

Factors influencing offspring traits in the oviparous multi-clutched lizard, *Calotes versicolor* (Agamidae)

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The determinants of offspring size and number in the tropical oviparous multi-clutched lizard, *Calotes versicolor*, were examined using both univariate and multivariate (path) analyses. In *C. versicolor* maternal snout-vent length (SVL) and body condition influence clutch mass and clutch size but have no significant influence on offspring size. The positive effect of maternal SVL and body condition on offspring number is counterbalanced by a negative effect of breeding time on egg mass. In fact, breeding time directly influences the offspring body mass and condition through variation in the egg mass. There is a trade-off between offspring mass and condition with offspring number, and breeding time influences both. Offspring hatched from the eggs of early (May–June) or mid (July–August) breeding periods invariably show lower mass and condition than those hatched from the eggs of late breeding season (September–October). Yet, there is no variation in offspring SVL among early, mid and late clutches. Thus, in *C. versicolor* offspring SVL is optimized while body mass and condition are not optimized.

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1. Introduction

Female body size, offspring number and offspring body size are basic components of an organism's life history. In many reptilian species clutch and offspring sizes are related to each other and to maternal body size. Natural selection may not always produce 'optimal' phenotypes because of trade-offs and mechanical constraints (Olsson and Shine 1997). Also offspring size and number are influenced by several other exogenous factors like food availability, breeding time and predator pressure (Seigel and Ford 1987; King 1993; Olsson and Shine 1997; Shanbhag *et al* 2000). However, the causal basis for these relationships remains controversial (Olsson and Shine 1997; Weatherhead *et al* 1999). A central problem in life history pertains to understanding how a female partitions her available energy for reproduction. A few studies show that when females have sufficient energy to invest in reproduction, they will partition an optimum amount of energy in each offspring. Thus, production of more offspring of similar size may maximize the female's fitness (Smith

and Fretwell 1974; McGinley *et al* 1987; Roff 1992). Yet a substantial variation in egg/offspring size is observed within a population, presumably due to non-optimization of egg size or offspring size (Smith and Fretwell 1974; McGinley *et al* 1987, McGinley 1989; Roff 1992; Stearns 1992). These studies have been carried out in species that breed either once in a breeding season or produce a small number of eggs in a reproductive bout.

Calotes versicolor, a multi-clutched oviparous lizard (Shanbhag and Prasad 1993; Shanbhag *et al* 2000), is a good model to elucidate factors controlling offspring size, number and reproductive investment because maternal investment in offspring is complete by the time the eggs are ovulated. The factors influencing clutch and egg sizes in *C. versicolor* have been described; maternal size influences clutch mass while breeding timing influences clutch and egg sizes (Shanbhag *et al* 2000). There is no optimization of egg size in *C. versicolor*. However, the relative indirect effects, and magnitude of causation of all possible factors influencing offspring size are not known. Hence, in the present study, data on offspring size, condition and

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number in *C. versicolor* were analysed by univariate as well as multivariate path analysis to determine the causal pathway among reproductive variables. The objective of the present study was to elucidate the complex interrelationships between various reproductive traits (such as egg size, egg mass and clutch size) and factors [such as maternal snout-vent length (SVL) and condition and breeding time and clutch mass] influencing offspring size and number, about which not much is known for any tropical multi-clutched lizards.

2. Materials and methods

Gravid *C. versicolor* ($n = 25$) were collected from a village 25 km away from Dharwad City ($15^{\circ}17'N$ and $75^{\circ}3'E$), Karnataka State, India during May–October 1998 from a single population exclusively for this study. Lizards were brought to the laboratory and housed in wire mesh terraria ($90 H \times 120 W \times 120 L$ cm, 3 or 4 lizards/terrarium) until oviposition stage. Oviposition was confirmed following the methods adopted by Shanbhag *et al* (2003). Each terrarium contained a few potted plants, dry sticks, hiding sites, water and a substratum of black soil obtained from the capture site. Food (grasshoppers/crickets/cockroaches) was provided *ad libitum* on alternate days. Since gravid females are found from May to October, they were categorized as early, mid and late breeders depending upon the capture date (following the procedure of Shanbhag *et al* 2000).

After confirming oviposition stage, body mass and SVL were recorded. The eggs were then stripped from the oviduct. The numbers of oviductal eggs, total clutch mass and individual egg mass were recorded. Eggs were incubated in moist soil at ambient temperature ($27 \pm 2^{\circ}C$) as described earlier (Radder *et al* 2002a). Eggs hatched after 70 ± 5 days of incubation and hatching success was $> 95\%$ for each clutch. At hatching, SVL, total length and

body mass of the offspring were recorded. The offspring were released in the botanical garden of the University 10 days later.

