

Metabolism, temperature relations, maternal behavior, and reproductive energetics in the ball python (*Python regius*)

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Summary. Thermogenic incubation has been documented in two large species of pythons, but the phenomenon has not been studied in small species with concomitantly large heat transfer coefficients. We describe behavior, metabolic rates, mass changes, and temperature relations for adult ball pythons (*Python regius*), the smallest member of the genus, during the reproductive cycle. Egg and hatchling metabolism and hatchling growth rates were also examined.

Rates of oxygen consumption (\dot{V}_{O_2}) of both gravid and non-gravid snakes showed typical ectothermic responses to changing ambient temperature (T_a). The Q_{10} for T_a 's of 20–35 °C was 2.2–2.3. The \dot{V}_{O_2} of gravid females was significantly greater than that of non-gravid snakes at all T_a . Maximum oxygen consumption (\dot{V}_{O_2} max) during forced exercise was about 12 times resting \dot{V}_{O_2} at $T_a = 30$ °C.

Eggs (5–6 per female) were laid in April. Total clutch mass was approximately 32% of the females' pre-oviposition mass. After oviposition, mother snakes coiled tightly around their clutches and remained in close attendance until the eggs hatched in June. Sudden decreases in T_a elicited abrupt but transient 2- to 4-fold increases in the \dot{V}_{O_2} of incubating females. Similar responses were not observed in non-incubating snakes. The steady-state \dot{V}_{O_2} of incubating females was independent of T_a . In no case was body temperature (T_b) elevated more than a few tenths of a degree above T_a in steady-state conditions.

The \dot{V}_{O_2} of developing eggs increased sigmoidally through the 58–70 day incubation period. Total oxygen consumption during incubation at $T_a = 29.2$ °C was about 3.6 l per egg. Young snakes quadrupled their mass during their first year of growth.

Compared to larger python species which are endothermic during incubation, ball pythons have similar aerobic scopes and higher mass-specific \dot{V}_{O_2} max. However, effective endothermy in ball pythons is precluded by high thermal conductance and limited energy stores.

Introduction

Pythons inhabit the tropical and subtropical regions of Africa, Asia, and Australia. All are oviparous and are among the few reptiles showing extensive parental care. Females coil about their eggs shortly after oviposition and attend them until hatching, a period that may exceed two months. At least two species, the Indian python *Python molurus* and the diamond python *Morelia spilotes*, are able to keep clutch temperature substantially warmer than ambient temperature (T_a) by means of physiological thermogenesis. Heat production is apparently accomplished by spasmodic muscular contractions ('shivering'), and is adjusted so as to keep body and egg temperature between 30 and 34 °C at T_a of 23–33 °C. At low T_a , rates of oxygen consumption of incubating females may be 10–20 times greater than those of similar-sized non-incubating individuals (Hutchison et al. 1966; Vinegar et al. 1970; Van Mierop and Barnard 1978; Harlow and Grigg 1984).

The occurrence, function, and evolution of thermogenic incubation in the python family are poorly understood. Endothermy is thought to be favored by large body size, with concomitant small surface to volume ratios that favor the retention

of body heat (derived metabolically or gained by solar basking). This concept is applicable to *P. molurus*, a robust species which begins to breed at a mass of 15–20 kg and attains masses in excess of 60 kg. However, *M. spilotes* is considerably smaller (3–6 kg). Other reports of shivering during incubation have concerned both large and small species: the blood python *P. curtis* (Vinegar et al. 1970), Timor python *P. timorensis* (Murphy et al. 1978), green tree python *Chondropython viridis* (Kratzer 1962; Van Mierop et al. 1983), and three Australian forms, *Aspidites melanocephalus*, *Liasus fuscus*, and *L. amethystinus* (Boos 1979). None of these papers contain data on metabolic rates during incubation, so the degree of heat production is uncertain. Somewhat paradoxically, two very large species, the African python (*P. sebae*) and the reticulated python (*P. reticulatus*), have not been reported to regulate clutch temperature by endothermic means, although they do coil around their eggs (Vinegar et al. 1970; Sclater 1862; Wall 1926).

A second hypothesis explaining the evolution of endothermy was proposed by Vinegar et al. (1970), who suggested that endothermic brooding allows pythons to extend their geographic range into regions where T_a 's are too low to support embryonic development. At least some species of pythons have eggs that require warm temperatures during incubation; for example, the eggs of *P. molurus* and *M. spilotes* fail to develop at temperatures below 27.5 °C (Vinegar 1973; Harlow and Grigg 1984). This model may explain the occurrence of endothermy in *P. molurus* and *M. spilotes*, both of which inhabit cool subtropical as well as tropical regions, and its apparent absence in *P. reticulatus* and *P. sebae*, which are restricted to warm habitats.

In this paper we describe the behavior, metabolism, and overall energetics of reproduction in ball pythons, *P. regius*. This African species is the smallest member of the genus *Python*, attaining an adult length of about 1.5 m and a body mass of about 2 kg. Because of its small size, and because its range is apparently limited to warm tropical lowlands (Pitman 1974), *P. regius* would not be expected to show a significant endothermic brooding ability. Nevertheless, as in larger pythons, female ball pythons closely attend their clutches. We felt that a study of the metabolic and behavioral responses of *P. regius* might produce data which, in conjunction with published information on *P. molurus* and *M. spilotes*, could provide useful insights into the evolution of python incubation behavior.

Materials and methods

Animals. Adult *Python regius* (6 gravid females, 2 non-gravid females and 3 males) were purchased in November 1983, shortly after their importation from Africa. The animals were housed individually in cages (1.5 × 0.45 × 0.45 m) which were kept in a large environmental room maintained at 29.1 ± 0.2 °C and 70–80% R.H. with a 12 h photoperiod. Drinking water was always available. Mice (*Mus* and *Peromyscus*) were offered to all snakes weekly.

