# DETERMINANTS OF REPRODUCTIVE OUTPUT IN FEMALE WATER PYTHONS (*LIASIS FUSCUS*: PYTHONIDAE)

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ABSTRACT: Female water pythons from a population in tropical Australia were captured shortly before oviposition and their eggs were incubated under constant conditions. We obtained data on 1605 eggs and 1285 hatchlings from 111 females to evaluate factors influencing reproductive output. Most reproductive traits varied only slightly between the 2 yr of the study. Successive clutches by the same female were consistent in mean egg and offspring sizes, hatching success, and oviposition date. Whether or not an adult-size female python reproduced in a given year was a function of her body size and her condition. The proportion of reproductive animals was highest at intermediate body sizes, apparently because the largest females could not gather enough energy for reproduction as well as maintenance costs. Clutch sizes and egg sizes were directly affected by maternal body length and maternal condition, but these effects were obscured by a strong indirect effect: a tradeoff between egg size and clutch size. Date of hatching depended primarily on date of oviposition (which in turn differed among subpopulations within our study area, apparently due to thermal factors), but incubation periods also differed between years. Maternal body condition after oviposition was influenced by pre-oviposition condition and relative clutch mass. Path analysis revealed a series of strong effects, both direct and indirect, in the relationships among reproductive variables.

*Key words:* Allometry; Fecundity; Path analysis; Python; Reproduction; Snake; Tropics

THE number and size of offspring that are produced each year, and the times at which they are produced, are important biological attributes. In terms of microevolutionary forces, these "reproductive output" variables contribute significantly to the individual fitness of reproducing females. The number, size, and time of appearance of offspring also influence the dynamics of population numbers. Despite this importance, there are relatively few detailed quantitative studies on factors influencing reproductive output. In the case of snakes, for example, there are abundant published data on mean clutch sizes and mean offspring sizes for numerous species, and many analyses of the influence of body size on reproductive output (e.g., Seigel and Ford, 1987; Seigel et al., 1986), but there is little detailed information, for any single population, on the determinants of reproductive output in a broader sense (but see King, 1993). Also, available published data on reproductive biology in snakes show strong biases with respect to phylogeny (most studies deal with species from a single lineage, the Colubroidea) and geography (tropical snakes remain almost unstudied, despite the fact that most species of snakes are primarily tropical in occurrence). In the present paper, we provide information on reproductive output

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in a tropical pythonid species as part of a longterm ecological study that we are conducting on these animals.

The reproductive output of an adult-size female snake in any particular year can be visualised as the result of a sequence of "decisions" (albeit, not conscious ones on the part of the female snake) that concern the following issues. First, will reproduction occur or will the female skip reproduction instead? How much of the female's available resources will she devote to the clutch? How will the investment be packaged (into a few large eggs or many small ones)? What will be the size and shape of the offspring that emerge from these eggs? When will oviposition occur? And finally, when will hatching occur?

There are many other influences on maternal fitness apart from these, but our data do not allow us to address them. For example, we have not attempted to assess offspring quality, nor have we measured survival rates of eggs in the field. A complete understanding of the evolutionary consequence of different female "tactics" would require data on these questions, as well as on potential "costs" of different levels of reproductive expenditure to the subsequent survival and growth of the reproducing female (e.g., Reznick, 1985; Stearns, 1976) and on the genetic basis of the observed phenotypic variation (e.g., Madsen and Shine, 1992). Unfortunately, lifetime reproductive success is difficult to measure under natural conditions in most long-lived animals (e.g., Clutton-Brock, 1988), and we thus focus on reproductive expenditure per annum in the present paper.

## MATERIALS AND METHODS

Water pythons (*Liasis fuscus* = *L. mackloti* of some authors) are large (to 3 m total length) nonvenomous snakes widely distributed through tropical Australia. Although their common name reflects their association with riparian habitats, our radiotelemetric studies show that many individuals of this species live far from water bodies for most of the year (Madsen and Shine, 1996). We have been studying the ecology of water pythons in the vicinity of Fogg Dam, 60 km southeast of the city of Darwin (Northern Territory) since 1987. Most analyses in the present paper are based on data from 2 yr (1991 and 1992) when we collected and maintained large numbers of reproductive female pythons. In 1993, we restricted our attention to "repeat females" (those that had already produced eggs in captivity during either 1991 or 1992) so that we could assess the degree of consistency in clutch characteristics produced by the same female in different years. Reproductive females (either gravid or close to ovulation, based on palpation of ovarian follicles) were hand-collected from three areas within a 5-km circle. These three areas, chosen because of the abundance of snakes and ease of collection, were: (1) the wall of the dam, (2) a paperbark tree (*Melaleuca cajuputi*) forest 2 km north of the dam wall, and (3) a complex of burrows constructed and inhabited by varanid lizards (*Varanus panoptes*) 4 km northeast of the dam wall. Our telemetered snakes often move >500 m/night, but interchange of individuals among these three nearby sites is low. For example, none of our "repeat females" moved from one area to another between reproductive episodes.

We collected most female pythons at night as they investigated potential oviposition sites (usually, varanid burrows or crevices among paperbark roots). Eggs are laid in these sites, with maternal attendance for varying periods of time by some but not all females (Madsen and Shine, unpublished data). Each female was weighed (±1 g) and measured (snout—vent length, SVL, ±1 cm), and individually marked by clipping a unique combination of ventral scales. Nonreproductive females were then released whereas reproductive females were retained. We housed them in wire-mesh cages (60 × 60 × 60 cm) in the shade (mean diurnal range in air temperature = 16–34 C) until oviposition. Water was available ad libitum, but no food was provided because female water pythons do not feed at this stage of their reproductive cycle (none of the 116 reproductive females contained prey items when collected). We provided dry grass in

each cage for cover. Cages were checked daily for eggs and these were then removed (usually in a single mass, because the eggs are strongly adherent), weighed, and placed into plastic boxes (30 × 35 × 14 cm) for incubation. The boxes were half-filled with moist vermiculite (50% water by mass) into which the egg mass was partly buried. We placed each clutch into a separate container, which was then sealed to retain moisture. The containers were kept in a constant-temperature room which maintained ambient temperatures within the range 29–32 C (monitored continually with a recording thermohydrograph). This temperature range is similar to thermal regimes that we have measured in natural nests (Madsen and Shine, unpublished data). We inspected the containers daily and added additional water if the eggs appeared to be desiccated.

