

## FEMALE PHENOTYPE, LIFE HISTORY, AND REPRODUCTIVE SUCCESS IN FREE-RANGING SNAKES (*TROPIDONOPHIS MAIRII*)

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**Abstract.** Life-history theory posits causal links from an organism's phenotype (e.g., its body size and condition) to its reproductive output (e.g., offspring size and number) and thence to its microevolutionary fitness. However, few empirical data are available to quantify such links, especially for tropical organisms. Oviposition by field-caught keelback snakes (*Tropidonophis mairii*, Colubridae) in tropical Australia yielded data on clutches from 318 adult females (3151 eggs) over three years, and mark-recapture studies (1804 nights surveyed) quantified survival rates of neonates from those laboratory-incubated clutches. Path analysis revealed strong links between maternal phenotype, reproductive output, and fitness, and these links were consistent among years and across different parts of the nesting period. Although all eggs were incubated at standard water potentials, clutches varied considerably in the amount of water uptake by eggs during incubation. Hatchling body length was influenced more by the egg's water uptake than by its initial mass. Larger offspring were more likely to survive their first year of life. Overall, the rate of water uptake by a female's eggs was more important for her reproductive success (number of surviving progeny) than were variables such as mean egg mass. Hence, this population experiences intense selection not only on "conventional" life-history traits such as egg size and number, but also on maternal nest-site choice and on the physiological mechanisms that control water exchange between the egg and the nest substrate.

**Key words:** Australia; colubrid snake; fitness; incubation; keelback snake; life history; offspring size; phenotype; reproductive success; reptile; selection; *Tropidonophis mairii*.

### INTRODUCTION

Darwinian selection ultimately relies on fitness differentials among individuals within populations. Measuring such fitness differentials and the factors that influence them thus are central to understanding the action of natural selection on phenotypic traits (Endler 1986, Grant 1986, Kingsolver et al. 2001). Unfortunately, logistical difficulties preclude such measurements for many types of traits in many types of organisms, so that evolutionary biologists often are forced to infer selective forces rather than measure them directly (Endler 1986). The problem should be minimized if we focus on phenotypic traits with causal links to organismal fitness, such as the size and number of offspring produced by a female (Pianka 1976, Roff 1992). Even for life-history traits such as these, however, available data primarily describe and interpret patterns of covariation in these traits (often via interspecific comparisons), rather than empirically testing for putative links between an adult's phenotype, its reproductive output, and its fitness (Charnov 1993, Roff 2002).

Because links between adult phenotypes, life-history traits, and fitness largely have been assumed rather than

empirically verified, critical assumptions underlying adaptationist interpretations of life-history variation remain poorly supported. For example, it is of central interest to know not only whether such links exist, but also the degree to which they fluctuate through time and space. Plausibly, variation in some life-history trait (such as offspring size) might have a major influence on maternal fitness in some years but not others, or in some seasons within a year but not others. Such variation would substantially complicate, and perhaps invalidate, any simplistic interpretation of the adaptive significance of life-history variation. Accordingly, we need field-collected data sets that test models of selection on the adult phenotype mediated via life-history variation (e.g., Endler 1986, Kingsolver et al. 2001). Importantly, such studies need to encompass a wide range of phylogenetic lineages and a wide range of habitats. In this regard, one weakness of the available studies on this topic is their strong taxonomic and ecological biases, with disproportionate attention having been paid to endothermic vertebrates living in relatively cool climates (e.g., Clutton-Brock 1988, Davies 1992). Our detailed field studies on tropical snakes provide an opportunity to address this deficiency. In this paper, we use path analysis to examine links between maternal phenotype and reproductive success, mediated via life-history traits, based on a long-term and unusually intensive field study of a tropical snake population.