Gravid females laid eggs in their cages in late April or early May. After oviposition the female and a subset of the egg clutch (usually 4 of 6 eggs) were placed into a plastic dishpan (0.33 × 0.30 × 0.12 m) containing 400 g of sterile particulate mica hydrated with 400 ml of chlorinated tap water. Copper-constantan thermocouples were positioned in the substrate at least 0.15 m from the female and also in the air ca. 0.10 m above the substrate. The eggs removed from each clutch were weighed, measured and incubated separately. Some of them were placed individually into 2 l respirometers containing 300 g of a 1:1 mixture of sterile mica and water. Other eggs were implanted with 36-gauge thermocouple wire with the couple positioned approximately in the geometric center of the egg. The leads were secured to the egg shell with methyl methacrylate adhesive. Thermocouple-equipped eggs were either returned to the maternal female or placed separately into plastic boxes (0.31 × 0.17 × 0.08 m) containing 400 g of 1:1 mica and water. Thermocouple implants did not affect subsequent embryonic development. Additional thermocouples were also placed in the substrate and air immediately adjacent to the egg. Eggs, and later hatchlings, were kept in the same environmental room as the adults.

Metabolic measurements. Oxygen consumption (\dot{V}_{O_2}) and in some cases carbon dioxide production (\dot{V}_{CO_2}) from post-absorptive animals were determined in open-circuit respirometer systems. Pythons were weighed to ± 0.1 g on a Mettler PC 4000 balance and placed into plastic metabolism chambers. Chamber volumes were 10.3, 17.6 and 0.8 l for adult snakes, incubating females with their eggs, and hatchlings, respectively. Metabolism chambers were placed into a large temperature-controlled (± 0.2 °C) environmental cabinet. Dry air was metered through Applied Materials mass flow controllers (model AFC-550), humidified to 50–80% R.H., and routed into the metabolism chamber via 2 side ports. A portion (40 ml/min) of the excurrent air from ports at the top of the metabolism chamber was dried (Drierite) and analyzed for CO₂ (Beckman LB-2 or Applied Electrochemistry CD-3A), then passed through CO₂ absorbent (Ascarite), redried and analyzed for oxygen (Applied Electrochemistry S-3A). The CO₂ analyzer was standardized against calibration gases daily and both analyzers were referenced against air diverted from immediately upstream of the flow controllers ca. every 5 min during measurements. Air flow was adjusted during experiments so that [O₂] was not less than 20.5% and [CO₂] did not exceed 0.5%. This required flow rates between 0.50–1.20 l/min for adults and 0.06–0.20 l/min for eggs about to hatch and for young snakes.

During metabolic measurements on incubating females, temperature data were collected from several thermocouples placed in the air surrounding the animal, affixed to its scales, and inserted within the egg mass. Body temperatures (T_b) of incubating snakes were determined using temperature sensitive radio-telemeters (Mini-Mitter, model XM) which were force-fed to the females prior to oviposition.

Steady-state metabolic measurements were obtained at ambient temperatures (T_a) between 20 and 36 °C. We assumed that an animal was in steady-state if it was quiescent and T_a ,

T_b and \dot{V}_{O_2} were stable during several hours of observation. During experiments data were recorded and stored by a computer. The \dot{V}_{O_2} and \dot{V}_{CO_2} were calculated using the equations in Hill (1972). For brooding animals, these values were corrected for the contributions of the eggs by subtracting egg \dot{V}_{O_2} (obtained from closed-system measurements as described below, and adjusted for the effects of temperature using a Q_{10} of 2.2) from the total oxygen consumption.

Maximum oxygen consumption ($\dot{V}_{O_2,max}$) was determined for non-gravid adult pythons in a 5.7 l chamber held at 30 °C with an air flow rate of 1.2 l/min. The $\dot{V}_{O_2,max}$ was elicited by continually turning the chamber so that the python struggled to right itself. This regime was maintained for 15 min. However, snakes were exhausted and unable to maintain a normal posture after 5–10 min of agitation. Because these \dot{V}_{O_2} measurements were short term, we computed ‘instantaneous’ \dot{V}_{O_2} according to Bartholomew et al. (1981). This procedure incorporates the washout characteristics of the metabolism chamber and therefore increases the resolution of short-term metabolic events. The ‘effective volume’ of the metabolism system during these measurements was 5.0–5.4 l, depending upon the size of the snake being studied.

The metabolism of two freshly laid eggs was followed throughout development. Egg respirometers and an empty respirometer used as a control were sealed with gas-tight lids equipped with rubber stoppers. A tube was attached to one stopper so that gas samples could be withdrawn from the immediate vicinity of the egg. Gas samples (60 ml) were removed as the respirometers were sealed and again after intervals (varying from 4–24 h) calculated to produce a reduction in initial oxygen concentration of about 1%. After each sample, the respirometers were opened and room air was drawn through the chambers with a vacuum pump to preclude gas trapping by the mica substrate. Duplicate 25 ml dry, CO₂-free sub-samples were assayed for O₂ with a Beckman E-2 paramagnetic analyzer. The CO₂ content of other sub-samples was determined using a Beckman LB-2 analyzer.

The metabolism of a third egg was measured by open flow respirometry during hatching. Observations began 12 h prior to pipping (the appearance of slits in the egg shell) and concluded 12 h after the snake emerged from the egg. When no activity within the egg was apparent, metabolic data were collected for about 30 min at intervals of approximately 60 min.