As soon as the offspring hatched, we counted, weighed, and measured them in the same way as we did for the adults. We sexed offspring from the last 48 clutches by eversion of hemipenes. We recorded the numbers of "infertile" eggs in each clutch; these were readily distinguishable by their small size and yellow color but may have included some fertile eggs in which embryos died very soon after fertilisation. The numbers of eggs failing to hatch were also recorded. Because we were unable to weigh eggs separately, and because of the potential statistical dangers in treating siblings as replicates, we have calculated means and variances for each of the traits for each clutch rather than using separate data for each individual egg or hatchling in our analyses. To obtain size-independent measures of maternal condition, we calculated residual scores from linear regressions of mass to SVL at different stages of the reproductive cycle (i.e., before and after oviposition).

We released all of the adult female pythons after oviposition and released all the hatchlings after marking. Thirteen of the reproductive adult female pythons were collected in two of the 3 yr of our study, so that we obtained data for two clutches from each of these 13 animals. To avoid non-independent sampling of the two main

years of the study (1991 and 1992), we deleted data for the second clutches of all of these "repeat females" from our path analyses. Thus, each female is represented only once in these analyses. Data for second clutches were used only to investigate temporal consistency in clutch characteristics for successive clutches from the same female.

Thus, we accumulated data on several variables related to reproductive output. Because many of these variables are highly inter-correlated, it is difficult to disentangle cause-and-effect. Two variables may well be highly correlated not because of a causal relationship, but because both are causally related to some third variable. In order to tease apart independent contributions of each variable to the patterns in our data sets, and to distinguish direct from indirect effects, we used path analysis. The use of this technique has been strongly advocated by many workers, because it can partition the correlations among variables (e.g., King, 1993; Kingsolver and Schemske, 1991). The technique is particularly useful where the data set includes a temporal component, so that cause-and-effect relationships between "earlier" and "later" variables can be superimposed onto an a priori model (the path diagram). Path coefficients are standardised partial-regression coefficients and can be used to predict the amount of change in a dependent variable (in units of standard deviation of that variable) due to change in an independent variable. Because we have large and virtually identical sample sizes from each of 2 yr (1991 and 1992), we were able to analyze data for each year separately, and thus examine the temporal consistency of the relationships among reproductive variables.

The structure of our path diagram (Fig. 1) was derived from probable causal links between variables. Thus, for example, we viewed clutch size as a causal influence on egg size, rather than vice versa, because clutch size is determined earlier in the reproductive process than is egg size (King, 1993). Because path analysis assumes that the relationships among variables are linear (e.g., Kingsolver and Schemske, 1991),
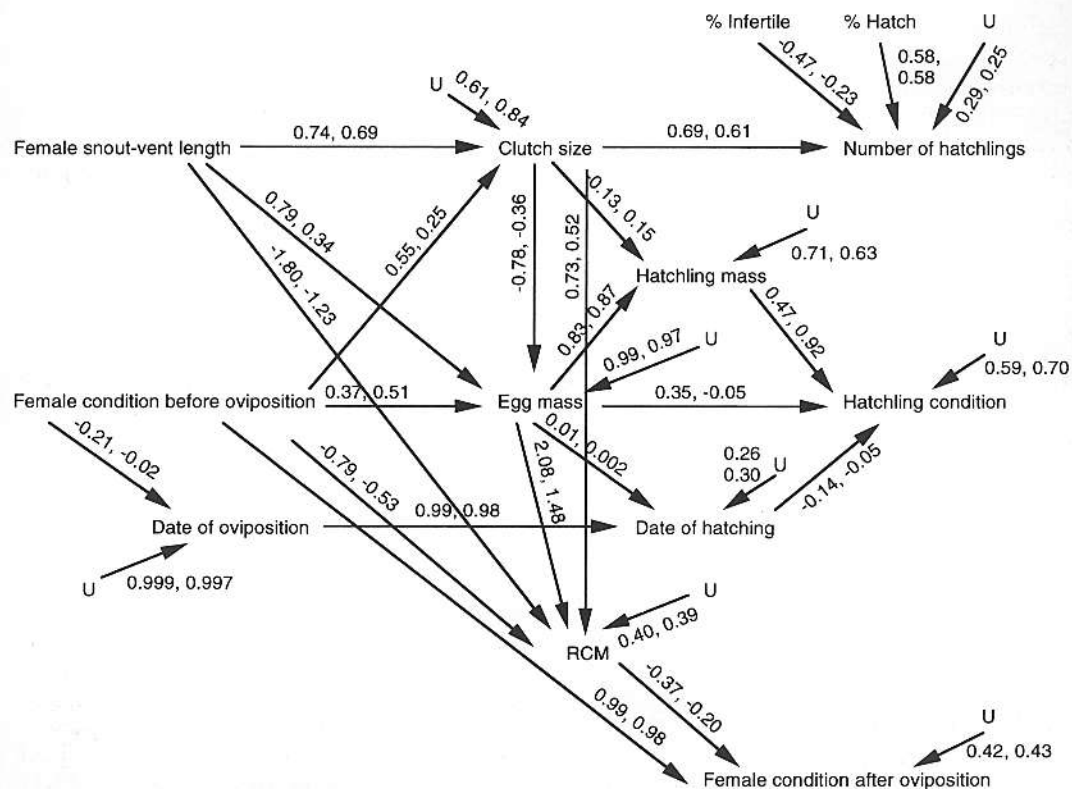
FIG. 1.—Path diagram for reproductive output in water pythons, with path coefficients (standardised partial-regression coefficients) calculated independently from data for 1991 and 1992. The first coefficient for each pathway is that based on the 1991 data, and the second is that based on the 1992 data. Unexplained variance ("U") calculated as $(1 - R^2)^{0.5}$, where R is the proportion of variation in the dependent variable that is explained by the model (Kingsolver and Schemske, 1991).

