

THE LIMITS TO REPRODUCTIVE OUTPUT: OFFSPRING SIZE VERSUS NUMBER IN THE SAND LIZARD (*LACERTA AGILIS*)

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Natural selection does not always (or indeed, usually) produce “optimal” phenotypes, because of a variety of phenomena including trade-offs (conflicting optimal phenotypes for different functions) and mechanistic constraints (e.g., physical limitations, lack of additive genetic variance, genetic correlations between the sexes). The importance of trade-offs and constraints has been widely appreciated (e.g., Gould and Lewinton 1979), but the ways in which such factors influence life-history traits remain obscure. One vigorous debate in reptilian biology concerns the determinants of reproductive output. In many reptile species, clutch sizes and offspring sizes are related to each other and to maternal body size. The causal basis for these relationships remains controversial, with two main approaches evident in the literature. One view stresses energy allocation, such that reproductive output (e.g., relative clutch mass [RCM] or reproductive effort [RE]) is determined by the optimal allocation of resources among maintenance, storage, growth, and reproduction (Tinkle and Hadley 1973, 1975; Congdon et al. 1982). Under this approach, the upper limit to reproductive investment is set by energy availability and, hence, is likely to vary in response to proximate factors (e.g., Ballinger 1983; James and Whitford 1994). An alternative approach suggests that physical constraints, such as the space available to hold the clutch in the female’s abdominal cavity, determine the upper limit for reproductive investment (Vitt and Congdon 1978; Shine 1988, 1992).

The central disagreement between the two approaches centers on which factor determines the upper limit to reproductive output: resources or physical constraints. The best evidence that such an upper limit does exist is the widespread occurrence of trade-offs between offspring size and clutch size: that is, larger clutches consist of smaller offspring (Elgar and Heaphy 1989; Madsen and Shine 1992, 1996; King 1993). The existence of such a trade-off implies that there is a fixed upper limit to investment. Interspecific comparisons suggest that RCMs are correlated with body shape, in keeping with the notion of physical constraints (if all females fill themselves with eggs, body shape should constrain reproductive output) (Shine 1992). However, the volume constraint hypothesis is difficult to reconcile with the high intrapopulational variance often observed in RCMs or the

phylogenetic shifts in RCM in morphologically conservative lineages (e.g., Qualls and Shine 1995). These latter observations support the idea that variation in reproductive output is due to variation in resources.

In the present note, we focus on the following question: Given that there is a trade-off between offspring size and clutch size, what factor determines the upper limit that enforces this trade-off—energy or space? To answer this question, we look in detail at the trade-off between offspring size and clutch size in a population of lizards exposed to variation in prey availability—both naturally (in the field, from year to year) and artificially (by food supplementation in the laboratory). If the upper limit to reproductive investment is set by energy availability, we would expect, first, that among-year differences in food supply would modify the position of this upper limit and, hence, alter the position of the trade-off lines and, second, that food supplementation in the laboratory would substantially modify (perhaps, remove) this trade-off. The alternative hypothesis—that the trade-off is enforced by a fixed upper limit of abdominal volume available to hold the clutch—predicts that the position of the trade-off line should not vary among years or in response to food supplementation, because maternal body shapes remain constant. Our study suggests that variation in reproductive output among females in this population is driven primarily by proximate factors (food availability) but within a maximum clutch volume set by physical limitations on abdominal space available to hold the clutch. More generally, although interspecific variance in reproductive output relative to maternal body size in reptiles may be due to variation in body shape (i.e., physical constraints), much of the intraspecific variance in this trait may be due to resource limitation.

MATERIAL AND METHODS

Field Study

We studied a natural population of sand lizards at Asketunnan, approximately 50 km south of Gothenburg on the Swedish west coast. The study site consists of a rocky coastline with stands of birch (*Betula verrucosa*), blackthorn (*Prunus spinosa*), and angelica (*Angelica litoralis*) as dominating plant species in a mosaic habitat. We visited the study every day in the spring and recorded the number of days that were too cloudy to permit lizard activity (heavy cloud for > 75% of the time between 0900 and 1900 hours). For each female lizard, we calculated the proportion of days that fell into this category from early spring (arbitrarily set at April 15) until the female was captured immediately prior to oviposition. This weather estimate provides an index of the availability of basking opportunities from emergence until egg laying.