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PLATE 1. Adult keelback snake (*Tropidonophis mairii*). Photo credit: G. P. Brown

## METHODS

### *Study area and species*

Fogg Dam is an artificial impoundment on the Adelaide River floodplain 60 km east of Darwin, in the Australian wet-dry tropics. The floodplain is dry for most of the year, but is shallowly inundated for up to five months with the onset of seasonal monsoonal rains (Madsen and Shine 1996b). In contrast to the highly seasonal precipitation regime, temperatures remain warm year-round (monthly mean maxima 31–34°C, minima 15–24°C). The region has a variety of soil types, with deeply cracking black soils on the floodplain and sandier red soil at higher, drier elevations (Madsen and Shine 1996b).

Keelbacks (*Tropidonophis mairii*) are medium-sized (to 0.8 m snout-vent length, 250 g) nonvenomous, natricine colubrid snakes (see Plate 1). Widely distributed in near-coastal habitats throughout much of tropical and subtropical Australia (Cogger 2000), these snakes feed primarily on frogs (Shine 1991). Female keelbacks produce multiple clutches within a single breeding season, with oviposition from April through November each year (Brown and Shine 2002). Two natural nests found at Fogg Dam were located in friable mixtures of soil and organic material (fine roots, leaf, or rotting wood) 10–22 cm beneath the soil surface. Thermal and hydric conditions during incubation strongly influence the phenotypic traits of hatchling keelbacks, including body size (Webb et al. 2001, Shine and Brown 2002, Brown and Shine 2004).

### *Methods*

Between June 1998 and December 2003, we surveyed the dam wall for snakes on 90% of the nights (total  $N = 1804$  nights surveyed). Throughout this period, we attempted to catch all keelbacks that we encountered on the dam wall; these individuals were then measured and individually marked prior to release at their capture site the following day. Gravid females were easily detectable by abdominal palpation, and were kept in captivity until they laid their eggs. Of the gravid females, 75% oviposited within one week of capture and 98% oviposited within two weeks. Postpartum females were reweighed and then released at their site of capture. Eggs were measured and weighed and then incubated under constant hydric conditions. Eggs from each clutch were placed together in plastic sandwich bags containing 22 g vermiculite and 22 g water, and were incubated in an insulated cooler.

These incubation conditions are similar to those experienced by keelback eggs in natural nests. Although water content in soil from natural nests and available nesting sites at Fogg Dam was much lower than in our vermiculite treatment (soil water content ranged from 2% in the late dry season to 11% in the wet season; Shine and Brown 2002), the levels were functionally equivalent in terms of rates of water uptake by eggs (a measure of water availability). Thus, water uptake increased with higher water content in both types of substrate, such that uptake rates from eggs kept in soil with 7% water by mass were similar to those seen in