it is inappropriate for some of the variables that we have measured (e.g., class variables, such as location or reproductive status). In such cases, we have used standard ANOVA and ANCOVA techniques. Also, we have examined univariate correlations among variables wherever they seemed to be of biological interest. For example, if our path analyses suggested that a high proportion of the variance in some trait could not be attributed to the influence of any of the traits incorporated in our path diagram, we examined other possible correlates of the relevant variable.

## RESULTS AND DISCUSSION

We obtained information on 55 reproductive females (>140 cm: Madsen and Shine, unpublished data) captured during the period July–September 1991, and 61 captured over the same period in 1992. With deletion of five "repeat females" (i.e., animals captured in both 1991 and 1992) from the 1992 data, we are left with similar numbers of reproductive females in each year (55, 56). The present analysis is based primarily on these 111 reproductive females, plus 129 nonreproductive adult-size females collected over the same time period. A further eight "repeat females" collected in 1993 are used for analyses of temporal consistency among females. All of the reproductive animals produced clutches, which contained a total of 1605 eggs. These in turn produced 1285 hatchlings. Female pythons were captured (and their mass determined) an average of 25.5 days before they laid their eggs (SD = 12.4,

TABLE 1.—Mean values and associated standard deviations (SD) for reproductive traits in water pythons collected in 1991 and 1992. Some variables are residual scores from linear regressions: "condition" = mass vs. SVL; Last two columns show results from unpaired $t$-tests (109 df) to compare between years. * = $P$ < 0.05. Note that all significant results remain at $P$ < 0.05 after sequential Bonferroni adjustment. The mean egg and offspring sizes are based on means from each clutch. If larger clutches generally contain smaller offspring, the overall mean figure derived in this way may be slightly smaller than the mean based on independent masses of each offspring.

| Trait | 1991 $\bar{x}$ ± SE | 1992 $\bar{x}$ ± SE | $t_{109}$ value | $P$ |
|---|---|---|---|---|
| Snout–vent length (cm) | 173.76 ± 2.11 | 176.34 ± 1.70 | 0.95 | 0.34 |
| Mass (pre-oviposition) (g) | 2253.29 ± 73.67 | 2393.23 ± 65.06 | 1.43 | 0.16 |
| Maternal condition after oviposition | −15.67 ± 20.89 | 8.21 ± 21.81 | 0.79 | 0.43 |
| Clutch mass (g) | 672.45 ± 25.73 | 723.8 ± 27.18 | 1.37 | 0.17 |
| Clutch size | 12.67 ± 0.44 | 13.14 ± 0.4 | 0.80 | 0.43 |
| % infertility | 6.91 ± 2.26 | 6.12 ± 1.73 | 0.28 | 0.78 |
| No. of fertile eggs | 11.8 ± 0.48 | 12.38 ± 0.46 | 0.87 | 0.39 |
| % eggs hatching | 85.07 ± 2.47 | 86.3 ± 2.89 | 0.32 | 0.75 |
| Relative Clutch Mass | 0.46 ± 0.01 | 0.47 ± 0.01 | 0.54 | 0.59 |
| Median date of oviposition | 1 October | 25 September | 4.58 | 0.0001* |
| Median date of hatching | 29 November | 2 December | 0.68 | 0.50 |
| Incubation period (days) | 58.53 ± 0.4 | 67.95 ± 0.39 | 17.05 | 0.0001* |
| Mean egg mass (g) | 55.28 ± 0.83 | 56.89 ± 5.85 | 1.35 | 0.18 |
| Mean hatchling mass (g) | 34.87 ± 0.54 | 35.87 ± 0.59 | 1.26 | 0.21 |
| Mean hatchling SVL (cm) | 43.78 ± 0.26 | 46.01 ± 0.22 | 6.59 | 0.0001* |
| Hatchling condition | 0.91 ± 0.42 | −0.96 ± 0.41 | 3.20 | 0.0018* |

extremes = 4–68 days). Ovulation is detectable by palpation in this species (ovarian follicles and shelled eggs are firmer than recently ovulated ova), and we found an average delay of approximately 30 days between ovulation and oviposition. Thus, about 70% of our females probably ovulated prior to capture, whereas the others ovulated while in captivity. Our analyses revealed no significant differences in reproductive characteristics between these two groups of females, so data were combined for all further tests. Similarly, although the number of days that snakes are held in captivity can affect reproductive traits (King, 1993), we detected no significant correlation between time in captivity and any of the traits that we measured ($P$ > 0.25 for all tests), so we did not include this variable in the analyses reported below.