Thermal conductance. Heating and cooling rates of females attending eggs were determined by equilibrating the females plus egg clutch in saturated air at constant T_a , and then rapidly heating or cooling the metabolism chamber to a new T_a . Body temperatures were measured by radiotelemetry. Air, substrate and intra-egg temperatures were measured by thermocouples. Simultaneous \dot{V}_{O_2} data were also obtained. Measurements were taken every minute, until T_a approximated T_b . Incubating females did not become active or change posture during these manipulations. Conductance was calculated from cooling rates and metabolic heat production, using a specific heat of 3.43 J/g and an assumed heat production of 20.1 J/ml O₂.

All gas volume data were corrected to STPD. Data are expressed as mean \pm standard deviation.

Results

Body mass and behavior of adults

Gravid *Python regius* weighed 1863 ± 123 g (range: 1744–2077 g; $n = 6$) in November, while non-grav-

id females and males weighed 1523 ± 136 g (range: 1427–1619 g; $n = 2$) and 1571 ± 146 g (range: 1408–1618 g; $n = 3$) respectively. Prior to oviposition, there was little difference in the behavior of gravid and non-gravid snakes. Both sexes were quiescent during the day, but considerable activity was observed at night. Non-gravid females and males ate sporadically throughout the period of observations. In contrast, all gravid and incubating females refused food until the eggs hatched in June. At this time all animals fed regularly.

In early April, prior to egg laying, the mass of gravid females had decreased to 1492 ± 149 g ($n = 4$). Oviposition occurred in mid-April and was observed in one instance, with eggs being deposited at ca. 40 min intervals. After egg deposition, mother snakes weighed 1085 ± 95 g ($n = 4$), or 28% less than non-gravid females. The mode clutch size was 6 eggs (range: 5–6; $n = 5$ clutches). Mean mass of the egg clutch shortly after oviposition was 485.0 ± 47.0 g ($n = 3$), or about 32% of the females’ pre-laying mass. Eggs were cream colored, ovoid, and smooth. Eggs weighed 85.5 ± 14.7 g ($n = 13$) and were 6.9 ± 0.3 cm through the longest axis and 4.2 ± 0.3 cm in diameter through the geometric center.

Behavior of incubating females

Females with eggs formed a turban-like coil around their clutches within an hour after oviposition (Fig. 1a). Their posture while coiled around the egg mass was unusual: the posterior third of the snake was rotated 90° so that the ribs and ventral scales projected laterally. The ventral body surface was concave and the eggs were partially accommodated within this cavity (Fig. 1b; see also Pitman 1974). In most cases (including periods when T_a exceeded 34 °C) the egg masses were completely covered by the females’ coils. Brooding females loosened their coils every 3–4 h, and appeared to inspect the eggs for 10–30 min before recoiling. Inspection activities consisted of nosing among the eggs accompanied by considerable tongue-flicking, but involved only the proximal third of the animal’s body and did not disrupt the arrangement of the eggs within the distal coils. Less frequently, a female would uncoil to the extent that the eggs were no longer in contact with her ventral surface. During uncoiling, movement by the female usually resulted in the repositioning of the eggs prior to the resumption of brooding posture. Occasionally an egg would become dislodged from the clutch during these maneuvers. A loose egg would be retrieved either by looping a coil over it or by

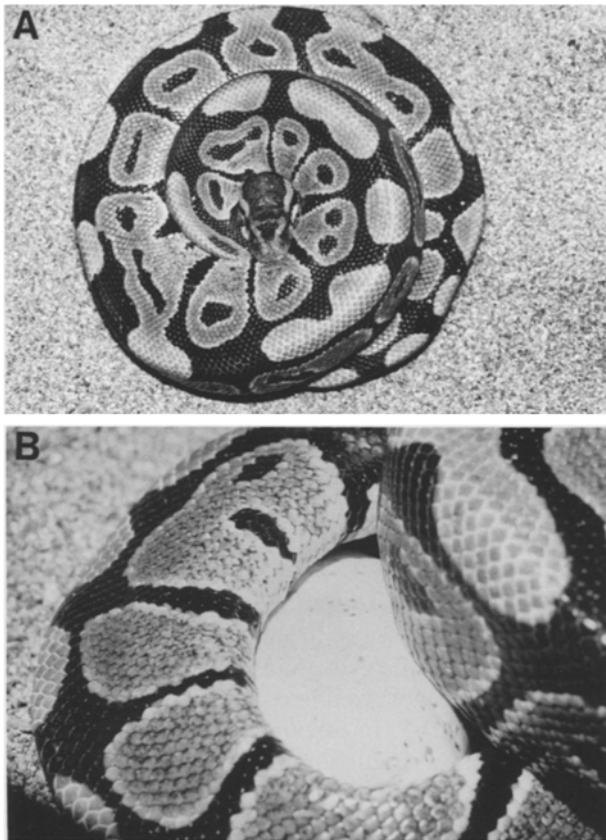


Fig. 1. **A** *Python regius* incubating a clutch of 5 eggs. The tight turban-like coiling posture was assumed by all incubating females. **B** Lifting the top coils reveals that the posterior third of the female is rotated and the ventral scales face laterally. Eggs fit within a cavity between the ribs

encircling the entire egg mass and recoiling. Females left their clutches unattended only to drink or shed. Excursions from the eggs were markedly less frequent at night.