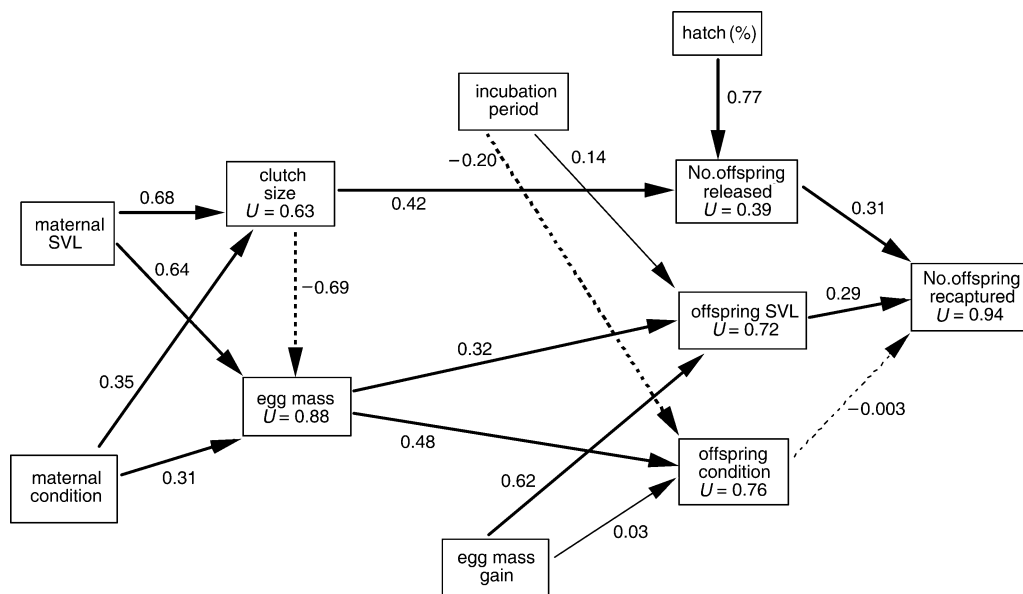


FIG. 1. Path diagram for links between phenotypic traits (body size and condition) of adult female keelback snakes, their reproductive output (size and number of hatchlings), the phenotypic traits of hatchlings, and the rate of survival to adulthood of hatchlings after release into the wild. SVL is snout-vent length; egg mass gain is the rate of water uptake by an egg during incubation. Numbers beside arrows show path coefficients based on the total data set; thick arrows show coefficients with  $P < 0.05$ ; condition is the residual score of mass relative to SVL;  $U$  is the proportion of unexplained variance. See *Analytical methods* for explanation.

eggs kept in vermiculite with 50% water by mass (Shine and Brown 2002). Temperatures inside the insulated cooler during incubation ( $23.6 \pm 3.01^\circ\text{C}$ , mean  $\pm$  SD; diel variation  $\sim 6^\circ\text{C}$ ) were similar to those measured in two natural nests ( $23.3 \pm 1.09^\circ\text{C}$ ; diel variation  $\sim 4^\circ\text{C}$ ; G. P. Brown and R. Shine, *unpublished data*).

Eggs were weighed monthly during the 8–9 week incubation period and water was added to each plastic bag to replace loss through evaporation. Within 48 hours of hatching, the neonates were measured, individually marked, and released at their mother's capture site. Many of these animals were later recaptured during our nightly surveys; the recapture period extended from November 1999 through August 2004. All data on female phenotypes and clutches in the present paper come from our studies over three calendar years (1999, 2000, 2001). Keelbacks mature quickly ( $<1$  year; Brown and Shine 2002) and are short-lived, with only 1% of individuals surviving for more than three years. Thus, our continuous capture effort extended over the expected life span of these three annual cohorts. Our analyses are based only on individuals that were marked at hatching and later recaptured as adults (based on minimum sizes at maturity of 44.5 cm SVL (snout-vent length) for females, and 27.5 cm SVL for males; Brown and Shine [2002]). These procedures provided extensive data on (1) attributes of females and their clutches; and (2) determinants (correlates) of offspring survival.

#### Analytical methods

To tease apart patterns of causation within a complex array of correlated variables, we used the multivariate technique of path analysis. This technique relies upon a specific a priori hypothesis about the nature and direction of causal links among variables, and these links are embodied in a path diagram (Fig. 1). The path coefficients that link variables are standardized regression coefficients showing the expected amount of change (in SD units) in a dependent variable that would be elicited by a change of one SD in an independent variable (Kingsolver and Schemske 1991). Coefficients for successive paths can be multiplied together to generate coefficients linking the first and last variables in that path, and the overall effect of one variable on another can be assessed by summing the coefficients along each path linking the two variables. Path analysis is well-suited to interpret relationships that are ordered in time: that is, where there is a causal relationship between "earlier" and "later" variables (Kingsolver and Schemske 1991). Previous authors have used path analysis to examine reproductive output in snakes (e.g., King 1993, Shine 1996, Weatherhead et al. 1999), including one species in the same study area (the water python *Liasis fuscus*; Madsen and Shine [1996a]). However, our analysis takes this procedure further by incorporating data on offspring survival rather than simply on maternal reproductive output.

We constructed a path diagram (Fig. 1) based upon the following well-established relationships in squamate reptiles.

1) The number and size of eggs that a female snake produces may be affected both by her body size (snout-vent length, SVL) and her body condition (mass relative to length), as represented by residual scores from the general linear regression of  $\ln(\text{mass})$  vs.  $\ln(\text{SVL})$ . The rationale for this statement is that reproductive output in snakes typically increases with increasing maternal abdominal volume and energy stores (Fitch 1970, Seigel and Ford 1991, Bonnet et al. 2001).