The lizards were marked temporarily by an adhesive tape with an individual number on their backs and permanently by toe clipping. Each lizard was measured snout to vent (snout-vent length [SVL] \pm 0.5 mm) and weighed (\pm 0.1 g) before being released at its place of capture. A representative sample of lizards was also stomach flushed (Legler and Sullivan 1979; eight lizards in 1987, 30 in 1988, and 25 in 1989), and we calculated an index of feeding rates based on the number of prey items per stomach.

When females became visibly distended with eggs (and hence were close to oviposition), they were brought to the University of Gothenburg. The lizards were kept separately in cages (each ca. 40 cm × 50 cm × 60 cm) with a 40-W spotlight mounted in each cage to allow thermoregulation, were fed mealworms ad libitum, and had fresh water available at all times. All females laid their eggs in moist sand under a flat rock in the cage. The cages were checked at least twice daily for recently laid eggs, which were transferred to a plastic 1.3-L container one-quarter filled with vermiculite and water in the volume ratio 10:1 (mL). The eggs were incubated at a constant $25^{\circ} \pm 1^{\circ}\text{C}$ and hatched after approximately 40 d.

Laboratory

The females kept in the laboratory study were animals that either had been caught at Asketunnan more than 2 yr prior to the collection of their eggs in captivity or were first-generation offspring from the field-caught females. All of these females were mated in captivity. They were housed in large communal cages until they became visibly distended with eggs, at which time they were transferred to oviposition cages as described above for females from the wild. All captive females were fed crickets (*Gryllus gryllus*, *Gryllus domesticus*) and mealworms (*Tenebrio mollitor*) in the communal cages and mealworms in the oviposition cages (as for wild-caught females). Vitamins and minerals were supplied at least once weekly. Thus, these females had been on a high-quality diet with food available ad libitum for at least 2 yr prior to oviposition. All oviposition and incubation procedures were identical to the ones used for the field-caught females.

Procedures Common to Field and Laboratory Clutches

For eggs from both groups, we removed the hatchlings and weighed and measured them (as for the adults) within 24 h after hatching. Data on hatchlings rather than eggs were used in analyses of trade-offs between clutch size and offspring size. Because water uptake of the egg begins immediately at oviposition, even minor differences in the time between oviposition and weighing can introduce significant errors into estimates of egg mass. Thus, hatchling mass is a more accurate estimate of a female's investment in individual offspring. To avoid statistical nonindependence of data, we used mean figures for each trait for each clutch instead of treating siblings as separate entities in our analysis. However, separate clutches from the same female were treated as independent, because our analyses showed that the magnitudes of variance in offspring traits were similar among females as within females; thus, this procedure should not introduce significant statistical artifacts (Leger and Didrichson 1994).

RESULTS

Field Study

When the data from all 5 yr of the study were combined, there was a strong negative correlation between clutch size and offspring size ($n = 140$, $r = -0.34$,

