Measures of reproductive allometry are sensitive to sampling bias

X. BONNET*‡†, R. SHINE‡, O. LOURDAIS*¶ and G. NAULLEAU*

Centre d'Etudes Biologiques de Chizé, CNRS, 79360, Villiers en Bois, France; ‡Biological Sciences A08, University of Sydney, NSW 2006, Australia; and ¶Université de Poitiers, 40 avenue du recteur Pineau, 86022 Poitiers, France

Summary

1. In both interspecific and intraspecific comparisons, maternal body size is the strongest predictor of fecundity for many kinds of animals. However, it has not been widely appreciated that the usual empirical descriptors of this relationship (correlation coefficient, slope and *P*-value of the linear regression between maternal body size and off-spring number) are sensitive to a factor that is very labile and subject to methodological bias: the degree of maternal investment, specifically the ratio of litter mass to maternal body mass (relative clutch mass, RCM).

2. Samples of females used to assess reproductive allometry may often be biased with respect to RCM. For example, RCMs may vary through time within a single population as a consequence of prey availability, or may vary geographically among populations. Also, females with low RCMs may be more difficult to capture, or may be discarded by researchers who do not realize that they are reproductive.

3. Our analyses on 173 litters of aspic vipers (*Vipera aspis* Linné) from central western France show that estimates of reproductive allometry are very sensitive to RCM: samples composed of high-RCM females show a positive and highly significant reproductive allometry, whereas samples composed of low RCM females do not. Conclusions also depend on the method of regression analysis used. This result has strong implications for methodology (i.e. selection of samples, choice of analytical methods and timescales of study) in this field of research.

Key-words: Reproductive effort, reptiles, snakes

Functional Ecology (2003) **17**, 39–49

Introduction

Organisms vary enormously in reproductive output, with clutch sizes ranging from one (e.g. in some seabirds and primates) to thousands (in broadcast-spawning fishes and trees). The factors responsible for this extraordinary diversity have attracted considerable scientific attention, and form a large part of life-history theory. Perhaps the strongest and most consistent correlate of fecundity, among as well as within species, involves maternal body size. Thus, many analyses of interspecific patterns in fecundity identify maternal body size as a primary axis of variation (e.g. Stearns 1983; Vitt & Seigel 1985; Reiss 1989). The same holds true for intraspecific comparisons: in virtually all populations in which adult females span a substantial range in body sizes, larger females tend to produce more or larger offspring (e.g. Ralls 1976; Vitt 1986). Given the importance of this relationship between maternal body size and fecundity – that is, it appears

to 'explain' a high proportion of variation in fecundity at both interspecific and intraspecific levels – it is important to understand factors that may influence conclusions from empirical studies on this relationship.

Reptiles provide appropriate study organisms in this respect. One of the most widespread generalizations in reptile biology is that clutch and litter sizes increase with maternal body size. This generalization appears to hold true for most species of lizards (Fitch 1970), snakes (Seigel & Ford 1987), turtles (Moll 1979) and crocodilians (Cooper-Preston & Jenkins 1993) that have been studied in this regard. The only clear exceptions are a series of lizard lineages that display invariant clutch or litter sizes (e.g. Andrews & Rand 1974; Shine & Greer 1991). Apart from these groups, a maternal size–fecundity relationship is so commonly reported that the absence of such a relationship is regarded as worthy of comment (e.g. Seigel & Ford 1987). Thus, the debate about the effect of female body size on reproductive output in reptiles has mostly been about the mechanism involved, rather than the existence of such a correlation (e.g. Shine 1992; Qualls & Shine 1995).

†Author to whom correspondence should be addressed. E-mail: bonnet@cebc.cnrs.fr

In the present paper, we examine one of the underpinnings of this literature. We do not dispute the fact that larger female reptiles often produce larger clutches than do smaller conspecifics, but we suspect that the strength of the size–fecundity relationship may have been overestimated in several field studies. Because of a potential methodological bias, the correlation between maternal body size and clutch size may not be as high as is often believed, and the slope of this relationship may be lower than is generally claimed. The main source of the problem involves the kinds of animals that are collected for investigations of reproductive biology. We suggest that these animals may sometimes constitute a non-representative or incomplete sample in terms of their level of reproductive investment relative to body size, and that variation in that level of investment will substantially affect not only mean values of reproductive traits, but perhaps also their 'allometry'.

RELATIVE CLUTCH MASS AND REPRODUCTIVE ALLOMETRY

In reptiles, the ratio of total litter mass to maternal postpartum mass (relative clutch mass, or relative litter mass; usually abbreviated as RCM) has often been used as a measure of relative reproductive investment (e.g. Tinkle 1969; Tinkle *et al*. 1970; Shine 1980; Cuellar 1984). Although there are statistical difficulties in using ratio measures such as the RCM for quantitative analyses (e.g. Seigel & Ford 1987), the ratio provides a simple measure of litter mass relative to maternal mass. It thus provides an index of how obviously a female's body is distended during pregnancy, and may also serve as an approximate index of the degree to which pregnancy impairs maternal mobility (e.g. Shine 1980; Seigel *et al*. 1987).

Why should biases in RCM affect the empirically determined relationship between maternal body size and litter size? The reason is as follows. Imagine a subset of females with identical, very high values of RCM. By definition, all of these females are equally 'full' of eggs. Thus, it must be true that larger females will contain more or larger eggs than do their smaller conspecifics. On the other hand, a wide range for RCM within a sample of females means that the correlation linking maternal body size to clutch size can potentially be much lower. The same is likely to be true for low mean values of RCM, because a low litter mass relative to maternal body mass means that minor variations in mass relative to body length in reproductive females can introduce substantial variation in reproductive allometry.

There are at least five plausible reasons why the animals upon which reproductive research is conducted may not accurately reflect the real distribution of RCM values in the population:

1. Temporal and geographical fluctuations in RCM levels. Females can respond to varying levels of prey availability by modifying patterns of repro-

ductive output (e.g. Seigel & Ford 1991). Similarly, geographical variations in food availability may translate into interpopulation variations in RCM values. If RCM shifts through time and geographically (Seigel & Fitch 1985), a study conducted in only 1 year or in a single population may well provide RCM values that are not representative of the long-term average value within that population or species. Along with RCM, any measure of reproduction (clutch size, breeding frequency …) is theoretically subject to fluctuations (Seigel & Fitch 1985; Seigel *et al*. 1986; Lourdais *et al*. 2002), hence the necessity to monitor the possible relationships between RCM and other reproductive parameters.

