

Reptilian endothermy: a field study of thermoregulation by brooding diamond pythons

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(With 4 figures in the text)

Miniature temperature-sensitive radiotransmitters were surgically implanted into free-ranging adult diamond pythons (*Morelia s. spilota*), which are medium-sized boid snakes of south-eastern Australia. Four female pythons oviposited during the study, and constructed incubation mounds. These apparently provided excellent insulation, and the snakes maintained high (approx. 31 °C) and relatively constant body temperatures throughout the two-month incubation period. They apparently maintained these temperatures primarily by endogenous heat production (shivering thermogenesis), but also basked briefly on most mornings.

Brooding females maintained a body temperature differential above ambient of about 9 °C, occasionally up to 13 °C; their temperatures were significantly higher and less variable than those of non-brooding females or males. The energetic cost of brooding must be high, but these costs may be outweighed by the benefits of rapid embryonic development and high embryonic survivorship.

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Introduction

The thermoregulatory systems of animals are conventionally divided into two categories: ectothermy (reliance upon heat from the environment) and endothermy (metabolic production of heat). This division also reflects phylogeny: the 'lower' animals are ectotherms, and endothermy is seen only in the 'higher vertebrates' (birds and mammals). Like most dichotomies, this concept is overly simplistic. Facultative endothermy (heterothermy) occurs in a diverse array of species, usually as a result of sustained muscular activity during locomotion (e.g. insects- Heinrich, 1974; Bartholomew & Casey, 1976; fishes- Carey & Teal, 1966; Emery, 1986; turtles- Frair, Ackerman & Mrosovsky, 1972; Heath & McGinnis, 1980). More rarely, muscular contraction independent of locomotion is used specifically for heat production, as in honeybees warming the hive (Heinrich, 1974). The most remarkable example of sustained endothermy in an 'ectotherm' is shivering

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thermogenesis by pythonine snakes. Captive females of several python species have been reported to coil around their eggs and maintain relatively high and constant body temperatures (and thus, egg temperatures) through rhythmic contractions ('shivering') of the body musculature (e.g. Hutchison, Dowling & Vinegar, 1966; Vinegar, Hutchison & Dowling, 1970; Harlow & Grigg, 1984; reviewed by Shine, 1988). Despite many studies of this phenomenon in the laboratory, there are no field data to indicate whether or not shivering thermogenesis: (i) is used regularly by brooding pythons in the wild; (ii) is effective in maintaining high and stable body temperatures, despite fluctuations in ambient temperature; and (iii) is combined with behavioural thermoregulation to maintain 'preferred' thermal levels, as has been suggested by laboratory studies. The present paper provides such data for the eastern Australian diamond python (*Morelia s. spilota*), a species which has been shown to use shivering thermogenesis during brooding in the laboratory (Harlow & Grigg, 1984). Our study demonstrates for the first time that some reptiles are able consistently to maintain high and constant body temperatures by means of endogenous heat production under natural conditions. The result is of particular interest in that diamond pythons are found further from the equator (and hence in cooler, more variable climates) than are any other python species.

Materials and methods

Adult diamond pythons (female snout-vent lengths 182 to 230 cm) were monitored by radiotelemetry in 2 small valleys near Sydney, New South Wales. Details of methods, and results on general ecology and thermal biology of these snakes are provided elsewhere (Slip, 1986; Slip & Shine, 1988*a, b*). Miniature temperature-sensitive transmitters (J. Stuart Inc., 152 mHz, TT-1U-1000, with a life of 10 to 13 months, reception range approx. 400 m) were surgically implanted in the peritoneal cavity under halothane anaesthesia. Transmitters measured 90 × 20 × 20 mm, weighed < 4% of the snake's mass, and apparently did not significantly affect movements, growth, feeding or reproduction of the radio-tracked pythons (Slip, 1986). Transmitters were calibrated ($\pm 0.1^\circ\text{C}$) at a range of known temperatures before implantation and after removal from the snakes.