2) An increased clutch size may necessitate a reduced mean egg size, because of finite energy stores or abdominal volume available for the clutch (Vitt and Congdon 1978, Qualls and Andrews 1999). Allometric engineering manipulations (e.g., follicle ablation) demonstrate a causal link between these components of reproductive output in squamate reptiles (Sinervo and Licht 1991, Sinervo et al. 1992, Du et al. 2005).

3) The body length of a neonatal snake may depend not only on the initial mass of the egg from which it emerged, but also on the amount of water taken up by the egg during incubation and the duration of embryogenesis (incubation period). Experimental incubation shows that keelback eggs in nests with higher moisture take up more water and produce larger hatchlings (Shine and Brown 2002). Similar links between incubation period and offspring size have been documented in other squamates (Olsson et al. 1996, Shine and Olsson 2003).

4) A hatchling's mass relative to length may vary as a consequence of its incubation conditions, as well as the initial egg mass (and thus, energy reserves; Shine and Harlow [1996], Shine et al. [1997], Deeming [2004]).

5) The number of neonates surviving long enough to be released in the study area (a few days post-hatching) will depend upon initial clutch size and the proportion of offspring that die before or shortly after hatching.

6) Lastly, the number of neonates recaptured after their release might be affected by the number released, their body sizes (SVLs), or their energy reserves (and thus, body condition; Weatherhead and Brown [1996]). Larger hatchling size enhances survival rates in some, but not all, reptiles (e.g., Fox 1975, Ferguson and Fox 1984, Warner and Andrews 2000, 2002).

Treating individual snakes as units of replication would violate the assumption of statistical independence, because siblings might resemble each other due to maternal (including genetic) effects. Thus, we used mean values per clutch as the unit of replication for all calculations, and all females were represented by only a single clutch (their first recorded). Although some female keelbacks lay multiple clutches within a single season (Brown and Shine 2002), this occurs rarely (in <2% of females). Also, few females survive long

enough to produce clutches in more than one year. Between 1998 and 2003, only 43 of 618 females produced clutches in more than one year, and only six reproduced in three or more years (G. P. Brown, *unpublished data*). Because most female keelbacks reproduce only once, our analyses based on a single clutch per female approximate lifetime reproductive success.

Prior to statistical analysis, values for all traits were standardized to mean = 0 and SD = 1. We calculated coefficients for the path diagram (Fig. 1) based on six data sets, representing different time periods during our study or combinations thereof. The analyses were run (1) on the combined data; (2) separately for each year of the study (1999, 2000, 2001); and (3) with all years combined, but looking separately at early (April to June) and late (July to November) clutches. We performed these multiple calculations to see if the relationships among variables were consistent through time. To quantify the degree of consistency, we used the path coefficients as dependent variables to calculate Pearson product-moment correlation coefficients. To evaluate the statistical significance of differences between pairs of path coefficients, we derived 95% confidence limits based on standard errors of the estimated coefficients. For coefficients based on paths containing more than two variables (i.e., obtained by multiplying together the successive coefficients for each segment of the path), we calculated standard errors by randomly generating 10 000 values based on the mean and variance of each coefficient, and multiplying these values together to obtain an estimate of the mean and standard error of the combined path coefficient.

## RESULTS

### *Collinearity*

Collinearity among independent variables can be problematic for multiple regression techniques, including path analyses (Petraitis et al. 1996). Diagnostic indices used to assess levels of collinearity include variance inflation factors (VIF) and the collinearity condition index (Petraitis et al. 1996). General rules of thumb are that VIFs should be <10 and condition indices should be <30. Because several of the measures that we used as independent variables were correlated with one another (Appendix A), we calculated VIFs and condition indices for all standardized regressions used in our path analyses to assess multicollinearity. The maximum VIF from all the regressions was 2.73 and the maximum condition index was 3.02, both well below levels at which collinearity may substantially bias estimates of path coefficients.