### How Much Do Reproductive Traits Vary from Year to Year?

Our results reveal a remarkable similarity in most reproductive traits in the two years (Table 1). For example, there were no significant differences between the two years in mean body lengths or masses of reproductive females, their mean Relative Clutch Masses (= RCMs henceforth; defined as clutch mass divided by maternal mass after oviposition), their mean clutch sizes, the means and variances of masses of eggs and hatchlings, the mean hatching success of fertile eggs, or the mean proportion of infertile eggs (Table 1). The only traits to show significant differences between years were oviposition dates, incubation periods, and offspring lengths: eggs from 1992 were laid earlier, took a longer time to incubate, and produced longer hatchlings than did those from 1991. This similarity in mean values of traits between years extends also to the sign and magnitude of relationships among traits. Comparison of path coefficients from 1991 data with the analagous 1992 coefficients revealed a very high correlation ($r$ = 0.94, $n$ = 28, $P$ < 0.0001: see Fig. 1 for coefficients).

### How Consistent Are the Reproductive Traits of Individual Pythons?

Our data on "repeat females" (those captured as reproductive animals in 2 yr)

TABLE 2.—Consistency between years in reproductive traits of the 13 female pythons that were recorded to reproduce in 2 yr. The Table gives values from one-factor analyses of variance, with female identification number as the factor. * = $P < 0.05$, but not significant after sequential Bonferroni adjustment for multiple tests; ** = $P < 0.05$, even after Bonferroni adjustment.

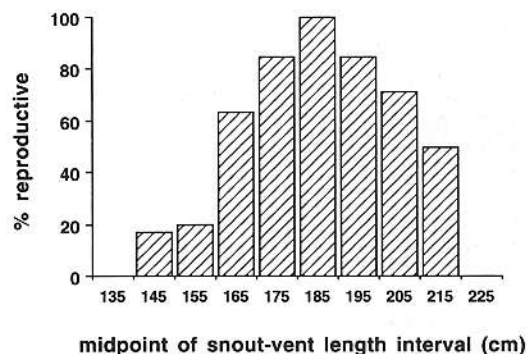| Dependent variable | F ratio (12, 10 df) | Significance level ($P$) |
|---|---|---|
| Relative clutch mass | 1.90 | 0.14 |
| Clutch size relative to body size (residual scores from linear regression) | 1.32 | 0.34 |
| Proportion of infertile eggs | 2.04 | 0.13 |
| Proportion of eggs hatching | 4.85 | 0.009** |
| Mean egg mass (g) | 6.29 | 0.003** |
| Mean offspring mass (g) | 3.35 | 0.03* |
| SD in offspring mass | 3.30 | 0.03* |
| Mean offspring SVL (cm) | 0.91 | 0.56 |
| SD in offspring SVL | 0.36 | 0.94 |
| Mean incubation period (days) | 0.88 | 0.59 |
| Maternal body condition after laying (residual scores from linear regression) | 1.52 | 0.26 |
| Oviposition date (days after 1 September) | 2.93 | 0.049* |



FIG. 2.—The percentage of female water pythons that were reproductive in the year that they were collected as a function of the body size of the snake. Based on 240 adult-size (>140 cm SVL) females captured over 2 yr.

enable us to evaluate the degree to which individual females are consistent from one year to the next in terms of their reproductive output. The question is an interesting one, because a high level of consistency in this respect would suggest that most of the intrapopulational variance is due to differences among females whereas a low consistency would implicate proximate environmental factors (or strong ontogenetic shifts) as a major source of variation. We analyzed the data on the 13 "repeat females" with one-factor analyses of variance, with female identification number as the factor. The variation between successive clutches from the same female (in terms of her RCM, the proportion of infertile eggs in her clutch, clutch size relative to body size, number of eggs hatching, mean or variance in offspring body length, incubation period, or the female's body condition after laying) was similar in magnitude to the variation among clutches from different females (Table 2). After sequential Bonferroni adjustment for multiple tests, the only vari-

ables that showed significant consistency were mean egg mass and the proportion of eggs that hatched successfully (Table 2).

## What Factors Determine Whether Or Not an Adult-size Female Python Reproduces in a Given Year?

Approximately half of the adult-size (>140 cm SVL) female pythons that we captured were reproductive, and the major influences on whether or not a female reproduced were her body length and her "condition" (= mass relative to length). That is, reproduction was most likely to occur if a female was large and was in good condition. Although these results seem intuitively reasonable, closer inspection suggests that the picture is more complex than it initially appears. Firstly, the relationship between maternal body length and the probability of reproduction is not one of simple linear increase. The proportion of reproductive animals rises to a maximum at around 180 cm SVL and then falls abruptly (Fig. 2); most very large females are nonreproductive. This pattern was apparent in both years of our study.

The other determinant of whether or not a female python will reproduce is her bodily condition: reproductive animals were much heavier than their nonreproductive counterparts at the same body length (Fig. 3: two-factor ANCOVA test; factors = reproductive status and year, co-
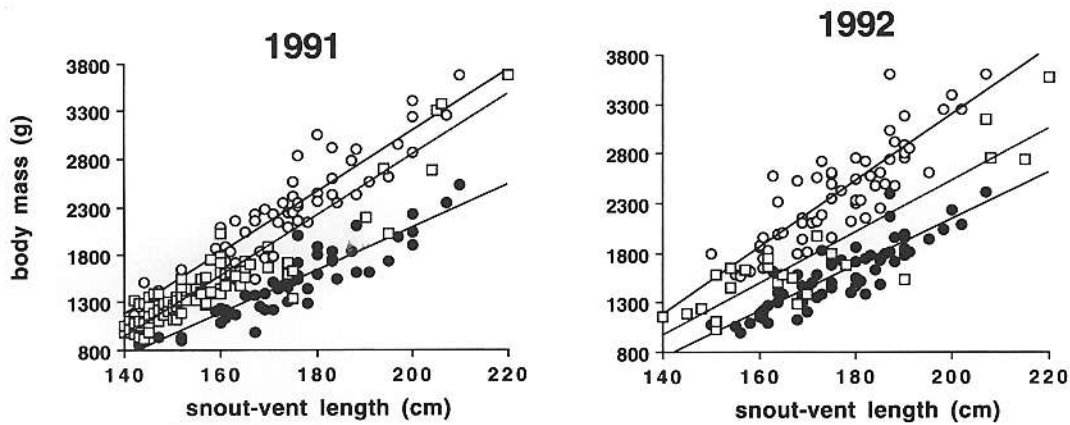
FIG. 3.—Body mass relative to snout–vent length in adult female water pythons. In both years of the study, reproductive females prior to oviposition (circles) were more heavy-bodied than nonreproductive females (squares). Post-oviposition reproductive females (filled dots) were much thinner than the nonreproductive animals. See text for statistical analyses of these data.

