under thermal conditions likely to be widespread over the geographical range of pythonid snakes, facultative maternal thermogenesis may substantially enhance offspring viability at very little cost to the energy balance of the reproducing female. It is easy to envisage maternal nest attendance evolving for initial benefits related to protection or hydration of the eggs, with the subsequent evolution of facultative thermogenesis because of further benefits to hatchling viability.

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