Metabolism of adults

Regressions of resting metabolic rates (measured as \dot{V}_{O_2} and \dot{V}_{CO_2}) as a function of T_a were computed for snakes in 4 reproductive categories: males and non-gravid females, gravid females, incubating females, and post-incubating females (mother snakes after their eggs had hatched). Within these categories, significant differences between individuals were observed only once (one non-gravid female's \dot{V}_{O_2} was slightly but significantly lower than the \dot{V}_{O_2} 's of the 4 other animals in this group; ANCOVA). Exclusion of data from this individual did not affect the significance of between-group comparisons. Accordingly, within-group data were lumped for further analysis.

The \dot{V}_{O_2} and \dot{V}_{CO_2} of males and non-gravid females, and of post-incubating females, showed responses to changing ambient (and body) temperatures that are typical for ectothermic vertebrates (Fig. 2a). Both \dot{V}_{O_2} and \dot{V}_{CO_2} increased exponentially with temperature with a Q_{10} of approximately 2.2. The regressions of $\log \dot{V}_{O_2}$ against T_a for non-gravid pythons and for post-incubating females were both significantly different from zero, but there were no significant differences in slope between these groups ($P=0.358$; ANCOVA). The respiratory exchange ratio (R) varied between 0.7 and 0.85.

Gravid females also showed a normal response to changing temperature, with a Q_{10} of 2.1 over the temperature range tested. However, they were characterized by mass-specific \dot{V}_{O_2} 's 27–33% greater than their own post-incubation values (Fig. 2b), or the combined data from males and non-gravid females. The differences are highly significant ($P=0.0003$ and $P<0.0001$, respectively; ANCOVA). Throughout the gestation period (December until April), \dot{V}_{O_2} was elevated by an increment that remained approximately constant.

In striking contrast to data from non-incubating snakes, the \dot{V}_{O_2} of female snakes incubating eggs and in thermal equilibrium with the environment did not show any relationship to T_a (Fig. 2c). The regression of $\log \dot{V}_{O_2}$ against T_a for incubating snakes has a slope not significantly different from zero. There is considerable scatter in the data due to both inter- and intra-individual variation. Nevertheless, the regression slope for incubating females is significantly different from the slopes for non-incubating adults (Fig. 2d; ANCOVA). In general, \dot{V}_{O_2} was elevated above the levels observed in non-incubating females of similar mass. The difference was particularly large at low T_a 's, where \dot{V}_{O_2} of incubating snakes was often more than twice that of non-incubating animals. Nevertheless, egg temperatures greater than 0.2 °C above T_a were never recorded during steady state-conditions. At high T_a (31–35 °C), the \dot{V}_{O_2} of incubating females was occasionally considerably less than for non-incubating snakes. We found no evidence suggesting that \dot{V}_{O_2} of incubating females followed a daily rhythm.

Incubating females also contrast with non-incubating animals in their metabolic responses to sudden drops in T_a . A typical pattern from a non-incubating snake is shown in Fig. 3a. This animal had been maintained at 31 °C for several hours and its \dot{V}_{O_2} was stable. Rapid reduction of T_a to 27 °C elicited a very small and transient increase in \dot{V}_{O_2} (after several hours both T_b and \dot{V}_{O_2} had

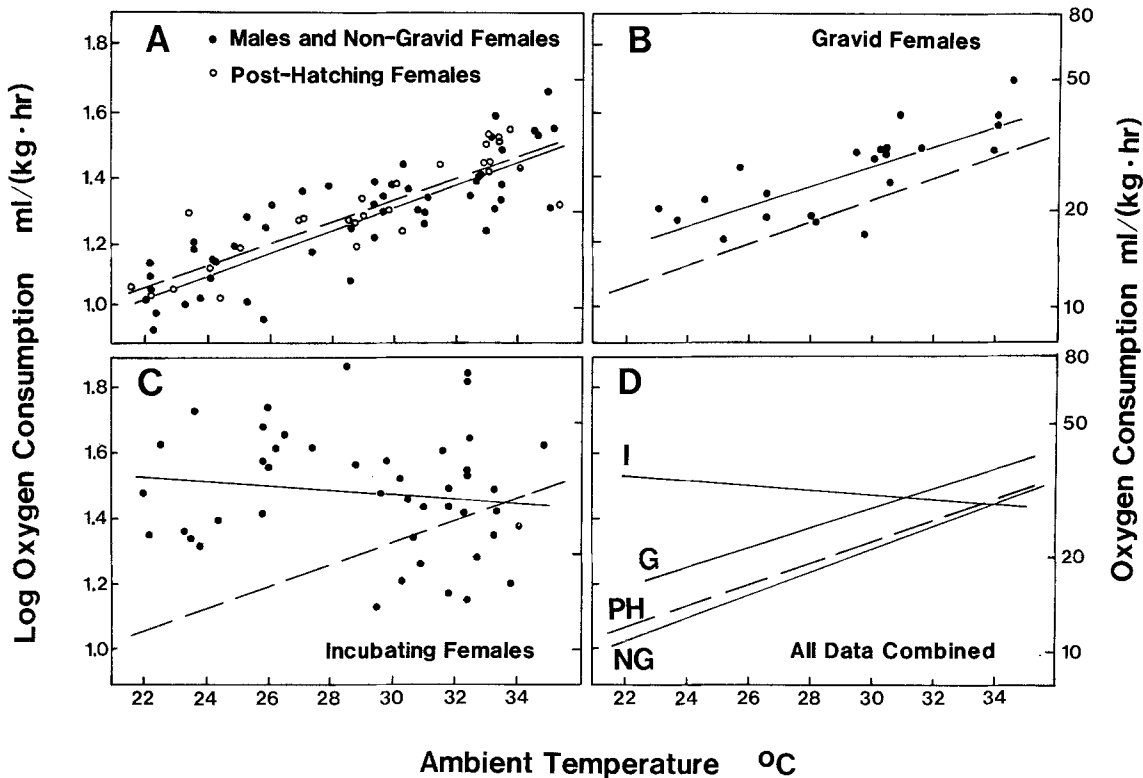


Fig. 2A–D. Oxygen consumption (\dot{V}_{O_2}) of adult ball pythons at different ambient temperatures (T_a). In all four plots, the dashed line is the regression for mother snakes after their eggs had hatched. **A** Non-gravid adults (solid regression line) and mother snakes after their eggs had hatched. The two regressions are not significantly different. **B** Gravid females (solid regression line). The slopes of the two regressions are not different but the intercepts differ significantly. **C** Incubating females (solid regression line). The slope for incubating females is not significantly different from zero. **D** Regression lines for non-gravid adults (NG), gravid females (G), incubating females (I), and mother snakes after their eggs had hatched (PH). The regression equations are:

$$\text{NG: } \log \dot{V}_{O_2} (\text{ml/kg}\cdot\text{h}) = 0.0350(T_a) + 0.2706; n = 53, r^2 = 0.690$$

$$\text{G: } \log \dot{V}_{O_2} (\text{ml/kg}\cdot\text{h}) = 0.0299(T_a) + 0.5461; n = 22, r^2 = 0.603$$

$$\text{I: } \log \dot{V}_{O_2} (\text{ml/kg}\cdot\text{h}) = -0.0063(T_a) + 1.6758; n = 53, r^2 = 0.017$$

$$\text{PH: } \log \dot{V}_{O_2} (\text{ml/kg}\cdot\text{h}) = 0.0332(T_a) + 0.3446; n = 27, r^2 = 0.766$$

declined). Similar small to nonexistent responses were characteristic of both males and non-gravid females. However, the \dot{V}_{O_2} of incubating females always increased markedly when T_a was lowered (Fig. 3b, 3c, 3d). The response occurred rapidly (ca. 3–6 min), even after small (ca. 0.4 °C) changes in T_a and before any decrease in T_b was apparent. In most cases \dot{V}_{O_2} increased by 100–300% within 10 min following a decrease in T_a , and began to decline about 40 min later. After several hours, \dot{V}_{O_2} remained elevated above the premanipulation level, even though T_b had decreased substantially. In no instance did we observe spasmodic muscular contractions or “shivering”, as reported for *P. molurus* and *M. spilotes*. No unusual metabolic responses occurred in any group when T_a was increased.

The $\dot{V}_{O_2 \text{ max}}$ of four adult pythons (1123 ± 159 g) at 30 °C was 257.4 ± 37.2 ml/kg·h ($n=4$) when averaged over 2 min and

219.6 ± 36.0 ml/kg·h ($n=4$) when averaged over 8 min. These values are approximately 11–12 times the resting \dot{V}_{O_2} of non-gravid animals at 30 °C. The \dot{V}_{O_2} and \dot{V}_{CO_2} remained elevated above normal resting levels for at least 40 min after exercise had ceased. However, pythons were able to locomote normally within 10–20 min after testing.

Thermal conductance

Adequate cooling curves ($n=4$) were obtained from three incubating females. The thermal conductance computed from these data showed considerable variation, possibly because of differences among the animals in the number of eggs and the arrangement of the coils. Values ranged from 0.43 to 1.02 W/[kg·°C], which is equivalent to 78 to 183 ml O₂/[kg·h·°C]. The mean conductance was 0.65 ± 0.11 W/[kg·°C].

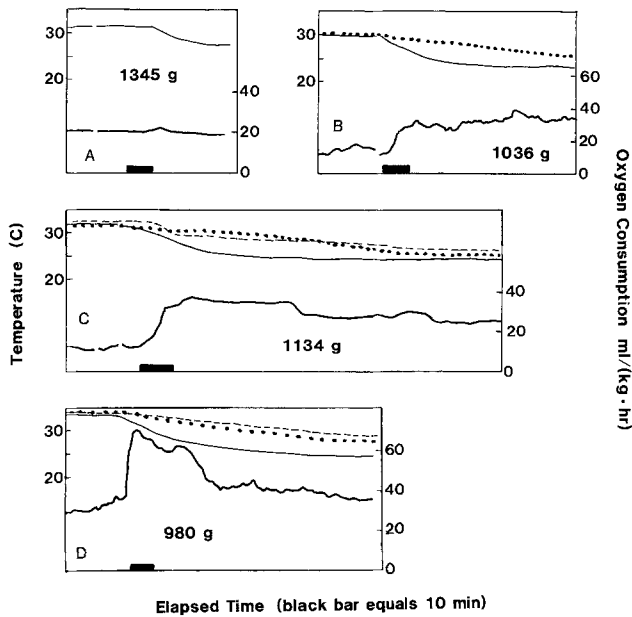


Fig. 3A–D. Responses to decreases in T_a . A Non-gravid adult, B–D incubating females. The black bars represent 10-min intervals. Heavy lower line is \dot{V}_{O_2} , thin upper line is T_a , dashed line is T_b (obtained from a telemeter in the digestive tract), and dotted line is T_{egg} (obtained from implanted thermocouples)

Egg metabolism and hatchling growth rates

Python regius eggs attended by the females pipped after 58–59 days ($n=7$), while those eggs isolated from the females required between 71–76 days ($n=8$) to pip at similar temperatures (29–30 °C). However, there was no differential mortality between the two methods of incubation.