2. Lower survival of females with high RCMs. If a greater relative litter mass substantially increases maternal mortality, high-RCM females may be under-represented in samples taken close to the end of gestation (which is the commonest method for collecting gravid females in the field). Because gestation extends over long time periods in many reptiles (Seigel & Ford 1987), and because it may entail very high survival costs in reproductive females (Madsen & Shine 1992), temporal variation in predation rates (e.g. due to fluctuations in predator abundance or vegetative cover) could modify the intensity of such an effect.

3. Catchability of gravid animals. Gravid female reptiles often modify their activity patterns in ways that change their catchability to humans. For example, gravid females of viviparous reptile species may reduce the extent of their movements, and spend more time in precise thermoregulation (e.g. Bauwens & Thoen 1981; Naulleau *et al*. 1996). If females with smaller-than-average reproductive investment show less behavioural modification (e.g. they remain in their usual habitats rather than moving to thermoregulatory sites where they are more easily collected), then capture rates will be non-random with respect to RCM. The same will be true if higher RCMs cause more substantial reduction in mobility of gravid females, as has been documented in both snakes and lizards (Shine 1980; Seigel *et al*. 1987). Under these conditions, animals with lower-than-average RCMs are less likely to be captured.

4. Selectivity of investigators searching for gravid animals. If researchers (either consciously or unconsciously) retain only those animals that are most obviously gravid (i.e. with abdomens distended by eggs or embryos), then they may sometimes miss animals that are not as 'full' of eggs (Fitch 1970). Because pregnancy may be difficult to detect in animals with very low RCM (unpublished ultrasonic scan and nuclear magnetic resonance, NMR, imaging data) this bias may have substantial consequences.

5. Sample size. For some of the reasons listed above, there is little chance for a small sample size (i.e. $N < 15$) to provide accurate mean values and to cover the range of variation of RCM.

Methods

A nine-year (1992–2000) field study on aspic vipers (*Vipera aspis*, Linné) in central western France provided data from which to evaluate the influence of RCM on reproductive allometry. Over this period we hand-captured, marked and released 469 adult reproductive and non-reproductive female vipers. As soon as the first parturition of the season in the field was recorded, all gravid females were retained in separate cages in the laboratory until they give birth. Neonates and their mothers were weighed immediately after parturition. Details on the study area, species and reproductive output have been given elsewhere (e.g. Bonnet & Naulleau 1996; Naulleau & Bonnet 1996; Bonnet *et al*. 2000, 2001, 2002; Lourdais *et al*. 2002). The important aspect for the current analysis is that every year we retained all females for which there was even the slightest chance (based on palpation of any objects in the abdomen) that they might be gravid. RCMs will depend not only on litter size, but also on neonatal mass and on the proportion of viable offspring (at constant energy content, infertile eggs or embryos that die early weigh much less, because they do not take up water during development: X. Bonnet *et al.* unpublished data). Thus, we excluded 18 females that produced only infertile eggs from one analysis (see Results). RCM was calculated in the usual way (litter mass divided by the postpartum maternal mass, %: see Shine 1980) and also using the residual values of the regression of litter mass against female postpartum body mass to control for statistical problems linked to ratios. Residuals provide negative and positive values that enable us to interpret for example variations among years within a single population in a straightforward way. However, the use of residuals has been strongly criticized for comparative studies (Garcia-Berthou 2001), thus we performed all the RCM comparisons using ANCOVAS with litter mass as the dependent variable, maternal postparturient mass as the covariable and years or populations as the factors. In practice, however, none of the results was altered using either residuals or ANCOVAS.

We have data on body sizes and reproductive output for 173 females from a closed population. We also collected data for reproductive females caught in three neighbouring populations 50–150 km away. Our large data set enables us to simulate some of the biases we discussed above, to see if they exert a significant influence on the relationship between maternal body size and litter size. We expect that analyses restricted to 'obviously gravid' animals (i.e. those with high RCMs) should demonstrate a stronger correlation between these two variables than would analyses based on the entire data set. To test this prediction, we calculated the Pearson correlation coefficients and slopes of the linear regression equations (both ordinary least-squares and reduced major axis) linking maternal body size (snout–vent length, SVL) to total litter size. This procedure was carried out for a series of subsets of our data, either by (1) looking separately at data for females with different RCM ranges, or (2) progressively excluding animals with relatively low (or relatively high) RCMs from the analysis.

The rationale behind this approach was to mimic the possible effects of year-to-year variations in food availability, interpopulation differences, or to simulate the situation where such biases occur without the investigator being aware of them (i.e. analyses based on all animals with RCMs greater or less than some cut-off). To evaluate the biological validity of such data selection, we also examine the degree of temporal and geographical variation in RCM manifested in natural conditions. For this purpose we used long-term data on a single population, as well as data recorded from neighbouring populations of the same species. Finally, we compared estimates of RCM and allometry obtained by two research teams who worked on the same study population but used different criteria to recognize reproductive females.

ANALYTICAL PROBLEMS

Sensu stricto, allometry refers to the form of the relationship between phenotypic traits (such as fecundity and body size). Hence it deals explicitly with statistical concepts of regression, not with correlation that quantifies the covariance of two (or more) variables. This methodological difference has often been ignored, leading to potential caveats when analysing the effect of body size on other traits. Almost all published reports on the relationship between maternal size and fecundity have calculated the correlation coefficient, the slope and the *P*-value of the linear regression between maternal body size and offspring number. To facilitate comparison, we also used these classical descriptors of reproductive allometry. Another method was also used to calculate slopes, however (see below).

There are two different questions that we can ask about the relationship between fecundity and maternal body size. The first is whether or not the two variables are non-randomly associated with each other. For this question, the correlation coefficient provides a simple measure of the covariation between traits, and we can assess whether or not it is greater than expected under the null hypothesis of random association. We thus use this method to assess the statistical significance of the relationship between maternal size and fecundity. Because correlation parameters are sensitive to sample size, we also calculated the power $(1 - \beta$ error) of each correlation and the associated sample size that would be necessary to reject H0 with both low α and β errors (0·05 and 0·10, respectively, as recommended in most statistical textbooks). Power analyses are primarily designed to plan experimental designs (i.e. to determine optimal sample size), and great prudence is necessary not to over-interpret their low ability to gauge the strength and the meaning of results (Gerard *et al.*

1998). However, they do provide useful information in retrospective analyses, notably to estimate how large a sample is needed to allow statistically reliable judgements (Forsman 1996; Steiger 1999). In this paper, we limit our interpretation within such boundaries.

