Ecology, 78(6), 1997, pp. 1713–1721

THE INFLUENCE OF NEST TEMPERATURES AND MATERNAL BROODING ON HATCHLING PHENOTYPES IN WATER PYTHONS

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Abstract. Previous work on phenotypic plasticity in hatchling reptiles has dealt almost exclusively with lizards and turtles from temperate zone habitats, in taxa where the only maternal control over incubation regimes is exerted via nest site selection. In contrast, water pythons (*Liasis fuscus*) in northern Australia are tropical snakes that show facultative maternal brooding, with shivering thermogenesis to warm the clutch. Thus, incubation temperatures of this species are influenced both by nest site selection and by maternal care. We experimentally simulated three thermal regimes typical of different types of natural nests in our study population. These were (1) hot, stable temperatures typical of nests laid in the burrows of varanid lizards (constant 32°C); (2) lower and more variable temperatures typical of nests laid inside tree root boles, either with maternal attendance (diel range 27.1°–32.9°C); or (3) in root boles but without maternal attendance (24.3°–32.9°C). We incubated 187 eggs from 15 clutches obtained from field-caught gravid pythons, using a split-clutch design to quantify influences on morphology (offspring size and shape), locomotor performance (swimming ability), and behavior (escape tactics, propensity to strike, willingness to feed in captivity). The thermal regime during incubation strongly affected incubation periods, body sizes, body shapes (mass and tail length relative to snout–vent length), initial growth rates, escape behavior, and willingness to feed. We also detected strong maternal effects on all of these traits, and interactions between maternal effects and incubation regimes. Thus, a female python's "decisions" as to where she lays her eggs and whether she remains and broods them through development have major consequences for the phenotypes of her offspring.

Key words: endothermy; hatchlings; nest site; parental care; phenotypic plasticity; python; Liasis fuscus; *thermogenesis; tropics.*

INTRODUCTION

A central assumption of many Darwinian analyses is the notion that phenotypic variation has an underlying genetic basis, and hence that natural selection on phenotypes has the potential to affect allelic frequencies. Under this view, phenotypic plasticity (i.e., modification of the phenotype by direct environmental influences, with no genetic underpinnings) may exist, but is so unimportant that it may be ignored. This view has come under increasing challenge in recent years, through a growing appreciation of the ubiquity and magnitude of phenotypically plastic responses (e.g., Via et al. 1995). For example, experimental studies on a wide range of reptilian species have shown that the physical conditions experienced by an embryo can substantially affect the size, shape, behavior, and performance of the resultant hatchling (e.g., Lang 1985, Miller et al. 1987, Webb and Cooper-Preston 1989). Hence, a high proportion of the phenotypic variance in hatchling reptiles may have no genetic basis (Vleck 1988). This result may have important implications for the ways in which natural selection can act on the population, and for the linkage of other traits (such as sex determination) to incubation conditions (e.g., Janzen and Paukstis 1991, Rhen and Lang 1995, Shine et al. 1995).

To date, almost all of the studies in this field have relied on constant temperature incubation in the laboratory, rather than on realistic simulations of thermal regimes in natural nests. The biological relevance of this approach is thrown into question by the recent discovery that thermal fluctuations can influence hatchling phenotypes and developmental rates, independent of mean incubation temperature (Shine and Harlow 1996). Also, previous work with reptiles has concentrated on turtles (e.g., Miller et al. 1987, De Souza and Vogt 1994) and lizards (e.g., Beuchat 1988, Van Damme et al. 1992), and has dealt almost exclusively with species from temperate zone habitats. Very little has been published on tropical reptiles, despite the fact that the majority of reptilian species are exclusively tropical. The only quantitative experimental work on thermal influences on hatchling phenotypes in snakes is based on a few temperate zone North American taxa, and relies on constant temperature incubation (Burger et al. 1987, Gutzke and Packard 1987, Burger and Zappalorti 1988, Burger 1989, 1990, 1991). We set out to examine phenotypically plastic responses of snake embryos in more detail, using fluctuating thermal regimes

Manuscript received 29 May 1996; revised 25 November 1996; accepted 6 December 1996.

FIG. 1. Water python (*Liasis fuscus*) on a paperbark root bole. These boles contain numerous small holes, some of which are used as oviposition sites by the pythons.

that closely mimic temperatures that we have measured in natural nests.