A sigmoid relationship of \dot{V}_{O_2} and \dot{V}_{CO_2} over time was obtained from two developing *P. regius* eggs (Fig. 4). During the first 50 to 55 days of incubation at 29.2 °C, the increase in \dot{V}_{O_2} and \dot{V}_{CO_2} with time was approximately exponential. Both \dot{V}_{O_2} and \dot{V}_{CO_2} plateaued for 10 to 15 days prior to pipping. Transient increases in gas exchange were observed during the plateau phase of \dot{V}_{O_2} prior to pipping; these may have been associated with embryonic activity. However, plateau-phase fluctuations produced at most an 8% increase in \dot{V}_{O_2} and \dot{V}_{CO_2} .

Respiratory exchange ratios (R) throughout incubation were about 0.73, which is indicative of fat catabolism. The total \dot{V}_{O_2} during the 70-day incubation period was about 3.6 l per egg at $T_a = 29.2$ °C, representing about 72 kJ of energy metabolism. Assuming that all of this energy was derived from fat, each egg metabolized about 1.8 g of fat over the interval from oviposition to hatching.

Several eggs were opened shortly after pipping.

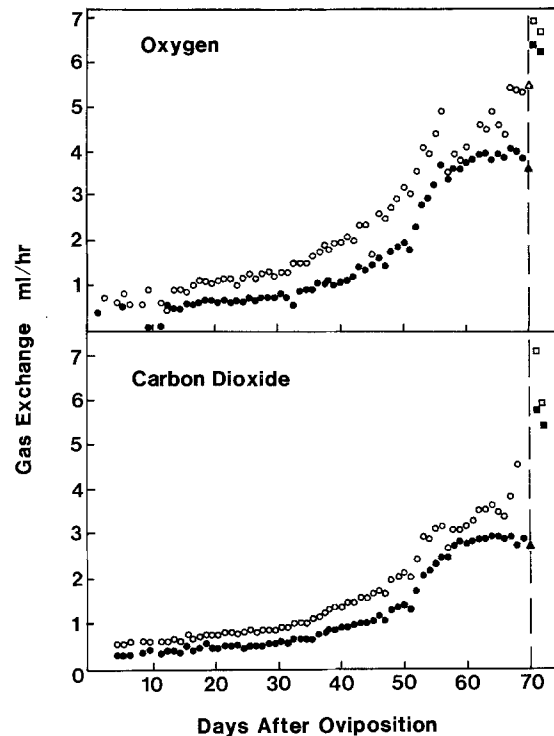


Fig. 4. Closed system \dot{V}_{O_2} and carbon dioxide production (\dot{V}_{CO_2}) during development in two eggs (open and closed symbols) maintained at 29.2 °C. Triangles indicate data gathered shortly after pipping (day 70). Squares represent data measured after emergence (day 71)

At this time the embryo was covered by fluids within the extra-embryonic membranes, which were engorged with extra-corporeal circulation (particularly those which adhered to the shell). No pulmonary breathing was evident. The \dot{V}_{O_2} and \dot{V}_{CO_2} at pipping were similar to the values measured during the last week of incubation. At 12 to 30 h after pipping, most of the fluids within the shell had evaporated, blood had coagulated within the extra-embryonic membranes, and pulmonary ventilation was obvious. The \dot{V}_{O_2} and \dot{V}_{CO_2} measured after the onset of lung breathing were about 40% greater than the rates present during pipping and correspond to the \dot{V}_{O_2} measured from emerged hatchlings. Table 1 gives metabolic data gathered in an open flow system from an individual egg and its hatchling.

Hatchlings emerged from the eggs 24–48 h after pipping. At this time, their mass was 50.2 ± 6.7 g ($n=15$) and they measured 39.6 ± 1.5 cm ($n=15$) from snout to vent and 43.0 ± 1.7 cm in total length. All but one of 15 young snakes refused food for 20–40 days after hatching and then began to feed avidly (the single exception fed on day 6). Six months after hatching, mass had increased

Table 1. Mass-specific \dot{V}_{O_2} , \dot{V}_{CO_2} , and respiratory exchange ratio (R) from an individual egg and its hatchling maintained at 29.1 °C. Gas exchange was measured using an open flow system. The hatchling weighed 59.5 g at emergence and 43.1 g at age 30 days. Values of \dot{V}_{O_2} and \dot{V}_{CO_2} (ml/[kg·h]) represent a series of measurements in each condition, and are given as the mean \pm standard deviation

Condition	\dot{V}_{O_2}	\dot{V}_{CO_2}	R
Pre-pipping	62.6 \pm 3.5	59.2 \pm 3.1	0.946
Pipping	69.4 \pm 4.1	61.2 \pm 3.5	0.882
Pipped, ventilating	91.2 \pm 6.2	87.5 \pm 2.5	0.959
Newly emerged	85.2 \pm 2.6	75.5 \pm 4.8	0.886
30 Days old, anorexic	61.9 \pm 2.6	47.2 \pm 4.0	0.763

4-fold to 207.4 \pm 29.8 g ($n=15$) while length increased less dramatically (snout-vent length: 57.2 \pm 2.3 cm, total length: 61.6 \pm 2.6 cm). We measured metabolism several times during the first year of growth. The \dot{V}_{O_2} of hatchlings declined steadily during the initial 20–40 day anorexic period (Table 1) and then stabilized when the animals began to feed. When combined with data from non-gravid, non-incubating adults, the \dot{V}_{O_2} data from snakes older than 40 days show a linear relationship between $\log \dot{V}_{O_2}$ and \log body mass. For data taken at $T_a=20$ °C and converted to the allometric form $y=aM^b$:

$$\text{ml O}_2/\text{h}=0.149 M^{0.575} \quad (1)$$

where: M =mass in g; $n=23$, mass range 43–1531 g; $r^2=0.83$; 95% confidence interval for $b=0.460$ – 0.691 . For $T_a=30$ °C:

$$\text{ml O}_2/\text{h}=0.264 M^{0.640} \quad (2)$$

where: $n=29$, mass range 43–1738 g; $r^2=0.92$; 95% confidence interval for $b=0.567$ – 0.712 .