The second question is how rapidly fecundity increases with an increment in maternal body size. This is a much more complex issue for several reasons. For example, one can measure maternal body size in various ways (SVL; total length; mass; volume) and fecundity in various ways (litter size; litter mass; litter volume; viable offspring only; offspring mass with or without associated embryonic fluids, etc.). We use SVL because snakes have a simplified and elongated morphology, and thus an animal's SVL should provide a robust index of the space available to hold the litter. In addition SVL is the standard body size measurement in snakes (Seigel & Ford 1988). We use total litter size as the most straightforward measure of current fecundity. Even with a given set of descriptors for maternal size and reproductive output, however, conclusions concerning the relationship between these variables (i.e. reproductive allometry) will depend upon the selection of regression models (Harvey & Pagel 1991). Most workers report ordinary least-squares (OLS) regression, which assumes that the independent variable (in this case, SVL) is measured without error. This is clearly not true for measures of body size; body lengths of living snakes are notoriously difficult to measure precisely. Alternative regression models (such as major axis [MA] and reduced major axis [RMA]) do not rely on this assumption and are more appropriate to calculate allometric slopes (LaBarbera 1989; Griffiths 1992, 1998); however, they introduce other difficulties. For example, MA slope estimates assume that error in Y and X is equal, an assumption impossible to meet in most field studies (follicular atresia and abortion entail error in the measure of fecundity). Such errors, combined with imprecision in SVL measurements, render MA regression inappropriate for our study (Harvey & Pagel 1991). Reduced major axis regression is more flexible, allowing independent errors in X and Y, but is also limited in application (see Results). Our analysis uses both OLS and RMA regression models to explore the effect of model selection on conclusions concerning reproductive allometry.

Our analysis on RCM involved two main phases: a simulation of variations in RCM values and an investigation of temporal and geographical variations. For the simulation we pooled data gathered over 9 years on individuals belonging to a single population. By doing this, we probably encompassed most of the variations that occur in natural conditions (see Bonnet *et al.* 2001 and Lourdais *et al.* 2002 for details). Then we cut up the data set in order to obtain a suite of isolated or cumulative subsets. Although the distribution of the raw data (SVL, litter mass, postparturient maternal mass, but not litter size – see below) was initially
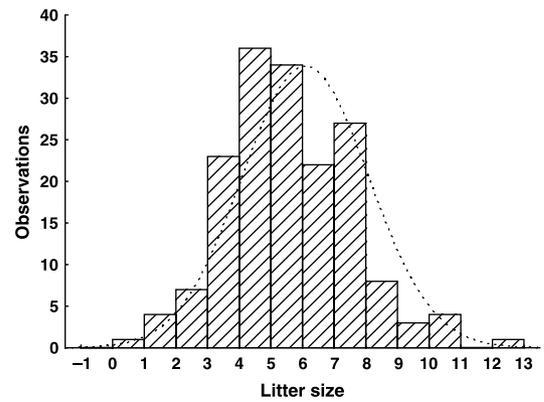
Fig. 1. Litter size is not normally distributed in the asp viper (Shapiro–Wilk $W = 0.967$, $P = 0.0004$), even after a ln-transformation (Shapiro–Wilk $W = 0.923$, $P = 0.001$). A graphic inspection show that the deviation from normality is not due to an over-representation of outliers in the data set, but rather to the effect of a body condition threshold for reproduction (Naulleau & Bonnet 1996; Bonnet *et al.* 2001) that favours relatively large litters, many females producing four to eight offspring (mean litter size being 6.08 ± 2.00).

normal in the complete data set, most of the subsets were truncated, especially the independent subsets. This may well have affected the shape of those subsets, and sometimes altered the distribution of the variables (see Table 1), consequently we did not compare subsets directly (i.e. through ANCOVA). However, although our truncation was arbitrary, our results suggest a non-random association between RCM and the descriptors of reproductive allometry (see Table 1 and Figs 2, 3 and 4). In addition, our simulation was contained within the range of natural variations in RCM (see Results). Importantly, the scope of these simulations was limited to explore a possible functional link between RCM and the maternal size/litter size relationship. Other analyses on geographical and temporal variations were all based on non-truncated data sets.

Continuous variables were tested for normality prior to analyses. Litter mass values were normally distributed without ln-transformation (Shapiro–Wilk $W = 0.986$, $P = 0.075$); by contrast, only after ln-transformation, the distribution of postparturient body mass (Shapiro–Wilk $W = 0.991$, $P = 0.375$) and maternal SVL (Shapiro–Wilk $W = 0.988$, $P = 0.144$) became normal. Litter size was not normally distributed, even after transformation (Fig. 1). However, the distribution of this variable was not biased by any over-representation of the extreme values; in addition the F-test is remarkably robust to deviations from normality (Lindman 1974). Overall, our results were not affected by ln-transformation of all the data, or combining transformed and non-transformed data.

In our main population, aspic viper show a marked trend toward semelparity (Bonnet *et al.* 2002), hence our analyses were almost free from pseudo-replication

Table 1. If we examine patterns of allometry separately for females with different levels of relative clutch mass (RCM), high-RCM females show a significant correlation between maternal snout–vent length (SVL) and litter size. These patterns disappear (and mean litter size decreases) as lower-RCM females are included. The table provides results from three separate analyses: one treating each RCM subset separately (a), one with high-RCM females progressively excluded (b), and one with 'low-RCM' females progressively excluded (c). RCM categories are given with the mean value (in brackets). N = sample size, r = Pearson product–moment correlation coefficient; slope = slope of the linear regression linking litter size to SVL. The power of the analysis was calculated as $1 - \beta$. The required sample size was calculated with $\alpha < 0.05$ and $\beta < 0.10$. Significant correlations are indicated in italics. Bold faces indicate cases where both α and β errors were low

(a) Independent subsets

RCM (mean)	<0·30 (0·20)	0·30–0·45 (0·38)	0·45–0·55 (0·50)	0·55–0·65 (0·60)	0·65–0·80 (0·72)	>0·80 (0·90)
N	35	26	32	30	34	16
P	0·72	0·20	*0·0002*	*0·005*	*0·0001*	*0·036*
Power	0·06	0·25	**0·97**	0·81	**1·00**	0·59
Required sample	2914	151	23	39	14	33
Female SVL (cm)						
Mean ± SD	49·6 ± 3·8	49·9 ± 3·7	48·8 ± 3·3	48·7 ± 3·3*	47·7 ± 3·2	49·4 ± 2·4*
Range	43–57	45–60	43·5–59	43·5–55·5	38·5–54·5	46–56
Litter size						
Mean ± SD	4·4 ± 1·7	5·1 ± 1·2*	5·7 ± 1·5*	6·6 ± 1·3*	6·6 ± 1·3	8·7 ± 2·4
Range	1–8	3–8	4–10	4–9	4–11	6–13