### *Range of variation in traits*

There was considerable variation in the sizes of female keelbacks and their clutches, eggs, and hatchlings (Appendix B). Fig. 1 shows the path diagram with coefficients calculated from the overall (combined) data



set, and Table 1 summarizes path coefficients calculated separately for each of the six data sets just described. In the combined data set, all path coefficients  $>0.15$  (i.e., 13 of 16 coefficients) were statistically significant ( $P < 0.05$ ), although in some cases, they explained only a small proportion of overall variation in the dependent variable (“ $U$ ” beneath each variable shows the proportion of unexplained variance  $\sqrt{1 - R^2}$ ).

#### *Temporal consistency*

Table 1 shows that, in general, the relationships among variables were consistent among years and similar between early vs. late clutches. Reflecting this pattern, the 15 pairwise comparisons among standardized path coefficient columns in Table 1 ranged from 0.35 to 0.98, and in 14 of these 15 comparisons, the coefficients were more highly correlated with each other than would have been expected by chance (in all of these cases,  $r > 0.53$ ,  $P < 0.003$ ). For example, patterns of covariation among variables were remarkably similar for early vs. late clutches ( $r = 0.82$ ,  $n = 29$ ,  $P < 0.0001$ ). The sole nonsignificant value was the comparison between coefficients in 1999 vs. 2001 ( $r = 0.35$ ,  $n = 29$ ,  $P = 0.06$ ).

#### *Links between maternal phenotype and reproductive output*

Increasing maternal body length resulted in a significant increase in clutch size and mean egg mass (Fig. 1, Appendix A). Changes in maternal body condition (mass relative to length) had a similar, but weaker, effect on the size and number of a female’s eggs (Fig. 1). Although larger, more heavy-bodied females thus produced both more eggs and larger eggs, the path diagram also reveals a strong trade-off between these two aspects of reproductive output: increases in clutch size were associated with decreases in mean egg mass (Fig. 1).

#### *Links between reproductive output and offspring phenotype*

The body length (SVL) of a hatchling keelback was affected by multiple factors. Most obviously, initial investment in the egg affected hatchling size: larger eggs produced neonates that were both longer and fatter than were those from smaller eggs. However, the egg’s response to its incubation environment also was important. Eggs that took up more water during incubation produced longer neonates than eggs that took up less water (Fig. 1). Indeed, water uptake had more effect on neonatal SVL than did initial egg mass (coefficients 0.62 vs. 0.32; see Fig. 1). In contrast, body condition (mass relative to length) of the hatchling snake was affected by initial egg mass, but not by water uptake of the egg during incubation (Fig. 1). Eggs that hatched after longer incubation generally produced thinner neonates (Fig. 1). Hatching success also varied

among clutches, generating considerable variation in the number of offspring released (Fig. 1).

#### DISCUSSION

Of the three traits that we examined, one (body condition at hatching) did not affect juvenile survival rates (Fig. 1). Both of the other traits were important, with most of the recaptured (i.e., surviving) offspring coming from clutches from which many offspring had been released, and where mean offspring size was large (Fig. 1).

#### *Overall links between reproductive output and maternal reproductive success (surviving offspring)*

In total, the path diagram indicates strong and temporally consistent links between a female keelback’s phenotype (i.e., her body size and condition) and her fitness (number of surviving offspring), mediated through her reproductive output (offspring size and number), but with important influences from developmental plasticity during embryogenesis (especially water uptake during incubation) also. In total, two main factors determine maternal reproductive success (i.e., how many surviving offspring a female keelback produced during the study period): (1) the number of her offspring that were released, in turn determined by her initial clutch size and by the hatching success of those eggs; (2) the body lengths of her offspring at hatching. Hatchling SVL was partly determined by initial egg mass, but was affected even more strongly by water uptake during incubation. Thus, the amount of water taken up by a female’s eggs was more important than the initial mass of her eggs in determining her fitness (0.18 vs. 0.09; see Table 1).