Four telemetered females oviposited during the course of this study, all in late December or early January. Ambient temperature was measured with a transmitter on the shaded substratum next to the incubation site. Ambient temperatures and body temperatures of incubating snakes were monitored at half-hourly intervals using a telemetry receiver (Telonics Inc., TR-2) and recorded on a Rustrak chart recorder. Each snake was monitored for at least 13 24-hour periods throughout the incubation period. Body temperatures of male snakes and non-brooding female snakes were recorded in the same manner at the equivalent time of year (difficulties with simultaneous reception of signals from different snakes made it impossible to record males, non-brooding females and brooding females over exactly the same days). During the periods of continuous recording, snakes were observed at least every 2 days. Movements were monitored by direct observation and by interpretation of signal amplitude from chart recordings.

Body temperatures were classified according to extent of cloud cover at the time records were taken, and total mean body temperatures were calculated from all temperature records for brooding females, males and non-brooding females on clear or cloudy days. Means were compared among classes of snake using the Games & Howell method for unplanned comparisons among means when variances are heterogeneous (Sokal & Rohlf, 1981). The variances associated with total mean body temperature were compared using Bartlett's test (Zar, 1974). Clear days and

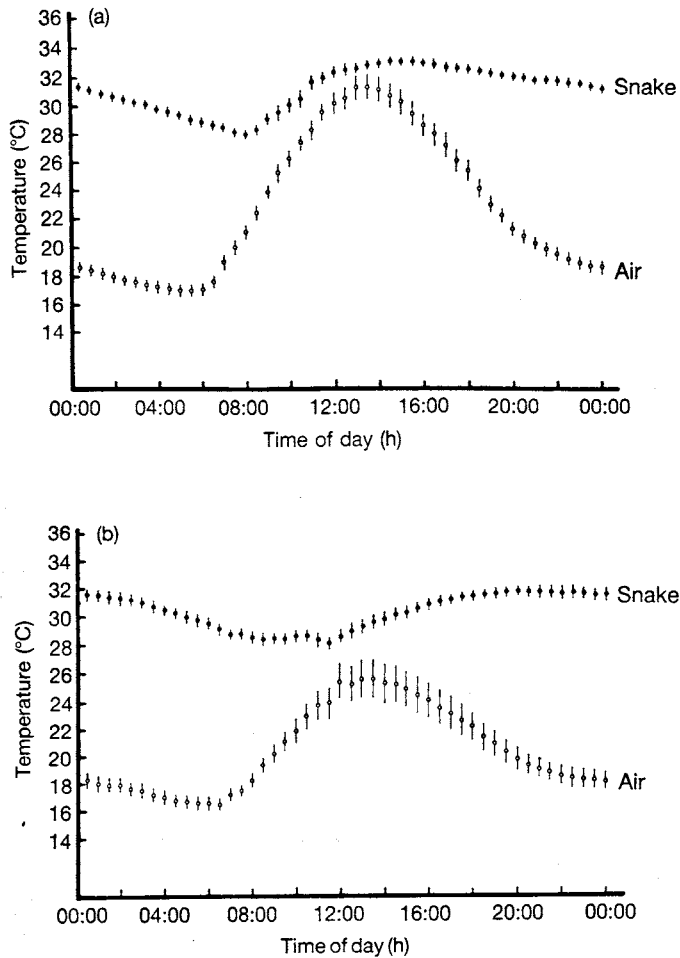


FIG. 1. Diurnal fluctuations in mean body temperatures and associated air temperatures for brooding female diamond pythons on clear days (a) and cloudy days (b). For each half-hourly mean N is between 29 and 32; vertical bars represent 95% confidence limits. Data combined from four snakes.

$d.f. = 3, P < 0.001$). Body temperatures of brooding females were less variable than those of males and non-brooding females on both clear and cloudy days (see Table I).