(b) Progressive exclusion of 'high-RCM' females

RCM	<0·30 (0·20)	<0·45 (0·27)	<0·55 (0·35)	<0·65 (0·41)	<0·80 (0·48)	All (0·52)
N =	35	61	93	123	157	173
P	0·72	0·71	0·09	*0·04*	*0·01*	*0·005*
Power	0·06	0·07	0·37	0·52	0·67	0·80
Required sample	2914	4198	359	320	287	234
Female SVL (cm)						
Mean ± SD	49·6 ± 3·8	49·8 ± 3·7	49·3 ± 3·6*	49·3 ± 3·5*	48·9 ± 3·5	48·9 ± 3·4
Range	43–57	43–60	49–60	43–60	38·5–60	38·5–60
Litter size						
Mean ± SD	4·4 ± 1·7	4·7 ± 1·5*	5·1 ± 1·6*	5·4 ± 1·7*	5·8 ± 1·8*	6·1 ± 2·0*
Range	1–8	1–8	1–10	1–10	1–11	1–13

(c) Progressive exclusion of 'low-RCM' females

RCM	All (0·52)	>0·30 (0·60)	>0·45 (0·65)	>0·55 (0·71)	>0·65 (0·78)	>0·80 (0·90)
N	173	137	112	80	50	16
P	*0·005*	*0·0001*	*0·0001*	*0·0001*	*0·0001*	*0·036*
Power	0·80	**1·00**	**1·00**	**1·00**	**1·00**	0·59
Required sample	234	68	34	28	17	33
Female SVL (cm)						
Mean ± SD	48·9 ± 3·4	48·8 ± 3·3	48·6 ± 3·2	48·5 ± 3·1	48·3 ± 3·0*	49·4 ± 2·4*
Range	38·5–60	38·5–60	38·5–59	38·5–56	38·5–56	46–56
Litter size						
Mean ± SD	6·1 ± 2·0*	6·5 ± 1·8*	6·8 ± 1·8*	7·3 ± 1·8*	7·3 ± 1·8*	8·7 ± 2·0
Range	1–13	3–13	4–13	4–13	4–13	6–13

*Cases when a deviation from normality was detected (Shapiro–Wilk's W-test < 0·05) in the SVL or litter size distribution.

effects; only 17 females contributed more than once in the data set. We repeated the statistics by deleting the few replicated data; none of the results was altered. This effect was expected because reproductive output is strongly influenced by environmental conditions, masking possible interindividual variations (Bonnet *et al.* 2001, 2002; Lourdais *et al.* 2002). Statistical tests were performed with Statistica 6·0.

Results

EFFECT OF MATERNAL BODY SIZE ON FECUNDITY AND RCM

One clear result is that the correlation between maternal body size and litter size is very low in our viper population (Fig. 2a). Whether or not it is statistically
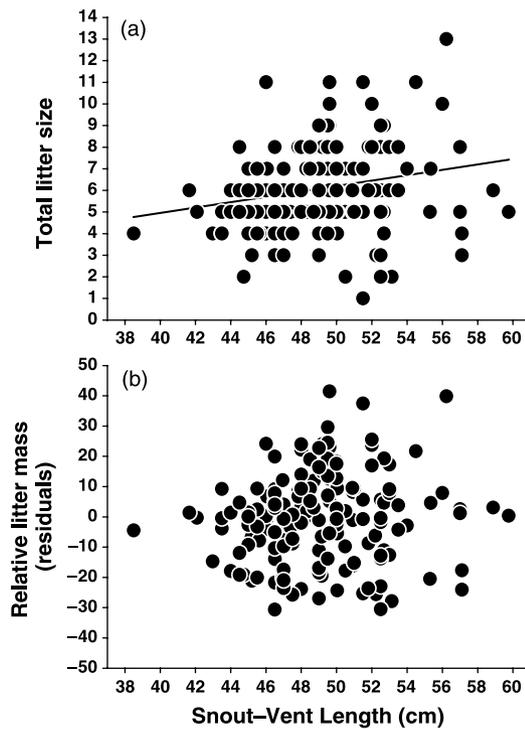
Fig. 2. Reproductive output relative to maternal body size in female aspic vipers. Larger females show a weak trend to produce more offspring per litter (a), but relative clutch mass is unaffected by maternal body size (b).

significant ($P < 0.05$) depends upon whether one includes abortive as well as viable offspring in the calculation (Bonnet *et al.* 2000). Maternal body size does not affect RCM in our aspic vipers ($r = 0.12$, $N = 173$, $P = 0.10$ using RCM as a ratio; and $r = 0.08$, $N = 173$, $P = 0.27$ using residual values of the regression of litter mass against female postpartum body mass; Fig. 2b).

EFFECT OF RCM ON THE RELATIONSHIP BETWEEN BODY SIZE AND FECUNDITY

Figure 3 and Table 1 present results from the OLS analysis of subsets of females with different ranges of RCMs. Maternal body size appeared to exert a very strong effect on litter size when analysis was restricted to females with high RCMs (>0.50 on average), but the ability to detect a significant correlation between these two variables fell rapidly if we based the analysis on low-RCM females instead. We also examined the effect of progressively excluding subgroups of females with either the highest or lowest RCM values (Table 1). The results were the same as for our analyses based on independent (rather than cumulative) subsets of the data. Regardless of whether we proceeded by progressive exclusion of the highest or lowest RCMs, the pattern was that both the slope of the OLS relationship and the coefficient of the correlation between maternal body size and litter size were highest for samples based on females with high RCMs. Perhaps the most important effect that emerged from our simula-
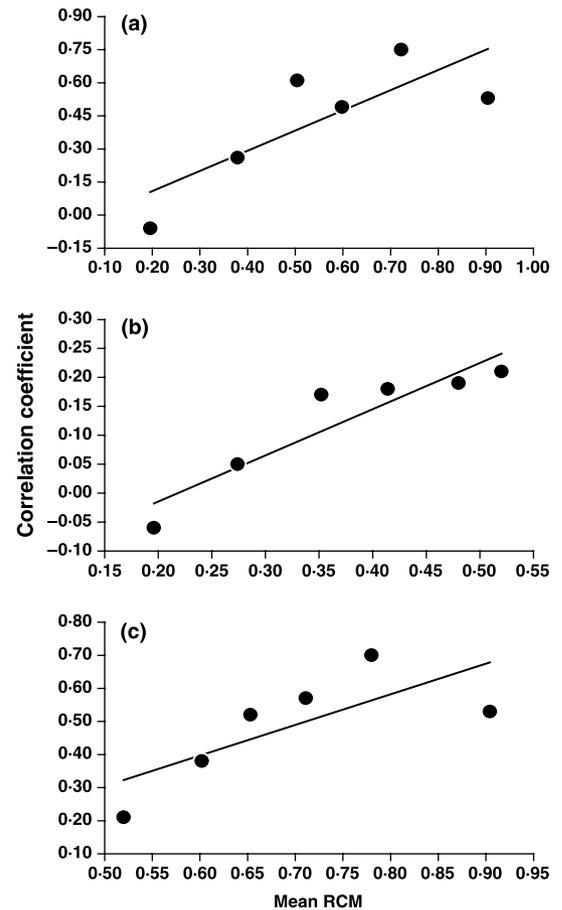