Overall, a female keelback’s body size was at least as important a determinant of her fitness as was her body condition (mass relative to length) (Table 1, coefficients 0.10 vs. 0.05; NS with randomization test). Indeed, reproductive output (clutch size and egg size) was enhanced more by greater SVL than by better body condition (Table 1). Thus, although many analyses of snake reproduction have focused on the role of maternal energy reserves (especially fat stores) in fuelling reproduction (e.g., Naulleau and Bonnet 1996, Bonnet et al. 2001), female keelbacks may enhance fitness more by allocating energy to body growth than by storing it as lipids. Perhaps reflecting this situation, female keelbacks attain much larger body sizes than conspecific males in our study population (Brown and Shine 2002).

Similarly, although intuition suggests that better body condition (greater energy stores) might contribute to neonatal survival, our field data suggest that a young snake’s body length played a more important role in this respect (Table 1, coefficients  $-0.003$  vs.  $0.29$ ;  $P < 0.025$  with randomization test). Again, this result suggests that juveniles may benefit from allocating energy to growth rather than storage, and indeed these young snakes display remarkably rapid growth and early maturation (five months in males, nine months in

TABLE 1. Path coefficients linking phenotypic traits (body length and condition) of female keelback snakes to their reproductive output (numbers and sizes of eggs), phenotypes of hatchlings and numbers surviving to be released, and numbers of offspring surviving to be recaptured.

| Source and pathway                       | All years     | 1999          | 2000         | 2001          | Early         | Late         |
|--|---------------|---------------|--------------|---------------|---------------|--------------|
| <b>Maternal SVL</b>                      |               |               |              |               |               |              |
| Clutch size–no. hatchlings released      | 0.089         | 0.129         | 0.043        | 0.060         | 0.106         | 0.092        |
| Egg mass–hatchling SVL                   | 0.059         | 0.046         | 0.044        | 0.083         | 0.075         | 0.068        |
| Egg mass–hatchling condition             | –0.001        | –0.074        | 0.003        | 0.099         | –0.055        | 0.016        |
| Clutch size–egg mass–hatchling SVL       | –0.044        | –0.037        | –0.026       | –0.073        | –0.070        | –0.033       |
| Clutch size–egg mass–hatchling condition | 0.001         | 0.058         | –0.002       | –0.087        | 0.051         | –0.008       |
| <b>Total</b>                             | <b>0.104</b>  | <b>0.124</b>  | <b>0.063</b> | <b>0.081</b>  | <b>0.107</b>  | <b>0.135</b> |
| <b>Maternal condition</b>                |               |               |              |               |               |              |
| Clutch size–no. hatchlings released      | 0.046         | 0.040         | 0.036        | 0.027         | 0.056         | 0.045        |
| Egg mass–hatchling SVL                   | 0.029         | 0.022         | 0.051        | 0.045         | 0.050         | 0.026        |
| Egg mass–hatchling condition             | 0.000         | –0.035        | 0.004        | 0.054         | –0.036        | 0.006        |
| Clutch size–egg mass–hatchling SVL       | –0.022        | –0.011        | –0.021       | –0.033        | –0.037        | –0.016       |
| Clutch size–egg mass–hatchling condition | 0.000         | 0.018         | –0.002       | –0.039        | 0.027         | –0.004       |
| <b>Total</b>                             | <b>0.052</b>  | <b>0.033</b>  | <b>0.067</b> | <b>0.054</b>  | <b>0.060</b>  | <b>0.057</b> |
| <b>Clutch size</b>                       |               |               |              |               |               |              |
| No. hatchlings released                  | 0.130         | 0.180         | 0.076        | 0.079         | 0.156         | 0.133        |
| Egg mass–hatchling SVL                   | –0.064        | –0.051        | –0.046       | –0.096        | –0.103        | –0.048       |
| Egg mass–hatchling condition             | 0.001         | 0.080         | –0.003       | –0.115        | 0.075         | –0.011       |
| <b>Total</b>                             | <b>0.067</b>  | <b>0.209</b>  | <b>0.027</b> | <b>–0.132</b> | <b>0.128</b>  | <b>0.074</b> |
| <b>Egg mass</b>                          |               |               |              |               |               |              |
| Hatchling SVL                            | 0.093         | 0.054         | 0.130        | 0.113         | 0.111         | 0.104        |
| Hatchling condition                      | –0.001        | –0.086        | 0.009        | 0.135         | –0.081        | 0.024        |
| <b>Total</b>                             | <b>0.091</b>  | <b>–0.032</b> | <b>0.140</b> | <b>0.248</b>  | <b>0.030</b>  | <b>0.129</b> |
| <b>Incubation period</b>                 |               |               |              |               |               |              |
| Hatchling SVL                            | 0.041         | 0.011         | 0.176        | –0.070        | –0.022        | 0.101        |
| Hatchling condition                      | 0.001         | 0.014         | –0.003       | –0.051        | 0.019         | –0.022       |
| <b>Total</b>                             | <b>0.041</b>  | <b>0.024</b>  | <b>0.173</b> | <b>–0.121</b> | <b>–0.003</b> | <b>0.079</b> |
| <b>Egg mass gain</b>                     |               |               |              |               |               |              |
| Hatchling SVL                            | 0.180         | 0.175         | 0.118        | 0.125         | 0.135         | 0.252        |
| Hatchling condition                      | 0.000         | –0.029        | 0.000        | 0.054         | 0.005         | 0.004        |
| <b>Total</b>                             | <b>0.180</b>  | <b>0.146</b>  | <b>0.118</b> | <b>0.179</b>  | <b>0.140</b>  | <b>0.256</b> |
| <b>Percentage hatch</b>                  |               |               |              |               |               |              |
| No. hatchlings released                  | <b>0.239</b>  | <b>0.189</b>  | <b>0.150</b> | <b>0.396</b>  | <b>0.213</b>  | <b>0.303</b> |
| No. hatchlings released                  | <b>0.310</b>  | <b>0.290</b>  | <b>0.200</b> | <b>0.440</b>  | <b>0.300</b>  | <b>0.370</b> |
| Hatchling SVL                            | <b>0.290</b>  | <b>0.180</b>  | <b>0.420</b> | <b>0.390</b>  | <b>0.270</b>  | <b>0.360</b> |
| Hatchling condition                      | <b>–0.003</b> | <b>–0.090</b> | <b>0.030</b> | <b>0.300</b>  | <b>–0.090</b> | <b>0.090</b> |