Our study took advantage of an unusual system revealed by longterm ecological studies on water pythons (*Liasis fuscus*) in tropical Australia. Most of the reproducing female pythons in our study population nest either in the burrows of varanid lizards (in which case, the nests experience high, relatively constant temperatures) or inside the root boles of paperbark trees (where the eggs are exposed to cooler, more variable temperatures: T. R. L. Madsen and R. Shine, *unpublished manuscript*; also see Fig. 1). Females using the hot varanid burrow nests deserted their eggs soon after laying (mean = 6.5 d), whereas cooler-nesting females in root boles attended their eggs throughout incubation (mean = 58.4 d: Shine and Madsen 1996; T. R. L. Madsen and R. Shine, *unpublished manuscript*). The cooler-nesting females warmed themselves each day by diurnal basking (generally for <60 min), and much of this heat was then transferred to the clutch when the female returned and coiled around it. Because we could not see the brooding snakes, we could not determine whether or not they also heated the clutch by muscular contraction (shivering thermogenesis), but two lines of evidence suggest that this behavior occurs. First, laboratory studies on brooding female pythons from this population reveal shivering thermogenesis at night (G. Bedford, *personal communication*). Second, our measurements of natural nest temperatures show a difference in nocturnal minimum temperatures between occupied and unoccupied nests (these measurements form the basis for the "hot" and "cold" treatments in Fig. 2).

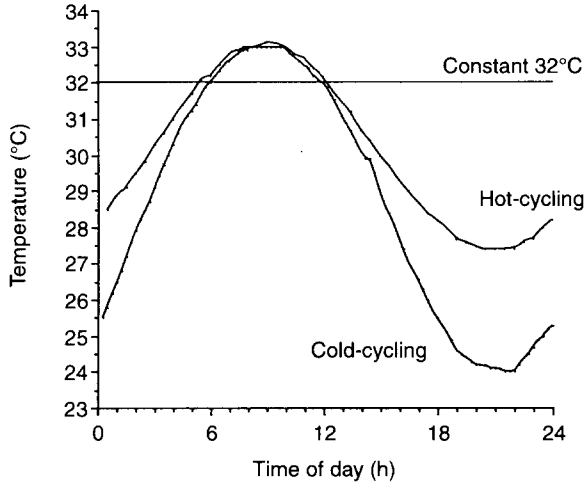

FIG. 2. Incubation regimes used in the experimental study. The graph is based on temperatures measured every 15 min over the entire incubation period. These temperatures were monitored by data-loggers inside water-filled condoms in incubation containers identical to, and adjacent to, those containing the eggs. Standard deviations around the mean were all <0.6°C at any time of day, for all treatments, and hence are not shown.

Hence, female pythons determine the thermal conditions experienced by their eggs through two "decisions:" which kind of nest they use, and whether or not they remain with the eggs and warm them throughout incubation. We explored the consequences of these "decisions" for offspring survival and phenotypes by incubating python eggs under these contrasting thermal regimes in the laboratory. We also included a treatment simulating a "cool" nest without maternal attendance (although we never observed such a nest in the field),

in order to examine the consequences of maternal attendance.

Materials and Methods

Our study area is on the floodplain of the Adelaide River, 60 km SE of Darwin in the Northern Territory of Australia. This area lies within the wet/dry tropics and is characterized by high, stable temperatures yearround. However, precipitation is highly seasonal (Shine and Madsen 1996). Female pythons were hand-collected within 3 km of Fogg Dam in August, 1995. Oviductal eggs were recognized by palpation, and gravid females were transferred to the University of Sydney where they were maintained until oviposition. The snakes were kept at a constant 32°C, in plastic tubs measuring 65 × 41 × 39 cm. The tubs were filled with straw, which was moistened regularly to prevent desiccation of eggs after laying. Because python eggs are strongly adherent, and we wished to separate eggs so that we could divide each clutch among incubation treatments, we attempted to find and separate eggs as soon as they were laid, before they adhered to each other. Hence, we checked each tub at least twice daily, and removed the eggs immediately after oviposition. In most cases, we were able to separate clutches into at least two sections. In four clutches, the eggs were so firmly adherent that we had to cut the egg mass into sections, thereby destroying some eggs.

Our incubation regimes were based on extensive field data, derived primarily from temperature-sensitive radiotransmitters that we surgically implanted in free-ranging female pythons prior to oviposition (Shine and Madsen 1996; T. R. L. Madsen and R. Shine, *unpublished manuscript*). We also monitored nest temperatures throughout incubation in the field (T. R. L. Madsen and R. Shine, *unpublished manuscript*). In the present study, we used three Clayson 10-step programmable incubators to simulate (1) varanid burrow nests (constant 32°C), (2) paperbark nests with maternal attendance (fluctuating in a sinusoidal curve from 27.4° to 32.9°C), and (3) paperbark nests without maternal attendance (fluctuating from 24.3° to 32.9°C) (Fig. 2). Henceforth, we refer to these treatments as "32°C," "hot," and "cold," respectively. The numbers of eggs allocated to each incubation treatment differed for two reasons: (1) As part of another study, we also examined the effects of applying hormones (estradiol and the aromatase inhibitor fadrozole) to five clutches of eggs kept at 32°C. Because our analyses showed no effects of these treatments on any of the dependent variables for the present study, we have included the data for these hormonally manipulated animals in our analyses (excluding them does not change any of our conclusions). Thus, we had more eggs at 32°C than at the other treatments. (2) We anticipated that eggs would be unable to complete development at the cold treatment, because python eggs are supposedly very sensitive to low temperatures (e.g., Vinegar 1974, Ross and Marzec 1990). Thus, we allocated fewer eggs to this treatment since we did not expect to obtain any information other than the demonstration of their low tolerance to cold. The end result was that we incubated 104 eggs at 32°C, 56 at the hot treatment, and 27 at the cold treatment.