Discussion

In many respects the thermal physiology and metabolism of ball pythons resembles that of other reptiles. Both adult and young ball pythons show regular increases of \dot{V}_{O_2} with increasing temperature, with Q_{10} values of 2.2–2.3. The \dot{V}_{O_2} increases as a function of mass^{0.575} at 20 °C and as mass^{0.640} at 30 °C (Eqs. 1 and 2). These values are not significantly different from each other or from 0.67 ($P > 0.2$, t -test). They are significantly different ($P < 0.003$; t -test) from the scaling to mass^{0.75} predicted by McMahon's (1973) model of elastic similarity, but are consistent with Heusner's (1982) prediction, based on dimensional analysis, of scaling to mass^{0.67}. Bennett and Dawson (1976) summarized the available data for sev-

eral families of snakes at 20 and 30 °C. Their equations indicate scaling to mass^{0.80}, which is significantly different from the b values we obtained. The Bennett-Dawson equations slightly underestimate the metabolism of a 40–50 g hatchling *P. regius*, but overestimate the metabolism of a 1 kg adult by about 2-fold.

Incubation periods and the overall rates of oxygen and carbon dioxide exchange for developing ball python eggs, isolated from the mother at oviposition and incubated separately, are similar to those reported for the eggs of other reptiles (Dmi'el 1970; Ackerman 1980). However, the ontogeny of gas exchange in *P. regius* eggs more closely resembles the sigmoid pattern reported during the embryological development of precocial birds (Vleck et al. 1980) and sea turtles (Ackerman 1980) than the exponential patterns reported for the eggs of other types of snakes (Dmi'el 1970). Presumably, embryonic growth proceeds most rapidly early in the incubation period and abates after days 50–55 (Fig. 4). Comparable data over the entire incubation period are lacking for other python eggs. However, Van Mierop and Barnard (1978) measured the \dot{V}_{O_2} of *P. molurus* eggs during the nine days that immediately preceded pipping; these eggs hatched synchronously with those in the maternally incubated clutch, but over this period no increase in \dot{V}_{O_2} occurred. These data suggest that a sigmoid pattern of metabolism with a plateau phase late in development may be the general case for python eggs. One striking (and unexplained) observation was the substantially shorter incubation period of *P. regius* eggs attended by female snakes (58–59 days) compared to that for unattended eggs (71–76 days).

Gravid ball pythons were behaviorally indistinguishable from other females, except that they did not eat. Nevertheless, they had rates of oxygen consumption that were disproportionately high when compared to non-gravid snakes, or to their own \dot{V}_{O_2} 's measured after their eggs had hatched (Fig. 2b). The \dot{V}_{O_2} appears to remain elevated over the entire five to six month course of egg production. During the ca. 150 day gestation period a fasting 1.85 kg gravid female resting quietly at 30 °C needs to metabolize about 91 g of fat. This represents roughly a 33% increase in fat utilization above that of a non-gravid snake of similar mass. The eggs themselves consist mainly of stored yolk and albumin and are presumably metabolically inactive throughout ovogenesis, but their mass contributes substantially to the total mass of the mother. This would be expected to depress estimates of maternal mass-specific \dot{V}_{O_2} , instead of ele-

vating it as we observed. The high \dot{V}_{O_2} of gravid ball pythons may reflect not only the maintenance metabolism of the mother but also the physiological events associated with egg production, including the conversion, mobilization and transfer of maternal resources into storage within nascent eggs.

The behavior of female ball pythons during incubation is quite similar to that seen in *Python molurus* and *Morelia spilotes*. All three species remain in nearly constant attendance on their clutches for two months or more, departing only when the hatchlings emerge. However, the metabolic responses to cold and the amount of thermogenesis in ball pythons are much less impressive than reported for the two larger species. Ball pythons were apparently unwilling or unable to produce enough metabolic heat to elevate body temperature more than a few tenths of a degree above ambient temperature. Several physiological, ecological, and evolutionary factors could account for the relatively poor performance of ball pythons, including small mass-specific \dot{V}_{O_2} or aerobic scope, high heat loss rates due to small size, inadequate energy stores due to small size, and lack of selection pressure for endothermic incubation in the natural habitat.

Our data suggest that ball pythons are capable of utilizing oxygen at mass-specific rates that compare favorably to those reported for incubating *P. molurus* and *M. spilotes*, at least for short periods. The comparison with *M. spilotes* is most germane, because that species is much closer in size to the ball python than is *P. molurus*. After adjusting for the metabolic contribution of the egg mass, the maximum \dot{V}_{O_2} observed during peak periods of thermogenesis was 1.47 ml/[kg·min] at 27 °C for a 3.8 kg *M. spilotes*, or about 22 times the resting level at that T_a (Harlow and Grigg 1984). When computed on the basis of body temperature (29.2 °C) instead of T_a , the factorial increase above resting \dot{V}_{O_2} was about 16. The maximum thermogenic \dot{V}_{O_2} of a 21 kg *P. molurus* was 2.82 ml/[kg·min], and the factorial scope (computed as described above) was 11.2 (Van Mierop and Barnard 1978). These values are similar to the factorial scope of 12 we observed in ball pythons exercised at $T_a = 30$ °C. Moreover, mass-specific \dot{V}_{O_2} max in ball pythons (3.5–4 ml/[kg·min]) was considerably greater than the mass-specific \dot{V}_{O_2} max in the other two species. From these data, and because the \dot{V}_{O_2} of incubating ball pythons never exceeded 25% of \dot{V}_{O_2} max, we conclude that the lack of significant thermogenesis in this species is not directly attributable to low aerobic scope. However, it should

be noted that \dot{V}_{O_2} max during short bouts of intense exercise may be higher than the \dot{V}_{O_2} max sustainable over long periods of thermogenic activity.