Fig. 3. The value of the rank-correlation between maternal body size (snout–vent length, SVL) and litter size depends upon the subset of females that is used for the analysis. The methods and data used to produce these figures are the same as those used in Table 1. The upper graph (a) shows calculated correlation coefficients based on subsets of females with different ranges of RCMs. The two lower graphs are based on progressive deletion of females with the highest RCMs (b) or the lowest RCMs (c), to simulate natural variations or biases that can occur in sampling. Statistical results: $N = 6$ in each case: (a) $r = 0.95$, $P < 0.005$; (b) $r = 0.95$, $P < 0.005$; (c) $r = 0.98$, $P < 0.001$.

tion was that the exclusion of the very low-RCM females ($N = 35$ among 173) leads to the deceptive conclusion that body size strongly influences fecundity, with both low α and β errors. Missing those apparently non-reproductive females provides robust, but biased, statistics. The subgroups of snakes with different RCMs also showed substantial differences in mean litter size (ANOVA with litter size as the dependent variable and RCM subsets as the factor; $F_{5,167} = 24.74$, $P = 0.0001$).

We found significant relationships between the range of RCM used, and the range of the usual descriptors (correlation coefficient and slope) of the correlation between maternal body size and fecundity (Figs 3 and 4). As mean RCM decreased by inclusion of low-RCM females (or exclusion of high-RCM females), this link between maternal body size and fecundity was weakened (Figs 3 and 4).
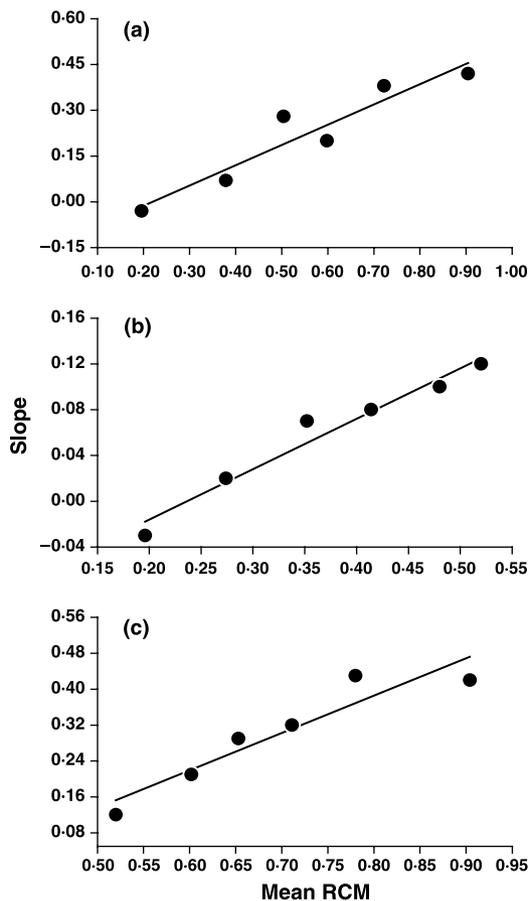
Fig. 4. The rate at which litter sizes increase with maternal body size (SVL) depends upon the subset of females that is used for the analysis. These data were obtained using ordinary least-squares (OLS) regression. If analysis is restricted to females with high relative clutch masses (RCMs), litter size increases rapidly with increases in maternal body size. However, analyses based on females with lower RCMs do not show such strong 'allometry'. The upper graph (a) shows the slope of the relationship between maternal SVL and litter size based on subsets of females with different ranges of RCMs. The two lower graphs are based on progressive deletion of females with the highest RCMs (b) or the lowest RCMs (c), to simulate natural variations or biases that can occur in sampling. Statistical results: $N = 6$ in each case; (a) $r = 0.98$, $P < 0.001$; (b) $r = 0.98$, $P < 0.001$; (c) $r = 0.99$, $P < 0.001$.

USING ALTERNATIVE REGRESSION MODEL

The above results were based on OLS regression. Analyses using RMA regression generated similar general patterns, although less clear-cut (Fig. 5). The link between maternal body size and fecundity was sensitive to the RCM of the females included in the analysis. The dispersion of the data in Fig. 4 compared with Fig. 3 is due to the characteristics of RMA slopes. Calculation of RMA regressions requires significant covariance between the variables tested; otherwise it can yield nonsensical results, such as the outliers in Fig. 4. When the error in X is not constant, OLS regressions generally underestimate the true value of the slope. This was apparent in our
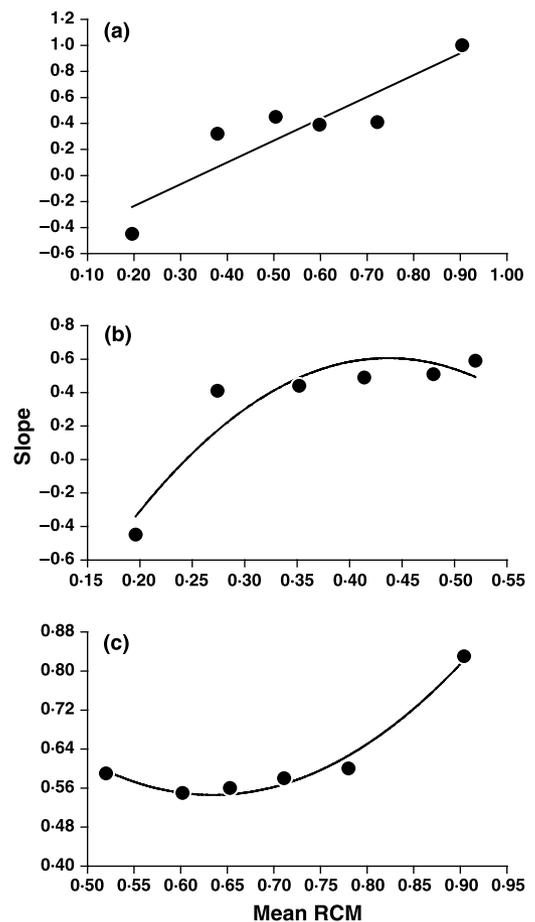

Fig. 5. The rate at which litter sizes increase with maternal body size (SVL) depends upon the subset of females that is used for the analysis (as in Fig. 3) when the slopes of the relationship between maternal SVL and litter size are calculated using reduced major axis regression (RMA) instead of the ordinary least-squares linear regression. Statistical results from the simple linear regression were, respectively: (a) $r = 0.90$, $P = 0.01$; (b) $r = 0.80$, $P = 0.054$; (c) $r = 0.79$, $P = 0.063$ ($N = 6$ in each case). Because data from (b) and (c) were better fitted by polynomial regressions, we also report the results of such analyses for (b), $r = 0.94$, $P = 0.044$; (c), $r = 0.99$, $P = 0.003$.

data, with mean slopes of 0.19 ± 0.16 ($N = 18$) and 0.44 ± 0.36 ($N = 18$), respectively, for OLS and RMA regressions (comparing each value obtained in the simulations, T-test for dependent samples was -3.75, df = 17, $P < 0.002$; Figs 4 and 5).