The pattern of diurnal fluctuations in body temperature of brooding females on clear days consisted of a steep rise between 08:00 h and 12:00 h, coinciding with a period of basking. Body temperatures then slowly declined until just after midnight, then fell slightly faster, reaching a minimum at around 08:00 h (Fig. 1). On cloudy days, no basking occurred but body temperature still rose. This rise was more gradual than on clear days and commenced later, around 11:00 h. There was a relatively stable period until just after midnight when body temperatures began to fall (Fig. 1). Body temperatures were maintained well above air temperature all day. Diurnal fluctuations in mean body temperature of brooding females on clear and cloudy days combined are presented in Fig. 2. Body temperatures were maintained above ambient, and generally were

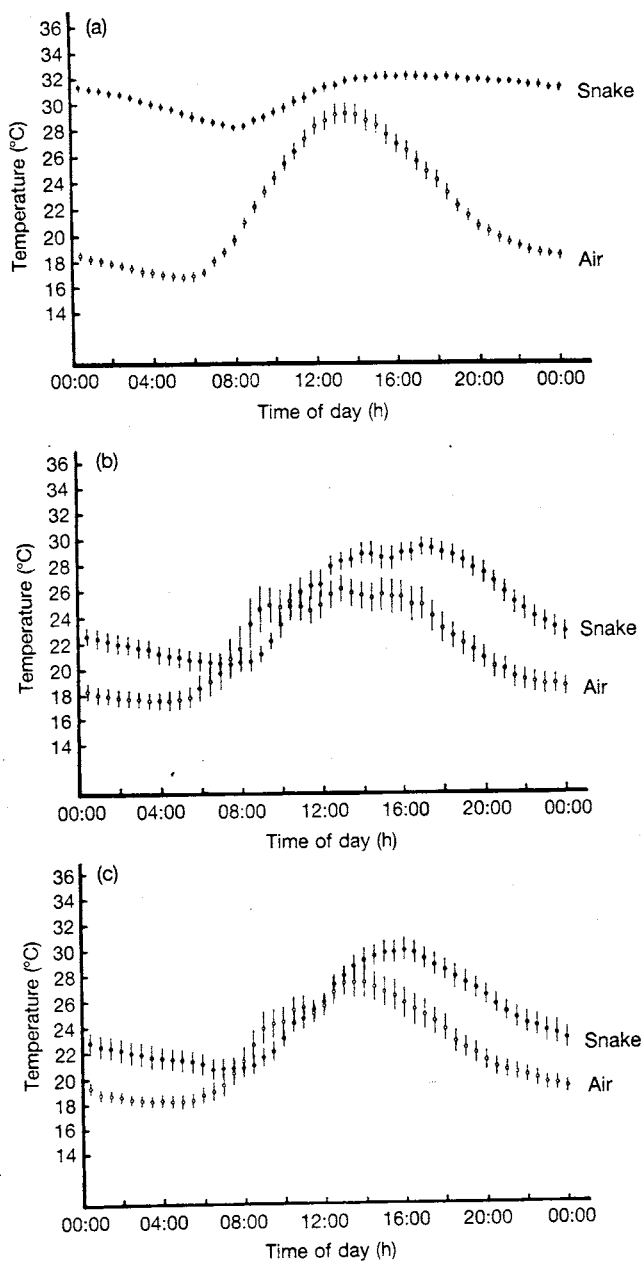


FIG. 2. Diurnal fluctuations in mean body temperatures and associated air temperatures for diamond pythons, combined for both clear and cloudy days. Vertical bars represent 95% confidence limits around each half-hourly mean. Data combined from four brooding females (a), three non-brooding females (b), and three males (c).