variate = SVL, dependent variable = mass; year $F_{1,279}$ = 2.93, $P$ = 0.09; reproductive status $F_{1,279}$ = 12.53, $P$ < 0.0005). The increment in mass of reproductive females consisted of energy stores destined for reproductive expenditure (in fat bodies, vitellogenic follicles, or eggs), because body masses were lower in post-oviposition females than in nonreproductive animals (Fig. 3). Clutch masses were high (RCMs averaged almost half of the female's post-oviposition mass: Table 1) so that females in poor condition simply did not have enough energy reserves to produce a typical clutch.

## What Factors Determine Clutch Size?

Clutch size increased with maternal size, as is almost universal in snakes (e.g., Seigel and Ford, 1987). Maternal condition also influenced the number of eggs produced by a female python in both years of our study (Fig. 1). These are separate effects, because the way in which we calculated our index of maternal condition (residual scores from the regression of body mass on maternal SVL) means that this score is not correlated with SVL.

## What Factors Determine the Mean Size of Eggs?

Some female pythons produced large numbers of relatively small eggs whereas others produced fewer but larger eggs. A tradeoff between egg size and egg number seems likely a priori given that a female has a finite amount of energy available for her clutch. The significant negative path coefficient between mean egg mass and clutch size offers evidence for such a tradeoff (Fig. 1) and has interesting implications for the relationship between maternal traits (body length and condition) and egg mass. Although the direct path coefficients show that egg mass is positively correlated with maternal body length and maternal condition, these latter variables are also positively correlated with clutch size (see above). Because of the tradeoff between egg size and egg number, increases in maternal body length or maternal condition have less effect on egg size than would be expected from the magnitude of their direct effects. For example, for 1991, the overall effect of an increase of 1 SD in maternal SVL is an increase of 0.74 SDs in mean egg mass (i.e., path coefficient = +0.74), whereas the indirect effect (mediated through clutch size) is +0.74 × −0.78 = −0.57. The overall effect coefficient is the sum of these two pathways, = +0.16. Similarly, the model predicts that an increase of 1 SD in maternal condition would yield an increase of 0.37 SDs in egg mass by the direct pathway, but that this effect would be reduced by 0.43 SDs (=0.55

TABLE 3.—Direct effects, indirect effects, and effect coefficients relating reproductive variables in *Liasis fuscus*. The values in this table are derived from the path diagram in Fig. 1 and show path coefficients calculated independently for data from 2 yr (1991, 1992). SVL = snout–vent length (cm); egg mass = mean mass of fertile eggs in the clutch (g); date of hatching = days since 1 September; Maternal condition after laying = residual score from linear regression of mass to SVL of females immediately after oviposition; RCM = Relative Clutch Mass. See text for explanation and more detailed definitions.

| | Clutch size | Mass of hatchling | Date of hatching | Maternal condition after laying | RCM |
|---|---|---|---|---|---|
| **Effect of maternal SVL** | | | | | |
| Direct | 0.51, 0.42 | | | | |
| Through clutch size | | −0.10, 0.10 | 0.008, 0.0007 | | −1.80, −1.23 |
| Through egg mass | | 0.66, 0.30 | | | 0.54, 0.36 |
| Through clutch size and egg mass | | −0.48, −0.22 | −0.006, −0.0005 | | 1.64, 0.50 |
| Through RCM | | | | 0.67, 0.25 | −1.20, −0.37 |
| Through clutch size and RCM | | | | −0.20, −0.07 | |
| Through egg mass, clutch size and RCM | | | | 0.44, 0.07 | |
| Effect coefficient | 0.51, 0.42 | 0.08, 0.18 | 0.002, 0.002 | 0.99, 0.98 | −0.82, −0.74 |
| **Effect of maternal body condition prior to oviposition** | | | | | |
| Direct | 0.38, 0.15 | | | 0.99, 0.98 | −0.79, −0.53 |
| Through clutch size | | −0.07, 0.04 | 0.004, 0.001 | | 0.40, 0.13 |
| Through egg mass | | 0.31, 0.39 | | | 0.77, 0.75 |
| Through RCM | | | | 0.29, 0.11 | |
| Through date of oviposition | | | −0.21, −0.02 | | |
| Through clutch size and egg mass | | −0.36, −0.08 | −0.004, −0.0002 | | −0.89, −0.13 |
| Through clutch size and RCM | | | | −0.15, −0.03 | |
| Through egg mass and RCM | | | | −0.28, −0.15 | |
| Through egg mass, clutch size and RCM | | | | 0.33, 0.03 | |
| Effect coefficient | 0.38, 0.15 | −0.12, 0.35 | −0.21, −0.02 | 1.18, 0.94 | −0.51, 0.22 |

× −0.78) by the indirect pathway. Thus, the overall effect of an increase in maternal condition would be a decrease in mean egg mass (0.37 − 0.43 = −0.06), rather than an increase as predicted from the direct effect. This example illustrates the utility of path analyses, because the influence of indirect pathways may often provide non-intuitive results such as these.