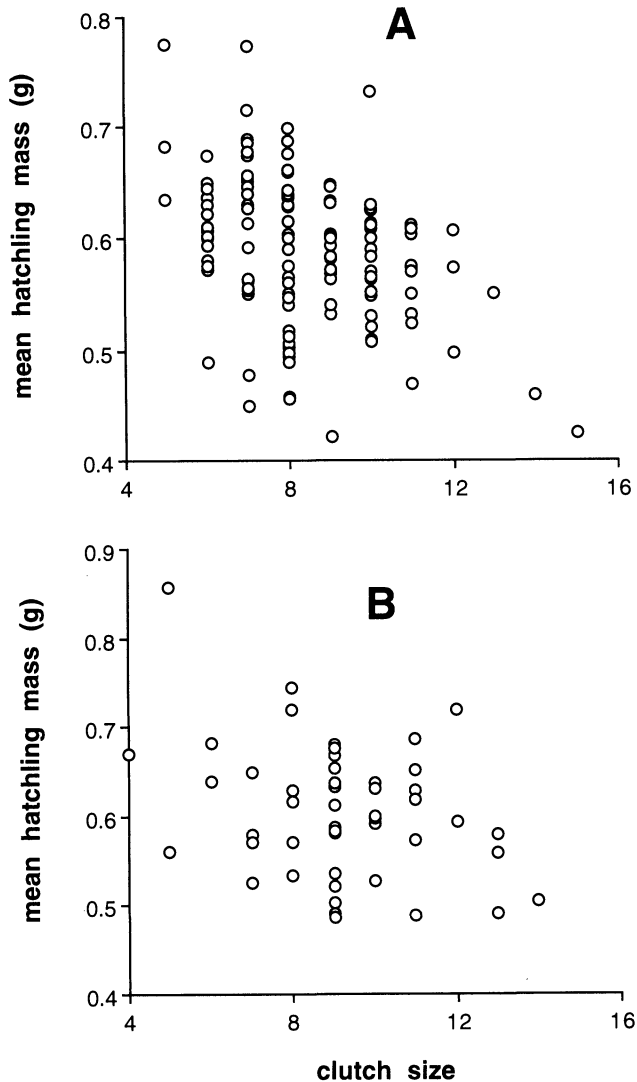


FIG. 1.—The negative correlation between clutch size and offspring mass in sand lizards (*Lacerta agilis*) from Asketunnan in southwestern Sweden. Larger clutches consist of smaller offspring, both from field-caught females (A) and females raised in the laboratory with food available ad libitum (B).

$P < .0001$; fig. 1). Clutch sizes also increased with maternal body size ($n = 153$, $r = 0.60$, $P < .0001$), which suggests that the decrease in offspring size in larger clutches might be an indirect effect of a decrease in offspring size with increasing maternal body size. However, multiple regression (with offspring mass as the dependent variable and clutch size and maternal SVL as the independent variables) was not consistent with this interpretation. Clutch size exerted a strong

negative effect on offspring size (standardized regression coefficient = -0.41 , $P < .0001$), whereas female SVL exerted a much weaker, positive effect (standardized coefficient = 0.13 , $P = .19$). To control for variance in absolute maternal body sizes in our analyses, we computed residual scores from the general linear regression of clutch size to female SVL. This size-corrected fecundity measure (hereafter, "relative fecundity") also showed a significant negative correlation with offspring mass ($n = 140$, $r = -0.34$, $P < .0001$), which reinforces our conclusion that the reduced offspring size in smaller clutches was a function of fecundity relative to body size and not maternal body size per se.

One important consequence of this relationship between relative fecundity and offspring size was that relative clutch mass (clutch mass divided by maternal body mass) did not correlate significantly with maternal body length ($n = 88$, $r = 0.08$, $P = .45$). That is, females with relatively large clutches tended to produce relatively small offspring, such that RCM remained constant with maternal size.

Because the two factors predicted to cause the "offspring size versus number" trade-off generated different predictions about temporal variation, we examined year-to-year variation in reproductive output in this population in more detail. Mean RCM remained relatively constant among the years of our study (one-factor ANOVA with year as the factor: $F = 1.42$, $df = 2$, 97 , $P = .25$), but we detected significant annual variation in mean maternal SVL ($F = 5.62$, $df = 4$, 147 , $P < .0003$), mean clutch size ($F = 2.61$, $df = 4$, 132 , $P < .04$), relative fecundity ($F = 3.90$, $df = 4$, 131 , $P < .005$), and mean hatchling mass ($F = 7.10$, $df = 4$, 132 , $P < .0001$). Female bodily condition also varied among years (condition prior to laying: $F = 10.19$, $df = 3$, 127 , $P < .0001$; condition after laying: $F = 5.85$, $df = 4$, 146 , $P < .0002$). When data for each of the 5 yr were analyzed separately, the trade-off between offspring mass and relative fecundity was evident in each year, but the position of the trade-off line varied considerably (fig. 2). One-factor ANCOVA confirmed that these among-year differences in the elevations of the lines were highly significant. With year as the factor, hatchling mass as the dependent variable, and relative fecundity as the independent variable, slopes of the regressions were homogeneous ($F = 0.03$, $df = 4$, 127 , $P = .99$), but elevations differed significantly ($F = 10.65$, $df = 4$, 131 , $P < .0001$). Thus, although the trade-off was evident each year, the exact value of offspring mass associated with any given level of relative fecundity varied markedly. For example, the mean hatchling mass of female sand lizards with "average" relative fecundity (horizontal axis of fig. 2 = 0) varied from 0.54 g in 1987 to 0.62 g in 1988 and 1991 (0.60 g in 1989 and 0.58 g in 1990; fig. 2).