The eggs were placed in 4-L buckets containing moist vermiculite (94% water by mass), and covered with plastic foodwrap to retain moisture but allow oxygen exchange. Incubator temperatures were monitored with data-logger probes inside condoms (inflated with 60 mL of water to simulate python eggs) in moist vermiculite in containers identical to those used for the eggs. Eggs were inspected daily, and hatchlings were removed as soon as they had fully emerged from the egg. Hatchlings were measured (snout–vent length [= SVL] and tail length) and weighed, and their sex was determined by manual eversion of hemipenes.

Hatchlings were maintained individually in plastic cages measuring 22 × 13 × 7 cm, with ad libitum water. All cages were housed in a temperature-controlled room at 30° ± 1°C. When the hatchlings were ~10 d old (± 1 d), we assessed two traits: their propensity to strike defensively at the experimenter, and their locomotor (swimming) ability. Propensity to strike was measured by tapping the young python with a small paintbrush on the top of the head, and counting the number of taps before a strike was launched. Swimming ability was measured in a pool 8.5 cm deep, with the water kept at 30° ± 1°C. Two vertical plastic walls formed a circular racetrack 1.0 m in diameter and 10 cm wide. When pythons were placed in the track, they immediately dived underwater and began swimming. A wire rod splashed into the water behind them encouraged their continued progress. We videotaped each trial and recorded the time taken to complete each circuit (converted to swimming speed in meters per second for analysis), the number of splashes needed to keep them moving in each circuit, and the number of circuits completed prior to exhaustion (indicated by a refusal to keep swimming). We also recorded the number of times the snake's head emerged from the water each lap, and noted cases in which the snake managed to crawl up over the plastic walls delimiting the track (thus bringing the trial to a premature end). All measurements of morphology, propensity to strike, and swimming ability were repeated when the young snakes were ≈1 mo old (29–31 d). The snakes were not offered food throughout this period; hatchling pythons frequently do not feed for the first few weeks of life, while they assimilate their abdominal yolk reserves (Barker and Barker 1994). Lastly, we assessed the 5-wk-old snakes' readiness to feed by placing dead mice in the snakes' cages and leaving them for up to 2 h. If a snake ate, it was not tested further. Thus, for each snake, we obtained a record of the number of times it was offered a mouse (up to three times for each snake) before it fed.

We analyzed the data for most of these traits using

TABLE 1. Effects of maternal identity and incubation treatment on phenotypes of young water pythons, *Liasis fuscus*. Table shows mean values for each trait for each incubation treatment, and results from statistical tests (two-factor ANOVAs) for effects of incubation temperature, differences among clutches, and the interaction between these two factors.