SOURCES OF VARIATION IN RCM ESTIMATES

Annual variation

In a single population, mean RCM varied significantly over time (ANCOVA with litter mass as the dependent variable, years as the factor and postparturient maternal mass as the covariable: $F_{8,143} = 2.40$, $P = 0.018$; 18 vitellogenic females that failed to produce at least one offspring (i.e. producing only unfertilized eggs) were excluded; Fig. 6). Comparing two contrasted years where sufficient data were available (1993 with a mean RCM
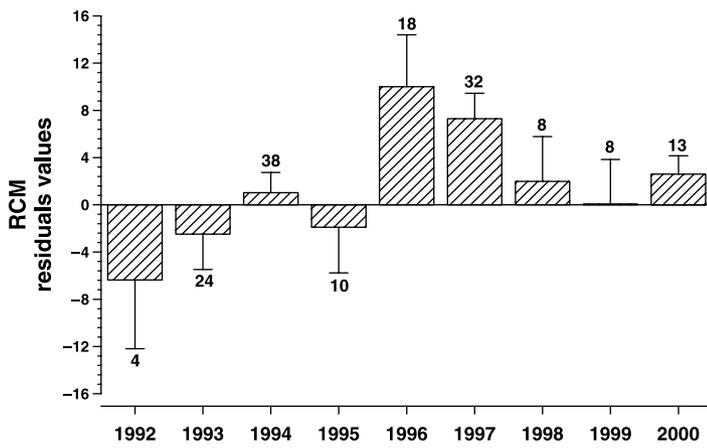
Fig. 6. Mean RCM (residual values from the general linear regression of litter mass against postpartum maternal; body mass) exhibits annual variation within a closed population of *Vipera aspis*. Mean values are expressed with standard error and sample size.
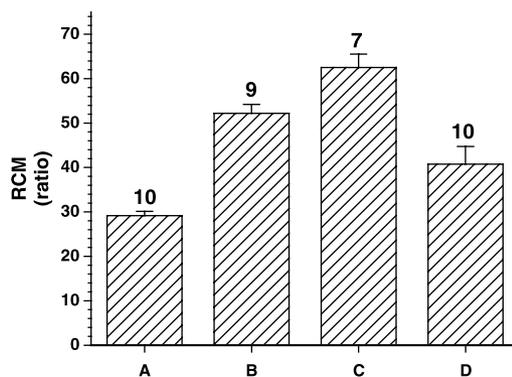

Fig. 7. Mean RCM (residual values from the general linear regression of litter mass against postpartum maternal; body mass) varies among neighbouring populations of *Vipera aspis*. Mean values are shown with standard errors and sample sizes. The four populations (A, B, C and D) were sampled in 1998 and 1999; they are situated in four districts extending from the south to the north of Poitou-Charentes in Western Central France: A, north of Charente Maritime; B, south of Deux Sèvres; C, west of Vendée; D, south of Loire Atlantique.

of 45 ± 3 [± SE], $N = 24$, 1997 with a mean RCM $= 60 \pm 3$, $N = 32$) reveals strong year-to-year variations in RCM (same design ANCOVA; $F_{1,52} = 8 \cdot 61$, $P < 0 \cdot 005$).

Geographical variation

We also found significant differences in mean RCM among four adjacent populations of vipers (ANCOVA with litter mass as the dependent variable, locality as the factor and postparturient maternal mass as the covariable, $F_{3,31} = 4 \cdot 97$; $P < 0 \cdot 007$; Fig. 7).

Selection of 'gravid' females

Are low RCM females sometimes discarded by researchers, thereby biasing data sets? Earlier studies on our viper population reported highly significant correlations between maternal SVL and litter size (Naulleau & Saint Girons 1981), whereas our later studies have not (despite a larger sample size: Bonnet *et al.* 2000). A comparison between the two data sets reveals higher mean values for litter sizes, and lower values for offspring sizes and the proportions of stillborn or unviable offspring, in the earlier study (Table 2). However, mean maternal body lengths were similar (Table 2, and see Table 1 for comparisons of mean litter sizes). In our study population, females with low RCMs tend to produce small litters of large offspring (Bonnet *et al.* 2001). Similarly, females with a high proportion of unviable neonates have low RCMs (because of the lower mass of these offspring) and hence, these animals tend not to display the distended abdomen thought to be typical of 'reproductive' females during pregnancy (Table 2 and unpublished data). These patterns fit well with the hypothesis that the earlier conclusions were in error because of frequent (albeit unconscious) exclusion of females with such low RCMs that they were not recognized as being reproductive. One of us (GN) who was involved in the earlier study recalls that he selected obviously gravid females, and thereby would have discarded low-RCM animals.

Discussion

At least in the aspic viper, there is a strong link between overall reproductive investment (as measured by RCM) and reproductive 'allometry' (the slope, the correlation coefficient and the statistical significance of the linear regression between litter size and maternal body size). Thus, empirical estimates of these relationships may be substantially modified by variations in RCM levels among samples. Such effects may arise from temporal variation of RCMs within a single population in response to prey availability, direct selectivity by researchers, or effects of RCM on a female's catchability or survival. Methods of analysis can also influence conclusions about reproductive allometry.