### What Factors Determine the Number of Eggs That Hatch?

The major influences on the number of eggs hatching, from a given number that are laid, are the proportion of infertile eggs and the probability of hatching experienced by fertile eggs (Table 3). This result is easily interpretable; once egg size and total allocation are fixed, infertility and failure to hatch are the only other factors that can influence effective clutch size. In our study, variance in hatching success was the more important of these two factors (Table 3). It is likely to be even more important in the field. By incubating our eggs in the laboratory, we removed any effects of physical and biological factors (e.g., desiccation, flooding, predation) that may increase the variance in hatching success among clutches in the field.

### What Factors Determine the Mean Mass of Offspring?

The path analysis shows that the mass of a hatchling python depends not only on the mass of its egg, but also on an interaction with clutch size. However, the nature of this effect differed between the two years of the study (Fig. 1), rendering it difficult to interpret this result. Mean incubation periods also differed between years, and the 1992 hatchlings emerging after prolonged incubation were longer and thinner than their equivalents in 1991 (combining data from both years: incubation period versus hatchling shape, $n = 114$, $r = 0.27$, $P < 0.003$). We hypothesise that the longer incubation gave more time for embryonic differentiation and thus resulted in offspring that had transformed more of their yolk reserves into hatchling tissues. This process would affect hatchling mass because of the greater metabolic costs

due to prolongation of development and the additional water content (and hence, mass relative to caloric content) of hatchling tissue compared to yolk (e.g., Shine, 1977; Vitt, 1978). The cause of the difference in incubation periods between years remains elusive and may relate to subtle differences in water potentials of the incubation media, as has been reported in turtles (Packard, 1991).

### What Factors Determine Offspring Shape?

Hatchling pythons from some clutches are shorter and fatter than those from other clutches, even when the mean hatchling mass is the same. This variation may be biologically significant (hatchling shape has been shown to influence the probability of survival in lizards: Olsson, 1992) and hence warrants attention. Heavier eggs produce larger hatchlings, but egg size also influences the shape of the offspring. In 1991, hatchlings emerging from larger eggs were more heavy-bodied (Fig. 1). The processes responsible for this effect remain obscure but may involve incubation conditions as suggested above.

### How Does the Sex of the Offspring Affect Other Aspects of Reproductive Biology?

Water pythons showed no sexual differences in body size (SVL, mass) or shape (mass relative to SVL) at hatching ($P > 0.10$ in all unpaired $t$-tests), and no correlation was evident between clutch sex ratio (% male offspring) and any other aspect of reproduction that we measured in this population ($P > 0.10$ in all comparisons). Hence, we can offer no insights into the adaptive significance (or lack thereof) of sex ratio variation in these animals.

### What Factors Determine the Time of Year at Which the Eggs Are Laid?

The time at which eggs are laid may have a considerable effect on the conditions under which they develop, and thus the success of incubation. Year-to-year variation in oviposition dates has been reported in many studies of reptiles, usually in response to climatic variation among

years (e.g., Pengilley, 1972). On average, oviposition in our water pythons occurred about a week earlier in 1992 than in 1991; this difference was highly significant statistically (Table 1, Fig. 4). However, the proportion of the within-year variance in oviposition dates explained by our path analysis was very low (Fig. 1). More detailed analysis provides a partial explanation for this variance. Our "repeat females" were consistent in terms of oviposition dates (see above), mainly because all "repeat" females oviposited in the same site in both years, and females from the different sites differed in oviposition dates. We scored each oviposition date in terms of number of days after 1 September. In 1991, animals collected from the varanid warrens laid their eggs earlier (mean oviposition date = 27.3, SD = 7.2, $n$ = 32) than did snakes from the wall of Fogg Dam (mean oviposition date = 41.8, SD = 8.0, $n$ = 20). The same pattern was evident in 1992, with snakes from the warrens ($\bar{x}$ = 17.7, SD = 8.1, $n$ = 21) ovipositing earlier than snakes from the paperbarks ($\bar{x}$ = 27.0, SD = 7.7, $n$ = 29) or Fogg Dam ($\bar{x}$ = 35.3, SD = 7.0, $n$ = 9) Two-factor analysis of variance of the data from Fogg Dam and the varanid warrens (with year and location as the independent variables, and date of oviposition as the dependent variable) confirmed the significance of these differences. Oviposition date was affected both by location and by year but with no significant interaction between these two main effects (location $F_{1,78}$ = 73.6, $P$ < 0.0001; year $F_{1,78}$ = 18.6, $P$ < 0.0001; interaction $F_{1,78}$ = 0.74, $P$ = 0.39). A nested two-factor ANOVA showed that the consistency in oviposition dates of the "repeat females" was due to their location ($F_{1,8}$ = 18.74, $P$ < 0.002), not to variation among individual females within each location ($F_{1,11}$ = 0.82, $P$ = 0.63).

### What Factors Determine the Length of the Incubation Period?

The timing of hatching and emergence from the nest can significantly influence hatchling survival, and thus may be of great biological significance (e.g., Ewert, 1979; James and Shine, 1985). All of the eggs
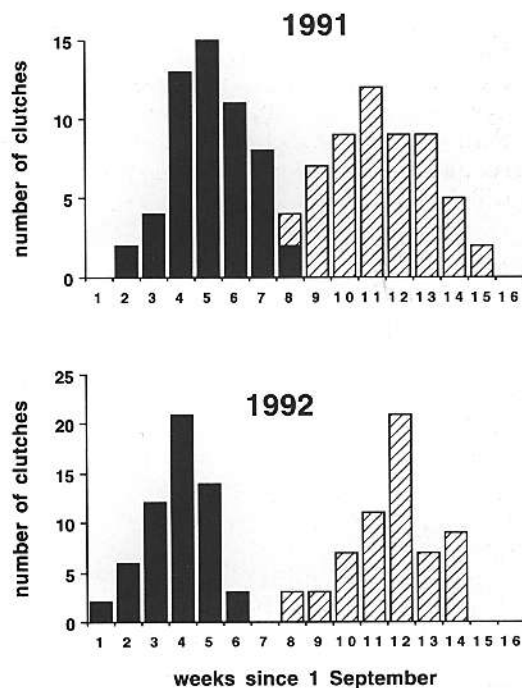