*Notes:* These standardized path coefficients are based on the path diagram in Fig. 1. The first column shows the independent variable for a pathway and the series of intermediate variables along specific pathways, and the totals (boldface) show the summed overall effect of the first (independent) variable on maternal fitness (number of surviving offspring). For example, analysis of the total data set (all years) yields a path coefficient of 0.089 linking maternal snout–vent length (SVL) to reproductive success via a path with two intermediate variables (clutch size and number of hatchlings released), and the overall path coefficient (summing coefficients for all the routes by which maternal SVL could affect reproductive success) was 0.104. Path coefficients were calculated separately for six subsets of the data or combinations thereof: all years combined (318 clutches); 1999 only (134 clutches); 2000 only (103 clutches); 2001 only (81 clutches); early season only (April–June, 152 clutches); and late season only (July–November, 166 clutches). SVL is snout–vent length.

females) in our study population (Brown and Shine 2002). There was substantial variation among years and between early and late clutches in the relative importance of hatchling condition and SVL (Table 1). However, there was no evidence of a trade-off between the two factors. Hatchling body condition was not more important during periods when the influence of hatchling SVL was low. Instead, there was a positive, but nonsignificant, correlation between the path coefficients of hatchling condition and hatchling SVL over the six analysis periods ( $r = 0.70$ ,  $P = 0.12$ ; Table 1).