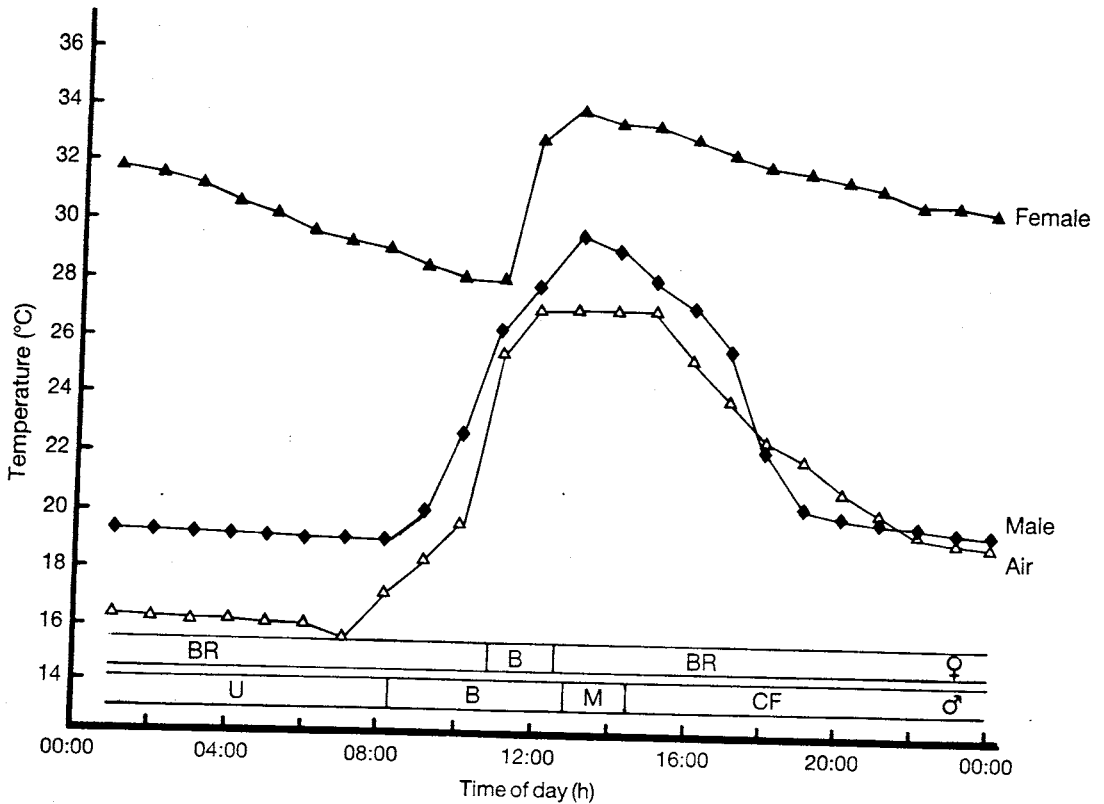


FIG. 3. Continuous record of body temperature of a brooding female diamond python (▲), a male diamond python (◆) and air temperature (△), recorded simultaneously on December 28, 1982. BR = brooding; B = basking; U = under cover; M = moving; CF = coiled under filtered cover.

within the range 28 to 33 °C. In contrast, mean body temperatures for non-brooding snakes fluctuated between 20 and 31 °C, falling to within a few degrees of air temperature by morning (Fig. 2). The ability of a brooding snake to maintain its body temperature well above that of a non-brooding snake and above the temperature of the surrounding air is demonstrated in Fig. 3. The body temperatures of a brooding snake and a male snake were monitored simultaneously. Although the male sought cover in a hollow log after 19:00 h, its body temperature was not maintained above air temperature.

To examine variability in mean body temperatures, temperatures were classified into four-hourly periods. The variances associated with mean body temperatures of brooding females were heterogeneous with time of day, both on clear days ($\chi^2 = 486.29$, $d.f. = 5$, $P < 0.001$) and cloudy days ($\chi^2 = 38.99$, $d.f. = 5$, $P < 0.001$). Body temperatures were most variable between 08:00 h and 12:00 h on clear days, and between 12:00 h and 16:00 h on cloudy days. Body temperatures were least variable between 16:00 h and 20:00 h (Table II).