FIG. 4.—Differences in the timing of oviposition (solid bars) and hatching (hatched bars) between the 2 yr of our study. Eggs were laid later in 1991 (upper graph) than 1992 (lower graph) but actually hatched slightly earlier in 1991. See text for statistical analyses of these data.

within a single clutch typically hatched over a relatively brief time period: usually, all hatched on the same day. Hatching dates were determined primarily by oviposition dates (Fig. 1, Table 3), but the observed variance in incubation periods (range 51–74 days) deserves attention. The year-to-year variation is particularly puzzling (Fig. 4).

### What Factors Determine RCM?

Relative clutch masses were affected in a complex way by direct and indirect paths. Unsurprisingly, increases in egg size and clutch size increased RCMs (as direct effects), but the tradeoff between egg size and clutch size meant that indirect pathways reduced or reversed the impact of the direct effects. Thus, increases in clutch size increased RCM by the direct pathway (in 1991, = +0.73) but decreased RCM even more via the tradeoff between clutch

size and egg size ($-0.78 \times 2.08, = -1.62$). The overall effect of changes in maternal body size and condition on RCM is thus very complex, depending as much upon indirect effects (mediated through clutch size and egg size) as on direct effects (Fig. 1, Table 3).

### What Factors Determine Maternal Condition after Oviposition?

The body condition of females subsequent to reproduction may be an important biological variable, and a useful indicator of "costs" of reproduction. For example, overly emaciated females may be less likely to survive or to reproduce again in the following year (e.g., Madsen and Shine, 1993). Our path analysis indicated that the most important determinant of a female's condition after oviposition was her condition prior to oviposition. Several pathways are important here (Table 3). Firstly (and unsurprisingly), there is a strong direct correlation between condition scores at the two stages. Secondly, females in better condition produce lower RCMs (see negative path coefficients in Fig. 1), presumably because some of their "condition" is due to tissues (e.g., fat stores or muscle) that are not lost at oviposition. Because lower RCMs correlate with better post-oviposition condition, the net effect of this indirect pathway is to reinforce the positive correlation between maternal condition scores before and after oviposition. However, other indirect pathways reduce the magnitude of this positive effect. The larger eggs and larger clutches of females in better condition result in higher RCMs, which tend to reduce maternal condition after oviposition. Yet another indirect pathway (via effects of clutch size on egg size, and effects of egg size on RCM) add an additional positive path coefficient. The end result is a positive correlation between condition scores at the two life-cycle stages (Table 3), but the underlying causal basis for this correlation is much more complex than might have been guessed at first sight.

### GENERAL DISCUSSION

In most cases, the patterns that emerge from our analysis of reproductive output in water pythons are consistent with intuition, and with published studies on other species of snakes. The large body size of adult water pythons means that they produce larger offspring and larger clutch sizes than do most other snakes, but in other respects, they appear to be fairly typical. Reproduction is strongly seasonal (Fig. 4), with an incubation period similar to that of many other python species (Ross and Marzec, 1990). The RCM for our pythons averaged approximately 0.46 (Table 1) and thus is very close to the mean value reported in a survey of other snakes (Seigel and Fitch, 1984; note that these authors calculated RCM by including clutch mass in the denominator as well as the numerator, so that 0.46 in our calculations equals 0.32 by their method of estimation).

Our extensive data set on water pythons lets us quantify sources of variation in reproductive output of these animals in more detail than has heretofore been possible with tropical snakes. We now focus on three aspects of particular interest: the relative magnitudes of direct and indirect effects of maternal traits on reproductive output, the determinants of hatching date, and the determinants of reproductive frequency. Firstly, what can our data tell us about the factors determining traits such as egg size, clutch size, RCM, or maternal condition after oviposition? The primary insight from path analysis is that indirect effects are likely to be at least as important as direct effects, and will often be much greater. Thus, even apparently "simple" results—such as the positive correlation between maternal condition scores before and after oviposition—can be the end result of a complex series of relationships, differing in sign as well as magnitude. King (1993) documented a similar effect in other snake species.

The seasonal timing of hatching may strongly influence maternal fitness, through influences on offspring survival and growth rates (e.g., James and Shine, 1985; Tinkle, 1967). The seasonal timing of reproductive activity in tropical snakes is poorly known but appears to be diverse (Fitch, 1982). Most tropical species have seasonal reproductive cycles, especially in areas with seasonal climates (e.g., Shine, 1985; Vitt and

Vangilder, 1983). Our study site experiences high and relatively constant temperatures over most of the year, but precipitation is highly seasonal (e.g., Shine, 1986). The water pythons in this area have a tightly synchronised reproductive cycle (Fig. 4), with mating during the dry-season (July–August), oviposition late in the dry-season, and hatching occurring shortly prior to the onset of the annual monsoonal rains (November–December). The timing of emergence of the young pythons coincides with peak availability of juvenile rats (*Rattus colletti*), the major prey resource for hatchling pythons in this area (Madsen and Shine, 1996).