Variable	32°C (mean ± 1 SD)	Cold (mean ± 1 SD)	Hot (mean ± 1 SD)	Effect of incubation temperature	
				$F_{2,93}$	P
Incubation period (d)	59.39 ± 1.94	80.00 ± 2.80	67.97 ± 1.68	11.3	<0.001
Morphology at hatching					
Mass at hatching (g)	32.93 ± 5.61	28.89 ± 8.08	32.27 ± 4.10	2.48	0.09
SVL at hatching (cm)	43.52 ± 3.04	41.53 ± 3.91	42.97 ± 2.18	0.31	0.27
Relative tail length at hatching (tail as a percentage of SVL)	14.09 ± 0.50	13.39 ± 1.13	13.89 ± 0.76	2.21	0.12
Body shape (mass/length)†	0.13 ± 2.34	−0.19 ± 1.89	−0.17 ± 1.62	1.02	0.36
Performance at 10 d of age					
Number of laps completed	11.18 ± 4.07	10.46 ± 5.30	11.72 ± 4.13	0.23	0.79
Laps relative to SVL†	−0.30 ± 3.90	−0.34 ± 4.40	0.46 ± 4.25	0.17	0.84
Number of tail taps per lap	14.32 ± 2.72	14.47 ± 4.51	13.42 ± 2.33	2.09	0.12
Tail taps relative to SVL†	0.43 ± 2.49	−0.11 ± 3.37	−0.71 ± 2.15	3.29	<0.042
Swimming speed (m/s)	0.38 ± 0.05	0.33 ± 0.07	0.38 ± 0.06	1.11	0.33
Speed relative to SVL†	0.01 ± 0.05	−0.03 ± 0.06	0.01 ± 0.06	0.81	0.44
Number of times surfaced per lap	4.70 ± 4.18	8.07 ± 6.42	9.49 ± 7.11	6.98	<0.002
Number of taps to elicit strike	8.37 ± 6.54	8.67 ± 7.06	8.73 ± 7.96	0.99	0.38
Taps relative to SVL†	−0.08 ± 6.31	−0.88 ± 6.60	−0.01 ± 8.08	1.39	0.25
Morphology at 20 d of age					
Mass (g)	30.52 ± 5.50	26.87 ± 7.44	30.59 ± 4.13	2.82	0.07
SVL (cm)	50.33 ± 3.25	47.08 ± 4.64	48.45 ± 2.63	8.23	<0.006
Relative tail length (tail as a percentage of SVL)	13.89 ± 0.45	13.24 ± 0.77	13.74 ± 0.73	3.40	<0.04
Body shape (mass/length)†	−0.86 ± 2.55	−0.20 ± 2.88	1.79 ± 2.22	6.21	<0.003
Morphology at 30 d of age					
Mass (g)	30.21 ± 5.52	26.14 ± 7.14	30.10 ± 3.92	3.50	<0.035
SVL (cm)	51.31 ± 3.42	47.73 ± 4.96	49.64 ± 2.49	9.56	<0.002
Relative tail length (tail as a percentage of SVL)	13.94 ± 0.45	13.34 ± 0.68	13.70 ± 0.79	4.25	<0.02
Body shape (mass/length)†	−0.74 ± 2.50	−0.06 ± 2.31	1.48 ± 2.29	6.76	<0.002
Performance at 30 d of age					
Number of laps completed	10.30 ± 3.93	10.70 ± 6.28	11.39 ± 4.21	0.31	0.73
Number of tail taps per lap	11.52 ± 1.95	12.23 ± 6.64	10.39 ± 0.98	0.94	0.39
Tail taps relative to SVL†	0.56 ± 1.95	0.23 ± 5.65	−1.02 ± 1.07	3.63	<0.031
Swimming speed (m/s)	0.41 ± 0.08	0.37 ± 0.10	0.43 ± 0.05	1.31	0.27
Speed relative to SVL†	−0.01 ± 0.07	−0.02 ± 0.08	0.02 ± 0.05	1.25	0.29
Number of times surfaced per lap	7.73 ± 4.72	6.33 ± 5.18	6.95 ± 4.92	1.19	0.31
Number of taps to elicit strike	5.82 ± 4.24	6.40 ± 5.67	4.30 ± 2.70	1.28	0.28
Taps relative to SVL†	0.79 ± 4.00	0.01 ± 4.49	−1.29 ± 2.74	3.51	<0.034

Note: SVL = snout–vent length; 32°C = constant 32°C incubation (n = 67 hatchlings); cold = diel range 24.3°–32.9°C (n = 15 hatchlings); hot = diel range 27.4°–32.9°C (n = 37 hatchlings). See *Materials and methods* for definition of performance variables.

† These variables are residual scores from linear regressions against SVL.

two-factor ANOVA, with clutch number and incubation treatment as the factors. The advantage of the ANOVA design is that it provides information on maternal × treatment effects (i.e., including gene × environment interactions), which may be important biologically. Hence, we can evaluate not only whether hatchling traits were affected by incubation temperatures and whether or not they differed among clutches, but also whether the offspring of different females responded differently to our incubation treatments. We used only a single measurement from each hatchling as the dependent variable in each test (so that, for example, we calculated mean swimming speeds rather than using replicate values for the same individual), to avoid spuriously inflating degrees of freedom in statistical tests. However, we carried out separate tests on data sets for hatchlings at each age tested, to see if incubation effects persisted (or emerged) through time. These tests are biologically meaningful (an incubation-induced difference may well become more or less pronounced through time) but are not independent, because they are based on the same individuals. Hence, we also carried out repeated-measures ANOVAs, to determine whether overall effects of incubation treatments were significant. We did not apply sequential Bonferroni corrections for multiple testing to the statistical results reported in Table 1, because of problems of subjectivity in identifying the class of common tests

TABLE 1. Continued.