The production of temperature gradients between body and environment depends on thermal conductance as well as thermogenic capability. We computed conductances of *P. molurus* and *M. spilotes* from \dot{V}_{O_2} and steady-state $T_b - T_a$ gradients reported by Van Mierop and Barnard (1978) and Harlow and Grigg (1984). In this respect, ball pythons are at a severe disadvantage compared to *P. molurus* and *M. spilotes*. Because of their small size (1–1.2 kg), incubating ball pythons have relatively high rates of thermal conductance: about 0.65 W/[kg·°C], as compared to 0.27 W/[kg·°C] for the 3.8 kg *M. spilotes* and 0.114 W/[kg·°C] for the 21 kg *P. molurus*. Even assuming it could sustain its proportionally high \dot{V}_{O_2} max for long periods, the ball python can maintain a $T_b - T_a$ gradient of no more than 2.1 °C at T_a 's near 30 °C. Because of Q_{10} effects the maximal gradient would be even smaller at lower T_a , where thermogenesis would presumably be most important for embryonic development. At the maximum \dot{V}_{O_2} we observed during metabolic responses to sudden drops in T_a (Fig. 3), brooding ball pythons produced about 0.18 W/kg of metabolic heat, which is sufficient to support a $T_b - T_a$ gradient of only 0.28 °C. Thus, surface-volume relationships, rather than metabolic scope, may be the principal physiological factor limiting the evolution of endothermic brooding in pythons.

Energy and material storage are also potentially limiting to the evolution of endothermic brooding in ball pythons. If the animals were capable of sustaining a high enough \dot{V}_{O_2} to maintain a modest $T_b - T_a$ gradient of 3 °C, a typical 1.1 kg post-oviposition female would need to metabolize about 4.7 g of fat per day to support this level of thermogenesis. The fat requirement over a long period of thermogenesis would be prohibitive – approximately 280 g over a 60-day incubation cycle.

Based on the above analyses, we would not expect to find significant endothermic brooding ability in pythons smaller than 2.5–3 kg. Exceptions to this rule may occur in hole-nesting species, which could benefit from the increased insulation provided by the walls of a wooden cavity. This may be the case for *Chondropython viridis*, a tree-cavity nester which has been reported to shiver while attending eggs despite a mass of less than 1 kg (Kratzer 1962; Van Mierop et al. 1983). Basking behavior may also be used to augment metabolic heat production. Early-morning solar basking was employed by an incubating 3.8 kg *M. spi-*

lotes to rapidly raise T_b . The warmed snake then returned to her clutch and the absorbed heat was transferred to the eggs. This behavior reduced the animal's need to produce heat endothermically (Harlow and Grigg 1984). Similar basking behavior at artificial light sources has been reported for incubating *P. regius*, but these small snakes (and their clutches) cooled rapidly when the heat source was removed (Van Mierop and Besette 1981).

Even though brooding female ball pythons are unable to conserve body heat, they increase \dot{V}_{O_2} considerably and rapidly when challenged with a decrease in T_a (Fig. 3). We have no satisfactory explanation for this response. Similarly, we cannot adequately explain the apparent temperature independence of metabolic rate in incubating females (Fig. 2c). One possible interpretation is that high \dot{V}_{O_2} at low T_a or following a sudden decrease in T_a simply reflect increased agitation or discomfort of the females at temperatures that may be too low for optimal egg development. Long-term incubation at T_a below 27.5 °C prevents development in the eggs of *P. molurus* and *M. spilotes* (Vinegar 1973; Harlow and Grigg 1984); presumably the development of *P. regius* eggs also requires warm conditions. However, we observed few if any behavioral manifestations of agitation when incubating females were held at low T_a . Alternately, the brooding ball python's elevated \dot{V}_{O_2} at low T_a may be an evolutionary relic from larger ancestral forms which were capable of effective endothermic brooding. Adequate evidence for evaluating these hypotheses is currently unavailable.

An obvious question concerns the evolutionary persistence of incubation behavior in the ball python despite the absence of any thermal benefit. A clear answer to this question requires a thorough knowledge of natural history, ecology, and evolution, which is unfortunately lacking for ball pythons. Nevertheless, it is unclear why females should undertake a 50–70 day incubation cycle, which is probably dangerous and is certainly energetically costly because of the concomitant fast, unless they achieve some selective advantage. The eggs comprise nearly one-third (32%) of the female's pre-oviposition mass and clearly represent a large fraction of the female's energy and material stores. Similar relative clutch masses have been reported for *P. regius* (31.1%; Van Mierop and Besette 1981), *P. molurus* (24.7%; Van Mierop and Barnard 1978) and *M. spilotes* (30.5%; Harlow and Grigg 1984). For a large snake, the additional cost of incubation and thermogenesis is proportionally small and presumably helps safeguard the large investment in the clutch by providing a more

optimal thermal environment for development. Ball python incubation confers no thermal advantage, but may help protect the vulnerable and valuable clutch against attacks by egg predators (e.g., small mammals or birds). Furthermore, our data suggest that parental attendance shortens the incubation period by 15–20% (compared to unattended eggs kept at identical temperatures), which would also help reduce vulnerability to predation. In an environment with substantial predator pressure, the additional investment in protective parental care might greatly benefit the female's fitness by increasing the probability the clutch will survive until hatching.

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