Intra- and interpopulation comparisons suggest that significant temporal and geographical variations in RCM occur in the asp viper (Figs 6 and 7, Table 2). Such variations have also been reported in other snake species (Seigel *et al.* 1986; Seigel & Ford 1987), probably because fluctuations in food availability strongly influence reproduction (Madsen & Shine 1999; Bonnet *et al.* 2001; Lourdais *et al.* 2002). Comparisons within and among populations suggest that several factors such as climatic conditions (Lourdais *et al.* 2002) can also influence reproduction, litter size and hence mean RCMs in the asp viper. Large sample sizes gathered in various situations are necessary to better appreciate the relationship between maternal size and fecundity. For logistical reasons, however, many studies reporting data on the body size/fecundity relationship have been carried out on small sample sizes, sometimes on captive animals only. For instance, in the important synthesis

Table 2. A comparison of the characteristics of reproductive female aspic vipers, *Vipera aspis*, between two studies carried out in the same area of western central France. The earlier study (Naulleau & Saint Girons 1981) was based on females collected because they appeared to be reproductive, whereas the present study included all females for which there was even a slight possibility that they might be reproductive (based on abdominal palpation). Despite the similarity in maternal body sizes (total body length), reproductive output was very different. The table provides mean values ± SD for most variables, with sample sizes in brackets. Unpaired two-tailed *t*-tests and chi-square contingency-table tests were used for these comparisons

Trait	Naulleau & Saint Girons (1981)	Present study	*P*
Maternal size (cm)	55·6 ± 4·6 (41)	55·7 ± 3·8 (173)	0·88
Litter size	6·7 ± 3·1 (57)	6·0 ± 2·0 (173)	0·056
Mean neonate mass	5·5 ± 0·9 (22)	6·3 ± 1 (158)	0·005
Living offspring (%)	92% (118)	78·2% (1050)	0·007
Correlation: length *vs* litter size	0·55 (57)	0·21 (173)	0·006

of Seigel & Ford (1987), less than 25% of the studies on reproductive output in snakes were based on sample sizes greater than 30; and, when available, temporal comparisons were based on short timescales (see Seigel & Fitch 1984; Seigel *et al.* 1986).

The contrast in allometric patterns (and mean RCM values, etc.) between the two studies on our aspic viper populations suggests that researchers sometimes mistakenly omit low-RCM females from studies of reproductive output. Such females may represent a 'hidden' component of the overall population, and specific attention must be paid during data gathering to include such animals. How widespread is the problem of inadequate sampling? Analyses based on preserved specimens in museums often reveal mean litter sizes much lower than those reported from field studies of the same taxa (e.g. Fitch 1970; Shine 1981a,b). This difference probably reflects the fact that museum collectors take specimens regardless of their perceived reproductive status, whereas researchers desiring reproductive animals often fail to collect females with lower-than-average reproductive output for their size. Such biases may be widespread, and we should be aware of them as we frame our research techniques. Otherwise, our knowledge of reproductive allometry will be disproportionately based on studies of high-RCM females, a group with allometries of reproductive output different from those of some other subsets of females within the same populations (e.g. Fig. 4). 'Low-investment' females may well have demographic tactics rather different to those of their high-RCM conspecifics, and we need to include both categories of females in our studies if we are to understand intrapopulational diversity of reproductive tactics.

We need to be aware of the potential for bias in selecting animals for study, and in determining the appropriate time and geographical scales for sampling. If we wish to quantify variables such as the mean litter size in a population, or the degree to which maternal body size influences reproductive output, we need to ensure that we are indeed taking a valid sample of the population. Ideally, studies on reproductive allometry should report the correlation coefficient and the slope of the OLS linear regression between maternal body

size and offspring number, and the mean RCM of the sample. Although broad trends in our results were similar using OLS and RMA regressions, the exact values of the slopes differed. In practice, such discrepancies between models increase as the coefficient of determination declines (Harvey & Pagel 1991). The range of RCMs that were included in the analysis also influenced the coefficient, suggesting that quantifying allometric relationships between maternal size and reproductive output is a more complex issue than is usually appreciated.

More importantly, we need long-term analyses of the relationship between reproductive output and maternal body size, especially in systems that experience fluctuating prey availability, in order to discern the degree to which reproductive allometries (as well as mean levels of reproductive output) shift in response to proximate variation in energy supply. Unfortunately, very few studies (Ford & Killebrew 1983; Ford & Seigel 1989; both on *Thamnophis* sp.) combine the classical descriptors of the body size–fecundity relationship (the correlation coefficient, the slope and the *P*-value) with measures of RCM; and we cannot evaluate to what extent published studies are based on particular subsets of females. One field study on European adders, however, found that litter size was correlated with maternal body size when food was plentiful, but not when food was scarce (Andren & Nilson 1983). This observation fits well with the patterns revealed by our analyses (e.g. Fig. 4), but more data are needed. If we are correct in our suggestion that published literature often overestimates the slope and strength of the link between maternal body size and litter size, then alternative influences on reproductive output may merit more attention than they have generally received. In addition, the sources and the consequences of variation in RCM deserve attention (Seigel *et al.* 1986; Dunham *et al.* 1988; Shine 1992).

Inc., the Conseil Général des Deux Sèvres, the Centre National de la Recherche Scientifique (France) and the Australian Research Council. Rex Cambag was a wonderful companion during field trips. Two anonymous referees provided useful comments.

References

Andren, C. & Nilson, G. (1983) Reproductive tactics in an island population of adders, *Vipera berus* (L.), with a fluctuating food resource. *Amphibia-Reptilia* **4**, 63–79.

Andrews, R. & Rand, A.S. (1974) Reproductive effort in anoline lizards. *Ecology* **55**, 1317–1327.

Bauwens, D. & Thoen, C. (1981) Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *Journal of Animal Ecology* **50**, 733–743.

Bonnet, X. & Naulleau, G. (1996) Catchability in snakes: consequences on breeding frequency estimates. *Canadian Journal of Zoology* **74**, 233–239.

Bonnet, X., Naulleau, G., Shine, R. & Lourdais, O. (2000) Reproductive versus ecological advantages to larger body size in female snakes (*Vipera aspis*). *Oikos* **89**, 509–518.

Bonnet, X., Naulleau, G., Shine, R. & Lourdais, O. (2001) Short-term versus long-term effects of food intake on reproductive output in a viviparous snake (*Vipera aspis*). *Oikos* **92**, 297–308.

Bonnet, X., Lourdais, O., Shine, R. & Naulleau, G. (2002) Reproduction in a typical capital breeder: costs, currencies and complications in the aspic viper. *Ecology* **83**, 2124–2135.

Cooper-Preston, H. & Jenkins, J.W.G. (1993) *Natural History of the Crocodylia. Fauna of Australia*, Vol. 2A. *Amphibia and Reptilia* (eds C. Glasby, G. Ross & P. Beesley), pp. 337–343. Australian Government Publishing Service, Canberra.

Cuellar, O. (1984) Reproduction in a parthenogenetic lizard: with a discussion of optimal clutch size and a critique of the clutch weight/bodyweight ratio. *American Midland Naturalist* **111**, 242–258.