Differences among clutches		Interaction between clutch number and temperature	
$F_{14,93}$	P	$F_{9,93}$	P
17.16	<0.001	2.37	<0.019
15.99	<0.001	2.54	<0.013
7.85	<0.001	4.34	<0.001
4.32	<0.001	1.53	0.15
6.13	<0.001	5.26	<0.001
2.65	<0.003	1.11	0.36
2.26	<0.011	0.75	0.66
3.81	<0.0001	1.06	0.40
2.61	<0.004	1.32	0.24
2.55	<0.004	0.82	0.60
2.17	<0.015	1.39	0.20
3.84	<0.001	1.32	0.24
1.12	0.35	0.86	0.56
1.24	0.26	0.80	0.62
15.91	<0.001	2.60	<0.011
10.04	<0.001	2.31	<0.022
2.90	<0.0012	1.80	0.08
10.17	<0.001	2.31	<0.02
15.49	<0.001	2.62	<0.01
11.28	<0.001	2.21	<0.03
2.02	<0.025	0.82	0.60
8.95	<0.001	1.50	0.15
3.17	<0.001	1.04	0.41
4.62	<0.001	0.62	0.78
4.15	<0.001	0.98	0.47
2.86	<0.002	1.23	0.60
2.88	<0.002	1.59	0.13
2.83	<0.002	0.89	0.54
2.14	<0.017	0.82	0.60
1.80	>0.05	0.71	0.70

to be corrected. In practice, this makes little difference to any of our conclusions: almost all of our statistically significant results remain at $P < 0.05$ even if Bonferroni corrections are applied to the entire analysis.

RESULTS

First, we examine the influence of offspring size (body length) and sex on other traits, for the following reason. If many of the variables we measured are correlated with absolute body size or associated with sex, it may be best to incorporate these additional sources of variation into analyses of the effects of our experimental treatments. The following analysis shows that body size is important in this respect, but that the sex of offspring has little effect on most of the variables that we measured.

Body size

Regressions against snout–vent length (SVL) showed that longer pythons were heavier at each age, and that locomotor performance was affected by body size. Longer pythons were faster swimmers ($n = 126$, $r = 0.41$, $P < 0.001$ at hatching; $n = 123$, $r = 0.41$, $P < 0.001$ at 30 d of age), needed fewer taps per lap to persuade them to keep swimming ($n = 126$, $r = -0.46$, $P < 0.001$ at hatching; $n = 124$, $r = -0.36$, $P < 0.001$ at 30 d of age), and swam more laps before becoming exhausted ($n = 126$, $r = 0.29$, $P < 0.001$ at hatching; but $n = 124$, $r = 0.16$, $P = 0.08$ at 30 d of age). The greater number of laps reflected the faster swimming speeds of the larger hatchlings, because time to exhaustion was not significantly affected by body size ($n = 101$, $r = 0.13$, $P = 0.20$ at hatching; $n = 91$, $r = 0.18$, $P = 0.08$ at 30 d). Longer snakes also required fewer taps before they launched a retaliatory strike ($n = 126$, $r = -0.23$, $P < 0.012$ at hatching; $n = 126$, $r = -0.31$, $P < 0.001$ at 30 d of age). Thus, for all of these variables, we examined the effects of incubation treatment in two ways: first by looking at the raw data; and second by looking at residual scores from the linear regression of that trait on SVL. This technique allows us to identify which effects are due to body size, and which are not.

Sex

In contrast to these body size effects, the sex of the offspring seems to exert little influence on its size, shape, or behavior. Two-factor ANOVA (with sex and clutch number as the factors) revealed no significant effects of offspring sex and no significant interactions on any of the traits we examined, except for the number of taps per lap for hatchling trials (interaction $F_{1,12} = 2.03$, $P < 0.031$). This is likely to be a spurious result, reflecting the large number of tests conducted, and may have no biological significance. Thus, our subsequent analyses did not incorporate sex as a factor.

Survival rate

Hatching success did not vary significantly among the three incubation treatments. Although the proportion of eggs hatching successfully was lowest in the cold treatment (16 out of 27, or 59%), this rate was not significantly different from that shown by eggs in the hot treatment (38 out of 56, or 68%) or at constant 32°C (75 out of 104, or 72%) ($\chi^2 = 1.70$, 2 df, $P = 0.43$). We also analyzed these data using a two-factor ANOVA on mean values for hatching success of eggs from each clutch under each incubation regime. This analysis also showed no significant effect of incubation temperature ($F_{2,14} = 0.79$, $P = 0.62$) or clutch number ($F_{14,14} = 1.84$, $P = 0.53$) on hatching success, and no significant interaction between these factors ($F_{1,14} = 2.71$, $P = 0.45$).

Incubation period

Eggs maintained in the hot treatment hatched significantly earlier than did eggs in the cold treatment; eggs held at a constant 32°C developed even faster (Table 1). Maternal effects were also highly significant, as was the interaction between clutch number and incubation treatment (Table 1). Thus, thermal regimes affected incubation periods differently in different clutches.

Morphology

Strong maternal effects were evident on the size and shape at hatching (Table 1). We did not detect any main effects of incubation temperature, but there were strong interactions between clutch number and temperature for both size and shape (Table 1). These maternal effects and interaction effects remained significant when measurements were taken at 20 d and at 30 d of age, while the main effects of incubation regime became stronger (and hence, statistically significant, see Table 1). For some traits, the rankings of hatchlings from the different incubation treatments remained consistent through time, even after significant growth (e.g., SVL, see Fig. 3), whereas other traits showed more complex ontogenetic shifts (e.g., body shape, see Fig. 3).