2.1 Data analyses

Data for all recorded variables were log transformed to meet the assumptions of regression and for facilitating biological interpretation and comparisons (King 2000). Maternal and offspring conditions were determined by generating residuals from the regression of log body mass on log SVL. In both univariate and multivariate analyses mean figures for each trait/clutch were used to avoid statistical non-independence and pseudoreplication. In univariate analysis Carl Pearson correlation and partial correlation methods were adopted while in multivariate analysis data were analysed by path analysis for assessing potential causative effects of multiple independent variables (Kingsolver and Schemske 1991; Mitchell 1992; Smith *et al* 1997). Path analysis has several advantage over multiple regression and partial correlation as it decomposes correlation between two variables in to: (i) the direct effect of the first variable on the second; (ii) indirect effect of the first variable on the second through other variables; (iii) spurious components due to common causation of the two variables by other variable; and (iv) unanalysed components due to unmeasured but correlated causes of the two variables (King 1993; Weatherhead 1999). Thus, direct and indirect causal components can be identified and distinguished from non-causal (spurious and unanalysed) components. Further, path analysis allows one to identify and to assess the magnitude of both direct and indirect effects, whereas only direct effects are assessed by multiple regression. To perform path analysis a visual representation of hypothesized causal pathways (path diagram) was generated (figure 1) based on data from our

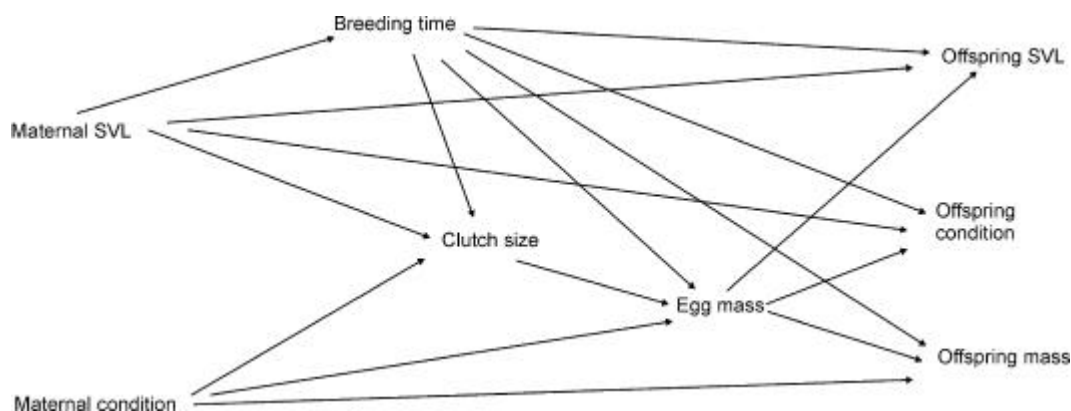


Figure 1. Hypothetical path diagram showing direct and indirect factors influencing offspring size in *C. versicolor*.

previous study on *C. versicolor* from the same population (Shanbhag *et al* 2000). This was our *a priori* model. Fresh data (the present study) was collected and used to test the model. Shanbhag *et al* (2000) showed that in *C. versicolor* breeding time is the prime factor that influences the clutch mass and egg mass. Clutch size is negatively correlated with breeding time. Maternal SVL is positively related to clutch size but not egg size. Also, in a given breeding time, gravid females of different SVL are encountered.

Based on this study (Shanbhag *et al* 2000), it is hypothesized that offspring traits (SVL, body mass and condition) are determined by breeding time, maternal SVL and condition and egg mass through clutch size. The path analysis was designed to assess the direct effects of maternal factors (size and condition), clutch size, egg size and breeding time on offspring traits (SVL, body mass and condition). The strength of direct effects (i.e. effect of one variable on another variable when all other variables are held constant) was determined by standardized partial regression coefficients obtained from multivariate regression using a forced entry procedure. Partial regression coefficients and their standard errors are considered equivalent to path coefficients (Li 1975; Pedhazur 1982; King 1993) which indicate the amount of change in the dependent variable to a change of one standard deviation in the independent variable(s). The strength of indirect effect was calculated as the product of direct effects between the variables involved. The overall effect of one variable on another (effect coefficient) was calculated by summing the direct effects between the two variables and all indirect effects operating through intermediate variables (Sokal and Rohlf 1981). Such analyses help to estimate relative magnitude and direction of effects.