Any reproducing female organism is faced with the allocation “decision” as to the balance between num-

ber and size of offspring; given finite resources (energy, abdominal space), any increase in offspring size must be penalized by a decrease in clutch size (Sinervo and Licht 1991, Olsson and Shine 1997). Our data on keelbacks bear upon this issue in two ways. First, the force of the trade-off between offspring size and number was stronger in terms of egg mass than offspring size, because much of the variance in hatchling size among clutches was generated after oviposition, by differential water uptake rates of eggs (Fig. 1). Water uptake not only differs among clutches incubated under standard hydric conditions, but also depends upon moisture levels in the soil: wetter substrates result in higher rates

of mass gain by eggs (Brown and Shine 2004). A female keelback thus may be able to partially escape the offspring size vs. number trade-off by depositing relatively dehydrated (and thus small) eggs into moist nest sites. In this way, a female might be able to achieve both a large clutch size and a large offspring size.

Second, optimality models predict that natural selection will fine-tune the balance between offspring size and clutch size such that the number of surviving offspring is maximized (Smith and Fretwell 1974). If so, we might expect that the path coefficients linking recapture rates to offspring size and number would be similar in magnitude. If this were not true, a female could enhance her fitness by changing her allocation rules to favor the attribute (size vs. number) that made a stronger fitness contribution. As predicted, path coefficients for the number of offspring released did not differ significantly from those for offspring body size in any of the three years of our study (randomization tests all have  $P > 0.05$ ), despite annual variation in the relative magnitudes of these pathways (Table 1). Year-to-year variations in the survival advantages accruing from larger offspring size have been reported in previous studies (Fox 1975, Ferguson and Fox 1984, Olsson and Madsen 2001).

In terms of actual reproductive output, the critical variables are egg mass and clutch size. Table 1 shows that in the first two years of our study (1999 and 2000), females benefited more from larger clutches than larger eggs (in 1999, coefficients 0.21 vs.  $-0.03$ ; randomization test with  $P < 0.001$ ; in 2000, 0.03 vs. 0.14;  $P < 0.05$ ), whereas in the third year (2001), maternal fitness was enhanced more by having larger eggs than by producing more eggs per clutch (coefficients of 0.25 vs. 0.13;  $P < 0.001$ ). Thus, these data reveal annual variation, but are consistent with the prediction of an equilibrium enforced by the trade-off between offspring size and number. Path coefficients linking clutch size and maternal reproductive success generally varied inversely with path coefficients linking egg mass and maternal reproductive success: that is, a greater role for one of these variables was associated with a lesser role for the other. Thus, a comparison of path coefficients over the six time periods used for our analyses revealed a significant negative correlation between the effects of clutch size vs. egg mass on maternal reproductive success ( $r = -0.98$ ,  $P = 0.0008$ ; Table 1).

Importantly, our path analysis showed that one of the strongest determinants of a female snake's fitness (number of surviving progeny) was the rate of water uptake by her eggs during incubation. These rates of water uptake were measured under constant water availability during laboratory incubation, and variation in this trait in the field would be significantly increased by spatial and temporal variation in soil water content (Brown and Shine 2004). The existence of strong (and fitness-relevant) variation in rates of water uptake among clutches in the present study means that factors other than sub-

stratum water potential also influenced hydric exchange between the egg and its environment. For example, interclutch variation in traits such as mean eggshell thickness and embryonic physiology may be significant in this respect, and warrant further study.

In summary, fitness of free-ranging female keelback snakes in tropical Australia bore strong and temporally consistent relationships to the phenotypic traits of those animals (especially maternal body size). The pathways that linked female phenotypes to reproductive success were relatively straightforward, with larger clutch sizes and larger offspring sizes being the best predictors of maternal fitness. However, the developmental plasticity of snake eggs in response to the hydric environment adds significant complications to this picture, suggesting that there will be strong selection both on maternal nest-site selection behaviors, and on the ability of eggs to sequester water from the substrate during embryogenesis. Maternal behavior and embryonic physiology thus play critical roles in the causal links between phenotypes and fitness in adult snakes.

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#### APPENDIX A

A table showing Pearson correlation coefficients between pairs of variables used in combined path analysis is available in ESA's Electronic Data Archive: *Ecological Archives* E086-148-A1.

#### APPENDIX B

A table showing overall mean values and associated standard deviations and ranges for variables used in the path analysis is available in ESA's Electronic Data Archive: *Ecological Archives* E086-148-A2.