The mean differentials of body temperature over air temperature were 8.9 ± 5.42 (S.D.) °C for brooding females (range -9.4 to $+18.0$, $n = 2492$), 3.1 ± 4.29 °C for non-brooding females (-14.2 to $+13.7$, $n = 701$), and 2.6 ± 3.89 °C for males (-12.5 to $+11.6$, $n = 1025$). The mean temperature

TABLE II

Diurnal fluctuations in mean body temperature (°C) and associated variance, pooled over four-hour intervals, of brooding and non-brooding snakes on clear and cloudy days

Time (h)	Males			Non-brooding females			Brooding females		
	N	Mean	Variance	N	Mean	Variance	N	Mean	Variance
<i>Clear days</i>									
00:00-04:00	139	22.9	21.34	83	22.9	7.26	248	30.8	1.35
04:00-08:00	121	21.8	23.17	88	21.3	9.65	224	28.9	5.14
08:00-12:00	113	23.3	18.87	89	23.4	18.22	232	30.0	7.56
12:00-16:00	103	30.0	8.05	86	28.3	11.43	272	33.0	1.70
16:00-20:00	121	29.6	12.49	96	29.3	5.83	261	32.8	0.76
20:00-00:00	132	25.3	17.86	87	25.7	10.29	250	31.8	0.90
<i>Cloudy days</i>									
00:00-04:00	45	20.5	10.09	29	20.1	3.04	160	31.3	3.17
04:00-08:00	67	20.0	6.47	31	19.1	0.37	184	29.6	3.68
08:00-12:00	59	21.8	6.61	29	22.3	12.20	181	28.5	3.69
12:00-16:00	42	24.9	6.33	29	27.8	12.31	160	29.7	4.08
16:00-20:00	40	25.3	14.76	26	26.3	10.22	160	31.5	1.79
20:00-00:00	43	22.5	9.45	28	22.8	11.06	160	31.9	2.25

N refers to the number of temperature records

differential of brooding females was significantly higher than that of males ($MSD = 0.54$) or other females ($MSD = 0.54$). The temperature differential for brooding females was largest at night, falling as air temperature increased over the day. Non-brooding snakes had a relatively constant temperature differential throughout the day. The highest temperature differential recorded for brooding females was 18.0°C when air temperature was 14.5°C at 01:30 h, while the highest differential for non-brooding snakes was 13.7°C when air temperature was 15.5°C at 20:00 h.

Regression analysis was carried out to examine the relationship between air temperature and body temperature for brooding females, other females and males on clear and cloudy days (Fig. 4). The slopes of each regression were significantly different from zero (see Fig. 4). A comparison of the slopes using covariance analysis revealed them to be significantly different ($F_{5,4206} = 701.53$, $P < 0.001$). A Newman-Keuls test revealed significant differences among the slopes of all regressions except those for males on clear and cloudy days. The elevations of the regressions for males on clear and cloudy days were significantly different ($t_{1021} = 6.88$, $P < 0.001$).

On clear days, brooding females were observed to leave the clutch and bask for 20 to 90 minutes. It was difficult to observe this accurately without disturbing the snakes, but analysis of continuous records of signal amplitude indicated that most basking bouts (29 of 35) lasted between 30 and 60 minutes. The time at which snakes emerged from the nesting mound varied among individual females (Table III). Snakes #2 and #3 generally emerged earlier than snakes #1 and #4. Snake #3 had a significantly lower body temperature at emergence than did the other three snakes ($F_{3,31} = 7.826$, $P < 0.01$), but there were no significant differences in mean body temperature on returning to the mound among the four snakes ($F_{3,31} = 0.620$, $P > 0.05$, see Table III).

Mean body temperatures for individual snakes calculated over the total recording period were significantly different ($F_{3,2488} = 86.21$, $P < 0.001$). A Newman-Keuls test revealed that mean body temperatures of females #1 ($30.4 \pm 1.75^{\circ}\text{C}$, range 27.2 to 35.1 , $n = 625$) and #3 ($30.2 \pm 2.72^{\circ}\text{C}$,