The path analysis was based on three independent variables (i.e. maternal SVL, condition and breeding timing) and five multivariate regressions: (i) Clutch size regressed on female SVL and condition, and breeding time; (ii) egg mass regressed on clutch size, maternal condition and breeding time; (iii) offspring SVL regressed on egg mass, female SVL and breeding time; (iv) offspring mass regressed on egg mass maternal SVL and breeding time; and (v) offspring condition regressed on female SVL, egg mass and breeding time.

Though one can derive path coefficients for any number of variables and paths through all possible ways using a path diagram and coefficients, we have not considered the paths that bypass breeding time or clutch size since both are the prime factors that influence clutch mass and egg mass. Further, we have also considered only five important paths so as to ensure reliability of analysis, because a general rule of thumb in path analysis is that sample sizes should be at least 5 to 20 times the number of estimated paths (Petraitis *et al* 1996).

Variation in clutch size among early, mid and late breeders was analysed by one way ANOVA followed by Scheffe's multiple range tests using residual clutch size. Residuals for clutch size were generated by regressing clutch size on maternal SVL. The use of residuals for comparative studies has been criticized previously (Garcia-Berthou 2001). However, recently it was reported that residuals provide negative and positive values that enable interpretation of the variations among years/seasons within a single population in a straightforward way (Bonnet *et al* 2003). For data analysis dates of collection of lizards were used as variables (for breeding time) to avoid the problem of using ordinal variables in regression analysis. Collinearity among variables was analysed by collinearity diagnostics using variation inflation factors (VIF). All variables met assumptions of normality and homoscedasticity. Significance was accepted at the 0.05 level in univariate analyses. Analyses were carried out using Statistical package for the Social Sciences (SPSS, version 6.1.3).

3. Results

All the recorded body and reproductive traits are summarized in table 1. The data on the relationship between maternal SVL, clutch size and egg size with respect to breeding season are presented in table 2. Egg and offspring traits (the variables of our interest) varied significantly between clutches. There was no relationship between maternal SVL and offspring SVL or mass or condition ($r = -0.28$, $P > 0.05$; $r = -0.34$, $P > 0.05$ and $r = -0.20$, $P > 0.05$, respectively, $n = 25$). Further, offspring body mass and condition increased with increase in the egg mass ($r = 0.60$, $P < 0.01$ and $r = 0.48$, $P < 0.05$ respectively).

3.1 Path analysis

The path diagram (figure 2) describes relationship among different reproductive variables. Path coefficients indicate the sign and magnitude of direct effects among variables.

Table 1. Summary of recorded traits of females and their offspring in *C. versicolor* ($n = 25$ clutches).

Variable	Mean	SD	Range
Maternal SVL (cm)	10.14	0.82	8.90–11.90
Maternal condition (log g)	0.00	0.08	–0.23–0.91
Clutch size	19.04	5.15	11.00–29.00
Clutch mass (g)	8.44	5.02	5.02–11.99
Egg mass (g)	0.47	0.09	0.35–0.72
Offspring SVL (cm)	2.70	0.18	2.50–3.00
Offspring mass (mg)	598.65	96.40	430.00–831.00
Offspring condition	0.00	0.05	–0.10–0.07
Offspring total length (cm)	7.70	0.27	7.15–8.17

Values along the arrows are direct-effect coefficients. Table 3 shows direct and indirect effects and effect coefficient related to offspring size and number in *C. versicolor*.

Analysis of a direct effect of one variable on another (holding all other variables constant) yielded the following results: (i) Clutch size was larger for larger females; female condition had a little positive effect. (ii) Clutches were large in early breeding season and decreased in late breeding season. (iii) Clutch size was negatively correlated with mean egg mass. (iv) Offspring condition and mass decreased with increase in clutch size (table 3).

Major indirect effects obtained by computing the product of coefficients along indirect paths (figure 2) from one variable to another were as follows: (i) Larger females produced bigger clutches with small sized eggs ($0.65 \times -0.68 =$

-0.44) especially during the early and middle part of the breeding season (figure 2). (ii) Offspring produced during the early and middle part of the breeding season were lighter than those produced in late breeding season. Thus, clutch size and egg mass were negatively correlated with breeding timing and lizards breeding later in the breeding season produced smaller clutches with heavier offspring ($-0.34 \times -0.68 \times 0.92 = 0.22$, figure 2) than early and mid breeders. Thus, a positive direct effect of maternal SVL on clutch size was balanced by negative indirect effects of breeding time on clutch size and egg mass. Similarly other indirect effects can be deduced from the path diagram for the remaining variables from table 3 and figure 2.