The increasing effect of incubation treatment on body sizes suggests that growth rates differed among hatchlings from the different incubation treatments. We tested this possibility by calculating residual scores from linear regressions linking body sizes at two successive ages (e.g., size at hatching vs. size at 20 d; or size at 20 vs. 30 d) as the measure of growth rate. Two-factor ANOVAs (with clutch number and incubation treatment as the factors and the residual score as the dependent variable) show significant effects of incubation treatments on growth rates in snout-vent length (0 vs. 20 d—main effect of incubation treatment $F_{2,94} = 12.99$, $P < 0.0001$, interaction $F_{9,94} = 3.93$, $P < 0.0003$; 20 vs. 30 d—main effect of incubation treatment $F_{2,94} = 3.35$, $P < 0.04$, interaction $F_{9,94} = 5.59$, $P < 0.0001$) and body mass (0 vs. 20 d—main effect of incubation treatment $F_{2,94} = 0.26$, $P = 0.77$, interaction $F_{9,94} = 2.61$, $P < 0.01$; 20 vs. 30 d—main effect of incubation treatment $F_{2,94} = 3.50$, $P < 0.04$, interaction $F_{9,94} = 0.60$, $P = 0.80$). Hence, our incubation treatments affected growth rates as well as body shape.

Locomotory performance

As with morphology, the strongest influence on locomotory performance was maternal identity: offspring from some clutches were faster swimmers than were conspecifics from other clutches, and these differences persisted until the young pythons were at least 30 d of age (Table 1). We did not detect any significant interactions between clutch number and incubation treatment for performance traits, and main effects of incubation regime were evident for only a few traits. The only consistent effect (i.e., evident at both 10 and 30 d of age) concerned the number of tail taps relative to SVL: at the same body length, pythons from eggs incubated at a constant 32°C required more taps to persuade them to keep swimming (Table 1). Repeated-measures ANOVA for this trait also yielded a significant effect of incubation treatment (see *Repeated-measures analyses*).

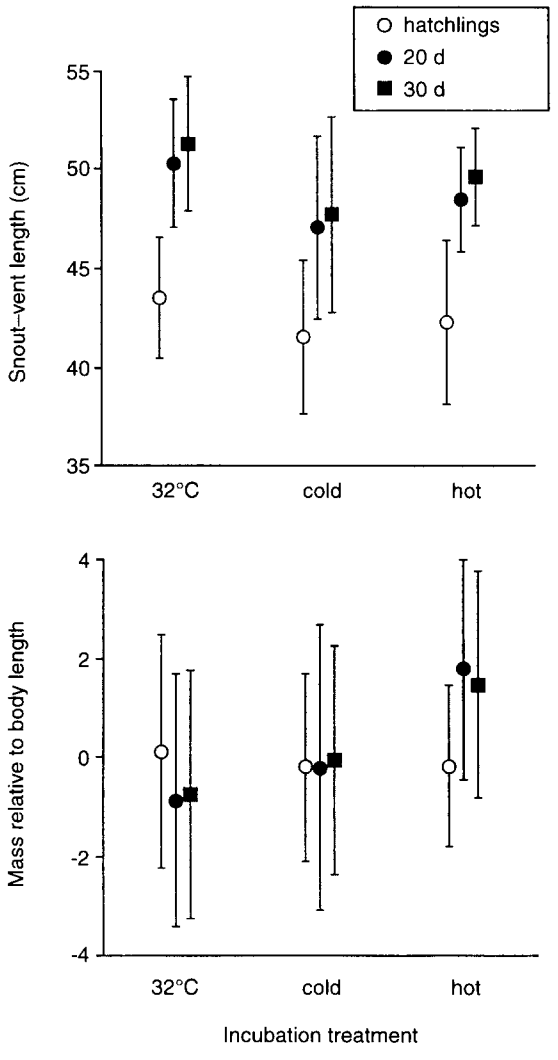

FIG. 3. The effects of thermal regimes during incubation on the morphology (size and shape) of young water pythons. The figure shows mean values, plus associated standard deviations, for pythons incubated under three different thermal regimes.

As noted above, some pythons actually crawled over the partition in the swimming pool during a trial, thus bringing that trial to an end. This behavior was more frequent in hatchlings from cold incubation (10 out of 16, or 63%) than from hot incubation (10 out of 37, or 27%) or 32°C (5 out of 73, or 7%) ($\chi^2 = 27.3$, 2 df, $P < 0.0001$). By 30 d of age, this difference had disappeared ("crawl-overs" recorded for 5 out of 16, or 31%

of cold; 9 out of 37, or 24% of hot, and 20 out of 73, or 27% of 32°C snakes: $\chi^2 = 0.3$, 2 df, $P = 0.87$).