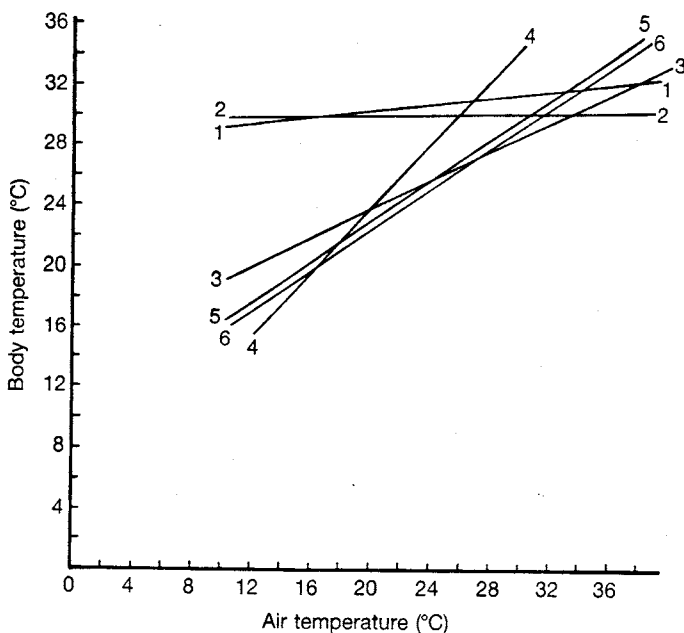


FIG. 4. The relationship between body temperature and air temperature for diamond pythons during the incubation period. Data are for brooding females on clear days (1) and cloudy days (2); non-brooding females, clear days (3) and cloudy days (4); males, clear days (5) and cloudy days (6).

TABLE III

Summary of the time when individual brooding female diamond pythons emerged from the nesting mound to bask, and their mean body temperatures (\pm S.D.) when emerging to bask and when returning following basking

Snake	N	Time of day when python emerged			Mean T_b at Emergence ($^{\circ}$ C)	Mean T_b on Returning ($^{\circ}$ C)
		Median (h)	Mode (h)	Range (h)		
#1	5	12:00	11:30	11:30-13:30	28.3 ± 0.83	34.2 ± 0.98
#2	13	10:00	10:00	09:30-13:30	28.6 ± 1.12	34.4 ± 0.69
#3	12	09:30	09:30	08:30-12:30	26.5 ± 1.49	34.0 ± 0.42
#4	5	12:00	12:00	10:30-13:30	28.5 ± 0.34	33.7 ± 0.49

N = number of observations

range 23.7 to 35.7, $n = 610$) were significantly lower than those of females #2 (31.6 ± 2.02 $^{\circ}$ C, range 27.4 to 35.2, $n = 635$) and #4 (31.5 ± 1.75 $^{\circ}$ C, range 27.3 to 34.7, $n = 622$). The two snakes with the lower mean temperatures also had longer incubation periods: 78 to 79 days for female #3, 57 to 59 days for female #1, compared to 53 to 57 days for female #4 and 47 to 50 days for female #2.

It is difficult to observe the phenomenon of shivering in the field, because of the sheltered nature of the nest site and the sensitivity of the female to disturbance: if the mound is adjusted to provide a better view, the snake will immediately cease shivering. Only one of the four sites (#3) provided the

opportunity for observations of shivering without disturbing the snake. Shivering was also observed in female #2, but could only be seen by modifying the surrounding vegetation. Shivering was observed most often in the late afternoon (07:00 to 16:00 h, shivering seen on one of 16 occasions; 16:00 to 19:00 h, shivering seen on five of seven occasions). The single record of shivering in the morning was at 09:30 h when the body temperature of the snake was 24.7 °C and air temperature was 23.0 °C. This was an overcast day as was the previous day and the snake had not been able to bask.

The temperature within a vacated nesting mound, measured less than two days after the eggs had hatched and the female departed, fluctuated between 20.5 and 24 °C over a 24-hour period when ambient air temperature ranged from 19 to 38 °C. Temperature within the mound rose slowly after 10:00 h and fell slowly after 18:00 h, remaining one to two degrees above the outside air temperature throughout the night.