The effect coefficients revealed that maternal SVL had very little or no overall influence on offspring SVL in *C. versicolor* ($-0.006 + 0.009 + -0.031 = -0.028$, figure 2, table 3). Likewise, the overall effect of maternal condition was least on offspring SVL and condition (figure 2, table 3). The effect coefficient that explains influence of breeding time through clutch size and egg mass on offspring traits revealed that it was least for SVL ($-0.05 + 0.16 = 0.11$, figure 2 and table 3), while it was larger with respect to offspring body mass ($0.08 + 0.21 = 0.29$, figure 2 and table 3) and condition ($0.14 + 0.11 = 0.25$, figure 2 and table 3). Further, all variables included in the path diagram had least influence on offspring SVL; the latter was maximally influenced by unexplained variation (0.96, figure 2). The important effect coefficients for other traits are

Table 2. Univariate relationships between the body and reproductive traits of *C. versicolor* ($n = 25$ clutches, computed by Carl Pearson correlation coefficient analyses).

Relationship between	<i>r</i>	<i>P</i>
Maternal SVL and clutch size	0.71	< 0.05
Maternal condition and clutch size	0.45	< 0.05
Maternal SVL and egg mass	0.31	> 0.05
Maternal condition and egg mass	0.33	> 0.05
Clutch size and egg mass	-0.57	< 0.01
Clutch size and breeding period	-0.47	< 0.05
Egg mass and breeding time	0.55	< 0.05

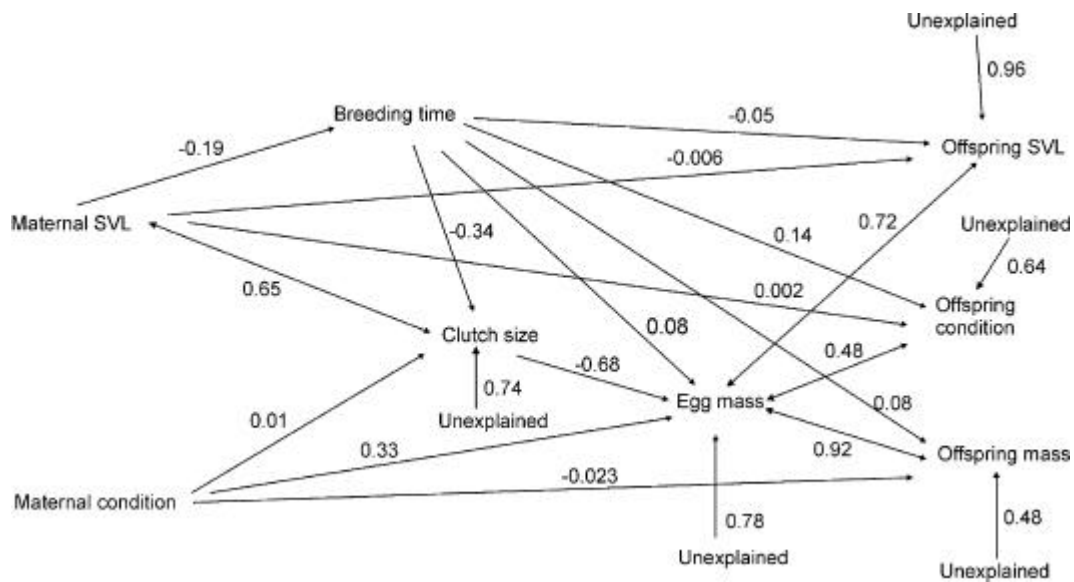


Figure 2. Path diagram showing observed relationships among reproductive variables in *C. versicolor*. Values along with the arrows are direct effects (standardized partial regression coefficients). Double headed arrows indicate sign and magnitude of correlation. Residual variation not accounted for by variables included in the path diagram is indicated as 'unexplained'. Path coefficients associated with 'unexplained' variation are equal to $(1-R^2)^{0.5}$, where R^2 is the variation explained by multiple regression for each dependent variable ($n = 25$ clutches).

shown in table 3 and figure 2. Interrelationships for other reproductive variables can be obtained from table 3 and figure 2.