Propensity to strike

Our analysis did not identify any influences on the propensity of hatchling pythons to launch a defensive strike (i.e., number of taps prior to strike), but trials at 30 d of age revealed significant effects of clutch number and incubation treatment. However, we note that neither of these results would remain significant after sequential Bonferroni correction, so their biological validity is dubious.

Willingness to feed

Of 73 mice offered to pythons incubated at a constant 32°C, 43 (59%) were immediately seized and swallowed, whereas only 34% (6 out of 16) of the mice offered to cold-incubated snakes were taken, and only 16% (6 out of 38) of the mice offered to hot-incubated snakes. These differences enable strong rejection of the null hypothesis of equal willingness to feed regardless of incubation regime (one-factor ANOVA with incubation regime as the factor, the percentage of mice eaten by each snake as the dependent variable—$F_{2, 123} = 15.21$, $P < 0.0001$).

Repeated-measures analyses

As noted above, our application of independent statistical tests on the same individuals at different ages violates the assumption of independence. Are these effects still significant overall, if this assumption is not made? We examined this question using single-factor repeated-measures ANOVA, with incubation regime as the factor. Sample sizes in each cell were too unbalanced for a two-factor ANOVA (incorporating clutch number as another factor), so we restricted our repeated-measures ANOVA to data from females that contributed eggs to at least two treatments. Thus, any biases due to maternal effects should be minimized. These analyses confirmed that incubation treatments significantly affected variables such as body shape (relative tail length—$F_{2,72} = 3.32$, $P < 0.042$; mass relative to length—$F_{2,72} = 4.07$, $P < 0.022$) and locomotor performance (number of taps per lap—$F_{2,72} = 3.75$, $P < 0.03$). Thus, our earlier results from single time periods are not artifacts of multiple nonindependent tests.

Discussion

Our data confirm and extend previous experimental studies that have demonstrated phenotypic plasticity in the size, shape, and behavior of hatchling reptiles in response to the thermal conditions which these animals experience as embryos (e.g., Lang 1985, Miller et al. 1987, Webb and Cooper-Preston 1989). This sensitivity clearly is also true for *Liasis fuscus,* despite the fact that this species is phylogenetically and ecologically very different from the temperate zone lizards and turtles that have been the basis for almost all previous studies on this topic. Hence, phenotypic plasticity in reponse to incubation conditions may prove to be widespread in reptiles. It is interesting to see significant plasticity in response to the relatively modest thermal differences among natural nest-types in a tropical species (e.g., compare cold and hot regimes in Fig. 2); thermal heterogeneity among nests is likely to be much greater in shallow-nesting temperate zone species of reptiles than in animals subject to the high, relatively stable temperatures of the tropics (e.g., Packard and Packard 1988, Shine and Harlow 1996). Thus, we expected a priori that we would see relatively little plasticity in the water pythons; in this respect, we were certainly wrong.

Radiotelemetric monitoring of gravid female water pythons immediately prior to oviposition suggests that they base their choice of nest sites on the variance in nest temperature, rather than mean temperature per se (T. R. L. Madsen and R. Shine, *unpublished manuscript*). However, our experimental design, whereby our incubation treatments mimicked natural nest conditions, does not allow us to identify which aspects of the thermal environment are responsible for the phenotypic effects we observed. Recent work has shown that both the mean and the variance of incubation temperatures can influence developmental rates and hatchling phenotypes of lizards (Shine and Harlow 1996). Similarly, we cannot separate out the strong maternal effects into genetic and nongenetic components. Presumably, some proportion of the intraclutch consistency in morphology and behavior (Table 1) represents genetic similarity between sibs, but a significant proportion of this consistency may also be due to environmental effects (e.g., maternal nutrition, parasite loads, etc.: see Shine 1995 for review). At least 30% of embryogenesis occurs prior to oviposition in most oviparous squamate reptiles, including water pythons, so there is ample opportunity for offspring phenotypes to be affected by maternal thermoregulation prior to oviposition (Shine 1995).

Our experimental treatments simulated actual thermal regimes in natural nests, so we believe that the phenotypic responses we detected are likely to be seen in natural nests as well. Whether these responses affect the individual fitness of reproducing females or their offspring is a much more difficult issue. Although the variables we measured (body size, shape, behavior, performance) were selected because of their plausible linkage with fitness (survival and growth rates) in the field, based on studies on a variety of other reptile species (e.g., see Shine 1995), we have no direct evidence of such a linkage for water pythons. Also, our data are based entirely on the first month of life. Water pythons are very long-lived (some individuals live for at least 12 yr: T. R. L. Madsen and R. Shine, *unpublished data*) and their phenotypic responses to incubation conditions may disappear with age. Nonetheless, other studies have suggested that these kinds of incubation-induced

phenotypic modifications may persist for long periods (e.g., Joanen et al. 1987, Shine et al. 1995), and our data show no suggestion that these effects decrease ontogenetically (compare the magnitude and significance of incubation-induced effects at 0 and 30 d of age: Table 1). Overall, though, the microevolutionary consequences of phenotypic plasticity in hatchling water pythons remain entirely speculative.