Discussion

The behaviour and body temperatures of the brooding pythons which we studied were very similar to those reported in a previous study of one captive female of this species (Harlow & Grigg, 1984), and to limited data on *M. s. variegata* brooding in the wild (Charles, Field & Shine, 1985). Harlow & Grigg (1984) found that their brooding female, when given the opportunity, would leave the eggs in the morning to bask in order to raise body temperature to around 33 °C, before returning to the eggs and maintaining body temperature between 32 and 33 °C for 8 to 12 hours each day. When the snake was given no opportunity to bask, body temperature rose and was maintained between 32 and 33 °C through shivering. Harlow & Grigg found that shivering rate fell soon after midnight, ceasing completely around dawn, and body temperature fell slowly but always remained above ambient temperature which was between 26 and 28 °C. Harlow & Grigg suggested that, under natural conditions, choice of a well-insulated nesting site could enable snakes to maintain high and stable temperatures. Our data confirm this prediction: although ambient temperatures in the field were much lower than those experienced by Harlow & Grigg's brooding female in the laboratory (minima of 13 °C vs. 26 °C); the well-insulated nature of the nest sites enabled the wild brooding snakes to maintain remarkably high and stable temperatures. The present study found that mean body temperatures of brooding females remained above 28 °C throughout the day, even on overcast days (Table II). The profile of mean body temperatures for clear days (Fig. 1a) is very similar to the daily pattern of body temperature reported by Harlow & Grigg when their snake was permitted access to basking, and the profile for overcast days (Fig. 1b) is similar to the daily pattern of body temperatures when they denied the snake access to basking.

The mean temperature differential of 8.9 °C above ambient was higher than the maximum differentials reported in laboratory studies of other pythons, presumably because the ambient temperatures to which our snakes were exposed were much lower. Harlow & Grigg (1984) recorded a maximum differential of 6.9 °C for *M. s. spilota* when the substratum temperature was 25.5 °C. Vinegar *et al.* (1970) recorded differentials of up to 5 °C for *P. molurus* and a maximum differential of 8.3 °C was reported by Van Mierop & Barnard (1978) for *P. molurus* when ambient temperature was 23 °C.

The body temperatures of brooding *M. s. spilota* on cloudy days suggest an impressive capacity of female pythons to maintain high body temperatures by endogenous heat production. On cloudy days, females remain coiled about the eggs and, hence, have no opportunity to raise body temperature through basking. None the less, mean body temperature increased throughout the

afternoon, and between 20:00 h and 24:00 h reached a mean body temperature no different from that reached after a clear day (compare Fig. 1a and 1b). There are three possible sources of heat: the decay of vegetation in the mound, embryonic heat production and endogenous heat production by the female. Heat of decomposition is a significant factor in the regulation of nest temperatures of some crocodylians (Webb, Sack, Buckworth & Manolis, 1983), although not in others (Magnusson, Lima & Sampario, 1985). Although diamond pythons may gain some heat from nest decomposition, the temperature of a vacant mound increased by only about 2 °C over the day, suggesting that decomposition contributes only a small proportion of the incubation heat. Embryonic heat production may be an important source of nest heat in some crocodiles (Webb *et al.*, 1983) and turtles (Bustard, 1972), although Magnusson *et al.* (1985) found that developing embryos of the crocodylian *Paleosuchus trigonatus* cannot maintain nest temperatures much above ambient. If embryonic heat is an important source of heat in the nests of *M. s. spilota*, it would contribute more heat towards the end of the incubation period as the relative mass of embryos increased. As increases in temperature occur without basking from early in the incubation period, it seems that endogenous heat production by the female is the most important source of heat. Based on our observations of shivering by brooding females in the wild, and the detailed laboratory work of Harlow & Grigg (1984), it seems very likely that muscular contraction (shivering thermogenesis) was the form of endogenous heat production used to elevate python temperatures.