4. Discussion

Path analysis was used to investigate the factors influencing the offspring number and quality, and the contribution of factors influencing offspring size in *C. versicolor*. The results provide no evidence for a direct relationship between maternal SVL and offspring size. One finding of the present study is that there is a trade-off between number and body mass and condition of offspring in *C. versicolor* and the trade-off varies with breeding timing via egg size. Early breeders produce large clutches but lighter offspring than produced by late breeders. These findings are consistent with those on other species of reptiles studied so far (Roff 1992; King 1993; Olsson and Shine 1997; Weatherhead *et al* 1999). In *C. versicolor* offspring SVL did not vary with respect to early, mid or late breeding. Also, the influence of various factors on offspring trait when considered independently as depicted in the path diagram showed least effects for the offspring SVL. A major contribution to the variations in offspring mass and condition arises from a negative relation between clutch size, egg mass and breeding time.

Hence, an increase in clutch size is accompanied by a decrease in offspring mass and condition, evidently due to a decrease in egg mass.

Optimal offspring size theory predicts that within each population, there exist optimally sized offspring. If so, all females in a population should produce offspring of similar size and no trade-off between offspring size and number should occur (Bernado 1996). In studies testing optimal offspring size theory in reptiles, the body mass of offspring is used as a criterion of body size rather than SVL. In fact SVL reflects linear growth of an individual. In the present study we considered both SVL and body mass independently as body size criteria. Interestingly, the optimal offspring theory holds good for offspring SVL in *C. versicolor* because that remained constant in the offspring of all clutches. However, this theory does not hold good when offspring body mass and conditions are considered as the parameters of body size in the lizard. The present study points out that both SVL and mass are to be taken into consideration while studying influence of egg size on offspring size in multi-clutched lizards.

It is believed that the offspring size varies with litter size in response to a variation in reproductive effort (Winkler and Wallin 1987). In *C. versicolor* reproductive effort (clutch mass), which as a function of body size remains comparable throughout breeding season though clutch size,

Table 3. Direct effects, indirect effects and effect coefficients relating to various reproductive traits in *C. versicolor* (analyses are based on log transformed data).

Effect of	Effect on clutch size	Effect on egg mass	Effect on offspring		
			SVL	Mass	Condition
Maternal SVL (direct)	0.650	–	– 0.006	–	0.002
Through breeding time	0.060	– 0.015	0.009	0.015	– 0.026
Through breeding time and clutch size	–	– 0.043	–	–	–
Through breeding time, clutch size and egg mass	–	–	– 0.031	– 0.04	– 0.021
Effect coefficient	0.710	– 0.058	– 0.028	– 0.025	– 0.045
Maternal condition (direct)	0.010	0.330	–	– 0.023	–
Through clutch size	–	– 0.006	–	–	–
Through clutch size, egg mass	–	–	– 0.005	– 0.006	– 0.003
Effect coefficient	0.010	0.324	– 0.005	– 0.029	– 0.003
Breeding time (direct)	– 0.340	0.080	– 0.050	0.080	0.140
Through clutch size	–	0.230	–	–	–
Through clutch size and egg mass	–	–	0.160	0.210	0.110
Effect coefficient	– 0.340	0.310	0.110	0.290	0.250
Clutch size (direct)	–	– 0.680	–	–	–
Through egg mass	–	–	– 0.490	– 0.630	– 0.320
Effect coefficient	–	– 0.680	– 0.490	– 0.630	– 0.320
Egg mass (direct)	–	–	0.720	0.920	0.480

Direct effects were determined by standardized partial regression coefficients from multivariate regression. Indirect effect was calculated as the product of direct effects between the variable involved and effect coefficient was calculated by summing the direct effects between the two variables and all indirect effects operating through intermediate variables.

declines in late breeding season (Shanbhag *et al* 2000). Therefore, in *C. versicolor* production of larger clutches or larger eggs does not burden the female in terms of its energy allocation strategy for reproduction. Production of heavier offspring in *C. versicolor* especially at the end of breeding season might enhance their survival as these have to compete with conspecific hatchlings of earlier clutches for food, shelter and so on. The hatchlings of *C. versicolor* of late breeding season internalize more residual yolk (reserve yolk) in comparison to those of the early and mid clutches (Radder *et al* 2002b).

In summary, in *C. versicolor*, the offspring number and size vary with several different factors. Among these, breeding time appears to place an upper limit on the offspring number and body size especially body mass and condition. *Calotes versicolor* females make adaptive decisions depending upon the breeding time for allocation of yolk to individual eggs that in turn matches offspring mass *vis-a-vis* energy reserves and is probably responsible for enhancing survival rate of offspring under varying ecological conditions.

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