Our study provides the first experimental evidence on the effects of nest attendance (and shivering thermogenesis) by female pythons. Female attendance at the nest throughout incubation has been described for many pythons and probably occurs in all species (e.g., Shine 1988, Ross and Marzec 1990). Shivering thermogenesis may also be ubiquitous in this group (Shine 1988, but see Ellis and Chappell 1987). Nonetheless, our results cast strong doubt on some widely accepted generalities for pythonid parental care. First, clutch attendance in water pythons is facultative rather than obligatory: females attend clutches only if they are laid in relatively cool nests (T. R. L. Madsen and R. Shine, *unpublished manuscript*). Second, the eggs of water pythons are capable of successfully completing development even if maintained at relatively low, variable temperatures throughout embryogenesis. Indeed, hatching success did not differ significantly among the three incubation treatments we used (although the difference in survival rates was in the expected direction). This result surprised us, and runs counter to the prevailing view that pythonid eggs can develop successfully only at high, stable temperatures. In contrast to previous reports of a high incidence of dead and/or malformed offspring from low-temperature incubation (e.g., Vinegar 1973, 1974, Ross and Marzec 1990, Barker and Barker 1994), we recorded only four malformed hatchlings, all of them from the same clutch and all incubated at high temperature.

If pythonid eggs are capable of developing at low and variable temperatures, why do female pythons typically stay with their eggs throughout incubation and expend vast amounts of energy on metabolic heat production to warm the clutch (Harlow and Grigg 1984, Slip and Shine 1988)? Clutch attendance may impose high costs to the female. In the water pythons of Fogg Dam, for example, nest attendance increases the risk of mortality for females, and delays production of the subsequent clutch (T. R. L. Madsen and R. Shine, *unpublished manuscript*). Our study suggests that maternal attendance may be favored because it accelerates incubation (and thus, enables hatching to occur earlier than would otherwise be the case), and may also affect offspring viability by inducing particular developmental pathways. Hatchling water pythons at Fogg Dam rely upon juvenile dusky rats (*Rattus colletti*) as their major prey, and in some years the numbers of these small rats decline dramatically during the course of the snakes' hatching season (T. R. L. Madsen and R. Shine, *unpublished manuscript*). Thus, juvenile pythons from late-hatching clutches experience much lower survival (T. R. L. Madsen and R. Shine, *unpublished manuscript*). By hastening hatching, maternal brooding may significantly enhance offspring survival. The substantial phenotypic modifications caused by maternal attendance (compare mean values of traits in Table 1 between cold and hot treatments) may also influence offspring survival and/or growth. Field studies would be needed to test this hypothesis, but it raises the interesting possibility that the initial selective advantage for reptilian endothermy (shivering thermogenesis) may have been the production of thermally induced modifications of developmental pathways during embryogenesis. A similar idea has recently been proposed for the evolution of reptilian viviparity (Shine 1995) and is equally plausible for maternal brooding.

In summary, experimental incubation of the eggs of a tropical python, under three different thermal regimes simulating natural nest-types, revealed a considerable sensitivity of embryogenesis to incubation temperatures. Both maternal nest choice and shivering thermogenesis can substantially affect not only the time at which the eggs hatch, but also the size, shape, and behavior of the hatchlings. Although the consequences of these effects for organismal fitness remain unclear, it seems unlikely that they are entirely neutral in this respect. Thus, biologically important aspects of the phenotype are determined by direct environmental factors, as well as by intrinsic (genetic?) causes. This conclusion has important implications. If a substantial proportion of the phenotypic variance in hatchling reptiles has little or no genetic underpinnings, then our current understanding of the relationship between natural selection and directional evolutionary (i.e., allele-frequency) changes may need to be reconsidered. On a more specific level, our study reinforces the emerging idea that physical conditions available in local nest-sites may be a major influence on the ecology of reptilian populations, and may have played an important role as selective forces for evolutionary shifts in traits such as parental care.

ACKNOWLEDGMENTS

We thank E. Cox and P. Fisher for assistance with python husbandry, and the staff at the Coastal Plains Research Station for logistical support in many ways. We are grateful to G. Bedford for his provision of unpublished data on shivering thermogenesis. Comments on the manuscript were provided by P. Doughty and F. Gregory. Financial support was provided by the Australian Research Council and the National Geographic Research and Exploration Fund.

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