Dunham, A.E., Miles, D.B. & Reznick, D.N. (1988) *Life Hitory Patterns in Squamate Reptiles. Biology of the Reptilia*, Vol. 16 *Ecology B, Defense and Life History* (eds C. Gans & H.F. Pough), pp. 441–522. Alan R. Liss, Inc., New York.

Fitch, H.S. (1970) Reproductive cycles in lizards and snakes. *University of Kansas Museum of Natural History Miscellaneous Publications* **52**, 1–247.

Ford, N.B. & Killebrew, D.W. (1983) Reproductive tactics and female body size in butler's garter snake, *Thamnophis butleri*. *Journal of Herpetology* **17**, 271–275.

Ford, N.B. & Seigel, R.A. (1989) Relationship among body size, clutch size, and egg size in three species of oviparous snakes. *Herpetologica* **45**, 75–83.

Forsman, A. (1996) An experimental test for food effects on head size allometry in juvenile snakes. *Evolution* **50**, 2536–2542.

Garcia-Berthou, E. (2001) On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *Journal of Animal Ecology* **70**, 708–711.

Gerard, P.D., Smith, D.R. & Weerakkody, G. (1998) Limits of retrospective power analysis. *Journal of Wildlife Management* **62**, 801–807.

Griffiths, D. (1992) Size, abundance, energy use in communities. *American Naturalist* **127**, 140–166.

Griffiths, D. (1998) Sampling effort, regression method, and the shape and slope of size–abundance relations. *Journal of Animal Ecology* **67**, 795–804.

Harvey, P.H. & Pagel, M.D. (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, New York.

LaBarbera, M. (1989) Analysing body size as a factor in ecology and evolution. *Annual Review of Ecology and Systematics* **20**, 97–117.

Lindman, H.R. (1974) Analysis of variance in complex experimental designs. Freeman, San Francisco.

Lourdais, O., Bonnet, X., Shine, R., DeNardo, D., Naulleau, G. & Guillon, M. (2002) Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake. *Journal of Animal Ecology* **71**, 470–479.

Madsen, T. & Shine, R. (1992) Determinants of reproductive success in female adders, *Vipera berus. Oecologia* **92**, 40–47.

Madsen, T. & Shine, R. (1999) The adjustment of reproductive threshold to prey abundance in a capital breeder. *Journal of Animal Ecology* **68**, 571–580.

Moll, E.O. (1979) Reproductive cycles and adaptations. *Turtles: Perspectives and Research* (eds M. Harless & H. Morlock), pp. 305–331. Wiley and Sons, New York.

Naulleau, G. & Bonnet, X. (1996) Body condition threshold for breeding in a viviparous snake. *Oecologia* **107**, 301–306.

Naulleau, G. & Saint Girons, H. (1981) Poids des nouveaunés et reproduction de *Vipera aspis* (Reptilia: Viperidae), dans les conditions naturelles et artificielles. *Amphibia-Reptilia* **2**, 51–62.

Naulleau, G., Bonnet, X. & Duret, S. (1996) Déplacements et domaines vitaux des femelles reproductrices de vipères aspic *Vipera aspis* (Reptilia, Viperidae) dans le centre ouest de la France. *Bulletin de la Société Herpétologique de France* **78**, 5–18.

Qualls, C.P. & Shine, R. (1995) Maternal body-volume as a constraint on reproductive output in lizards: evidence from the evolution of viviparity. *Oecologia* **103**, 73–78.

Ralls, K. (1976) Mammals in which females are larger than males. *Quarterly Review of Biology* **51**, 245–276.

Reiss, M.J. (1989) *The Allometry of Growth and Reproduction*. Cambridge University Press, Cambridge.

Seigel, R.A. & Fitch, H.S. (1984) Ecological patterns of relative clutch mass in snakes. *Oecologia* **62**, 293–301.

Seigel, R.A. & Fitch, H.S. (1985) Annual variation in reproduction in snakes in a fluctuating environment. *Journal of Animal Ecology* **54**, 497–505.

Seigel, R.A. & Ford, N.B. (1987) Reproductive ecology. *Snakes: Ecology and Evolutionary Biology* (eds R.A. Seigel, J.T. Collins & S.S. Novak), pp. 210–252. Macmillan, New York.

Seigel, R.A. & Ford, N.B. (1988) A plea for standardization of body size measurements in studies of snake ecology. *Herpetological Review* **19**, 9–10.

Seigel, R.A. & Ford, N.B. (1991) Phenotypic plasticity in the reproductive characteristics of an oviparous snake, *Elaphe guttata*: implications for life history studies. *Herpetologica* **47**, 301–307.

Seigel, R.A., Fitch, H.S. & Ford, N.B. (1986) Variation in relative clutch mass in snakes among and within species. *Herpetologica* **42**, 179–191.

Seigel, R.A., Huggins, M.M. & Ford, N.B. (1987) Reduction in locomotor ability as a cost of reproduction in snakes. *Oecologia* **73**, 461–465.

Shine, R. (1980) 'Costs' of reproduction in reptiles. *Oecologia* **46**, 92–100.

Shine, R. (1981a) Ecology of the Australian elapid snakes of the genera Furina and Glyphodon. *Journal of Herpetology* **15**, 219–224.

Shine, R. (1981b) Venomous snakes in cold climates: ecology of the Australian genus *Drysdalia* (Serpentes: Elapidae). *Copeia* **1981**, 14–25.

Shine, R. (1992) Relative clutch mass and body shape in lizards and snakes: is reproductive investment constrained or optimized? *Evolution* **46**, 828–833.

Shine, R. & Greer, A.E. (1991) Why are clutch sizes more variable in some species than in others? *Evolution* **45**, 1696–1706.

Stearns, S.C. (1983) The influence of size and phylogeny on patterns of covariation among life-history traits in mammals. *Oikos* **41**, 173–187.

Steiger, J.H. (1999) *Statistica Power Analysis*. University of British Columbia and StatSoft R & D Department, StatSoft, Inc., Tulsa.

Tinkle, D.W. (1969) The concept of reproductive effort and its relation to the evolution of life histories of lizards. *American Naturalist* **103**, 501–516.

Tinkle, D.W., Wilbur, H.M. & Tilley, S.G. (1970) Evolutionary strategies in lizard reproduction. *Evolution* **24**, 55–74.

Vitt, L.J. (1986) Reproductive tactics of sympatric gekkonid lizards with a comment on the evolutionary and ecological consequences of invariant clutch size. *Copeia* **1986**, 773–786.

Vitt, L.J. & Seigel, R.A. (1985) Life history traits of lizards and snakes. *American Naturalist* **125**, 480–484.