On clear days during incubation, body temperatures were least variable between 16:00 h and 24:00 h which corresponds to the time of maximum shivering rate found by Harlow & Grigg (1984). Body temperatures were most variable between 08:00 h and 12:00 h, which corresponds to the time when most basking took place (Table II). On cloudy days, body temperatures were most variable between 12:00 h and 16:00 h, which probably reflects the time when shivering began. Between 16:00 h and 20:00 h, body temperatures became least variable (Table II), which may correspond to the period of maximum shivering rate on cloudy days.

When body temperature is plotted against ambient temperature, a regression line with a slope of zero indicates perfect homeothermy, while a slope of one indicates total passivity, with a range of thermoregulatory strategies available between these two extremes (Huey & Slatkin, 1976). Brooding females have a regression slope very close to zero on both clear and cloudy days, while non-brooding snakes have a slope much closer to one (Fig. 4), thus indicating that non-brooding snakes show a higher degree of thermoconformity than brooding snakes.

Producing and incubating the eggs is costly to the female, with up to 48% of pre-oviposition body mass lost by the time hatching occurs (see Slip & Shine, 1988a). The decrease in maternal mass due to egg-laying (30%: Slip & Shine, 1988a) is similar to results from laboratory studies (references below). The mass loss attributable to incubation (15%), however, was much greater than that found by Harlow & Grigg (1984) for *M. s. spilota*, by Van Mierop & Barnard (1978) for *P. molurus* and by Van Mierop & Bessette (1981) for *P. regius* (4 to 7%). The difference is probably due to the lower ambient temperatures experienced in the wild, requiring brooding animals in the field to shiver more often or at a higher rate, thus expending more energy. Brooding females in these laboratory studies refused food and did not produce urine or faeces during the period of incubation. Similarly, examination of the area surrounding each of the oviposition sites in the present study revealed no faecal material or the very obvious uric acid deposits associated with urination.

The opportunistic use of basking seems an obvious way to reduce the metabolic costs of incubation. These costs are high: oxygen consumption at maximum shivering represents a 22-fold

increase over the resting rate (Harlow & Grigg, 1984). Basking during brooding has been reported in *M. s. spilota* both in the wild (Cogger & Holmes, 1960) and in captivity (Harlow & Grigg, 1984), and in wild brooding *M. s. variegata* (Charles *et al.*, 1985) and captive *M. amethystinus* and *L. fuscus* (Boos, 1979). Captive brooding *P. regius* also leave the eggs for short periods to bask (Van Mierop & Bessette, 1981), but *P. molurus* may not do so (Hutchison *et al.*, 1966; Van Mierop & Barnard, 1976, 1978; Van Mierop & Bessette, 1981).

Shivering has been recorded in all genera of Australian pythons and it is likely that all species have the capacity to shiver. However, the extent to which shivering is utilized under natural conditions may vary, as tropical species may not encounter ambient temperatures low enough to require regular shivering. Shivering may be used as a fail-safe mechanism and be initiated only when ambient temperatures drop to critically low levels. Thus, the costs of incubation in terms of body weight loss may be lower in tropical species. This may allow more energy to be devoted to egg production, or enable females to reproduce every year in tropical species.

Given the high metabolic costs, what are the advantages of brooding as a reproductive strategy? There is evidence that development time of embryos is increased at low temperatures in *P. molurus* (Vinegar, 1973) and *P. sebae* (Branch & Patterson, 1975). Limited data on *M. s. spilota* suggest that hatching success is reduced at incubation temperatures below 30 °C, and that the optimum temperature for development is around 33 °C (Harlow & Grigg, 1984). Hence, this species may reproduce successfully in temperate climates only because of the favourable thermal environment in the nest created by maternal endogenous heat production (Harlow & Grigg, 1984). The female may face a trade-off between maximizing embryonic developmental rate and minimizing her own weight loss due to metabolic expenditure. By reducing the incubation period, the female would have more time to increase body mass before the next mating season. This would be of particular importance to a temperate-zone species, such as *M. s. spilota*, which may be constrained by temperature to a short feeding season. The hatchlings would also have a longer growth period before the following winter which may improve their chances of avoiding predation and ultimately allow them to reach maturity earlier.

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