The date that a clutch of eggs hatches is a function of the date that it is laid and the duration of incubation, and our data show interesting patterns of variation in both of these attributes. The strongest source of variation is geographic location; in both years of the study, snakes from Fogg Dam laid their eggs about a week later than snakes from the varanid warrens. Our radiotelemetric monitoring of snakes in these areas ($n > 50$ snakes over 5 yr) suggests that thermal factors may be responsible for this difference. Snakes living in Fogg Dam spend most of their daylight hours in the water, which is relatively cool (approximately 25–30 C) whereas pythons from the varanid warrens must perforce spend their time in much hotter terrestrial environment (air temperatures >30 C within the warrens). These higher temperatures should accelerate vitellogenesis and embryogenesis, and thus result in earlier oviposition in the terrestrial snakes. This hypothesis also fits the data for oviposition dates of snakes in the paperbark forest, an area that experiences an intermediate thermal regime because it offers shaded terrestrial habitats. The significant difference in mean oviposition dates between the 2 yr of our study may also reflect thermal history: mean daily air temperatures over the month preceding oviposition were significantly lower in the year (1991) when oviposition occurred later (August daily mean minima and maxima = 13.8–32.9 C in 1991, versus 17.7–34.1 C in 1992: records of Coastal Plains Research Station).

We turn now to the question of repro-ductive frequency, which a recent review identified as the most important (and poorly understood) variable in the reproductive biology of snakes (Seigel and Ford, 1987). It is a common observation in snake populations that many adult-size females are nonreproductive (e.g., see review by Shine, 1986). Low reproductive frequencies seem to be particularly common in large snake species (e.g., Shine, 1977), and field and laboratory studies on other python taxa have generally revealed less-than-annual reproduction by adult females (e.g., Ross and Marzec, 1990; Shine and Slip, 1990; Slip and Shine, 1988). Thus, it is somewhat surprising that female pythons in our population are capable of reproducing in two successive years (as was true for at least 11 of our snakes in the current study; the other two "repeat females" reproduced in 1991 and 1993). Given the dramatic decrease in maternal mass at oviposition, and the consequent large increment in mass required before the next reproductive episode (Fig. 3), this high reproductive frequency indicates that rates of food intake in these animals must have been very high during the year between these two clutches. Our mark-recapture studies of rodents in the study area confirm this supposition, with very high population densities and biomass throughout the 2 yr of this study. Similarly, a high proportion of the pythons captured over this time contained freshly ingested prey, suggesting that food was readily available. These 2 yr may not be typical of long-term averages, however, as rodent densities show extreme annual variation in this area. In some years, snakes are in very poor condition and rarely contain food (Shine, 1993). Presumably, reproductive frequencies of the pythons decline at these times.

Even within these years of high prey abundance, only about half of the adult-size female pythons produce clutches (Fig. 2). Many of the females that forgo reproduction are relatively thin animals that have presumably failed to gather enough resources to produce a full clutch (Fig. 3). Interestingly, RCMs are relatively high in all females. Thin females tended to produce low RCMs in 1992, but the opposite was true in 1991 (note the effect coeffi-

cients for maternal condition on RCM in Table 3). This difference between years was due primarily to indirect effects (Fig. 1, Table 3). Why do not thin females reproduce with smaller clutches, instead of skipping reproduction entirely? Life-history theory suggests that such a strategy is unlikely to enhance fitness if reproduction entails significant costs that are unrelated to the level of reproductive output (e.g., Bull and Shine, 1979). In the case of water pythons, a female producing a small clutch would still face considerable energy expenditure (and, perhaps, risk of predation) if she remained to protect her eggs and to warm them by shivering thermogenesis (e.g., Slip and Shine, 1988). Under such conditions, the most efficient life history tactic may be for the female to delay reproduction until she has gathered enough energy to produce a large clutch.

There is a second group of nonreproductive females, however: those that are fat enough to reproduce but fail to do so (Fig. 3). There are many heavy-bodied females that fail to reproduce, especially at relatively small body sizes. Our analyses of growth rates in this population suggest that a heavy-bodied adult-size female that forgoes reproduction can thereby grow very rapidly. Due to the consistent increase in clutch size with maternal body size, this large increment in maternal body size greatly enhances the female's output when she finally does reproduce.

Nonetheless, total reproductive output does not necessarily increase with increasing maternal body size. At SVLs >190 cm, the proportion of reproductive females declines sharply (Fig. 2). This result offers a strong contrast to several reports of a consistent increase in reproductive frequency with increasing maternal size in other species of snakes (e.g., Blem, 1982; Seigel and Ford, 1987; Shine, 1986). Why does reproductive frequency decline in larger female water pythons? One obvious possibility is senescence, but the rapid growth rates of marked females in this population (Shine, 1991, 1993) suggest that many of these large females are relatively young. Instead, we favor a hypothesis based on increasing maintenance costs and reproductive capacity with increasing body size.

The absolute energy allocation for a large female's clutch is much greater than that required by a smaller animal. Unless the rate of food intake increases rapidly with maternal size, large females may be unable to gather enough energy for an entire clutch.

This situation may be particularly likely to occur in our population of *Liasis fuscus*, because these animals feed almost entirely on a single species of relatively small rodent (the dusky rat, *Rattus colletti*; mean adult mass = 80 g) that can be ingested by even the smallest adult python. In many other species of snakes (and perhaps in other populations of *L. fuscus*), larger snakes can locate and ingest larger prey items, and thus substantially increase their rate of food intake. In our population, however, this option is unavailable because of the scarcity of larger prey. Thus, reproductive output in female water pythons in this area may be reduced rather than enhanced by continued growth. Simple calculations show this effect clearly. A female water python of 185 cm SVL has a mean clutch size of 14.6 eggs and a probability of reproduction of 100% whereas a 205-cm female has a larger mean clutch size (17.7) but a lower probability of reproduction (71%). Thus, the overall mean annual reproductive output is actually lower ($17.7 \times 0.71 = 12.6$, versus $14.6 \times 1.0 = 14.6$) for the larger snake. Hence, the demonstration that mean clutch size increases with maternal body size does not necessarily mean that larger body size enhances a female's reproductive output.

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