Our data on the Asketunna sand lizards enable us to tease apart some of the possible causes of this variation. The most likely influence is the weather, because of its effects on food availability and the amount of time for lizards to feed prior to ovulation. The Asketunna site experiences considerable year-to-year variation in weather conditions, with consequent effects on feeding rates of lizards. One-factor ANOVAs with year as the factor revealed significant differences among years in the proportion of days in spring (April 15 to oviposition) that were warm and sunny enough for lizard activity ($F = 48.49$, $df = 4$, 55 , $P <$

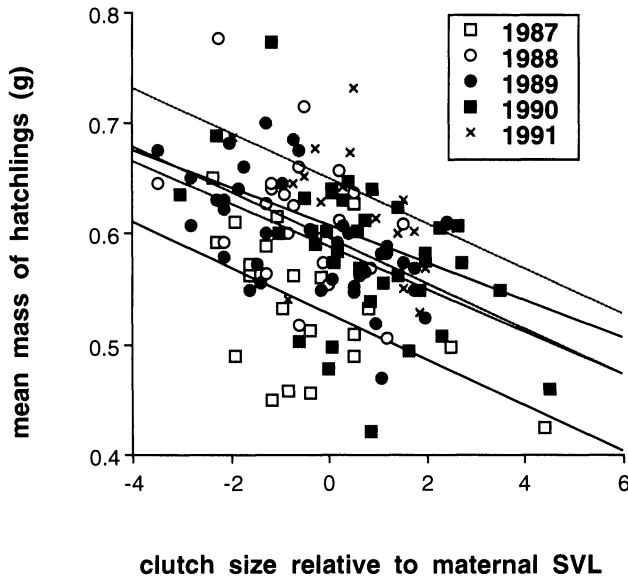


FIG. 2.—Year-to-year variation in the form of the trade-off between mean offspring mass and relative fecundity (residual score from the linear regression of clutch size on maternal SVL). There was a trade-off between offspring size and relative fecundity in each year of the study, but the position of the trade-off line varied significantly among years. See text for statistical analyses.

.0001), the feeding rates of the lizards (mean number of prey items per lizard stomach, adjusted for volume differences in prey: $F = 4.20$, $df = 2, 60$, $P < .02$), and the mean dates of first capture ($F = 8.95$, $df = 4, 139$, $P < .0001$) and oviposition ($F = 77.79$, $df = 4, 141$, $P < .0001$). Egg laying was delayed for approximately 30 d in the coolest year (1987) compared with the warmest one (1990). Weather conditions (identifying the proportion of days suitable for activity) were significantly correlated with the dates that female lizards emerged from hibernation and the dates that they laid their eggs (Olsson and Shine 1996).

This climatically induced variation in opportunities for basking and feeding apparently influenced female bodily condition and, hence, reproductive output. Combining data from different years, we see that variation in mean hatchling mass is related to maternal bodily condition. Females in better condition produced heavier offspring (hatchling mass vs. maternal condition after laying: $n = 135$, $r = 0.21$, $P < .015$) but did not produce larger clutches relative to their own body size (relative fecundity vs. maternal condition after laying: $n = 151$, $r = 0.12$, $P = .13$). Females exposed to a higher proportion of cloudy days prior to laying produced hatchlings that were smaller (proportion of cloudy days vs. mean SVL of hatchlings: $n = 58$, $r = -0.39$, $P < .003$). The same pattern is evident from comparisons of annual mean values for weather conditions (identifying the proportion of cloudy days in spring) and mean offspring size that year ($n = 5$ yr, $r = -0.95$, $P < .012$). These results suggest that weather-induced annual varia-

tion in food intake in spring influenced maternal bodily condition and, hence, induced annual variation in reproductive output.

Comparison of Laboratory and Field Data

The females raised in captivity were similar in body size to the field-caught females (mean SVL = 81.8 mm for 152 wild females vs. 81.02 mm for 43 laboratory-raised animals; unpaired $t = 0.80$, $df = 193$, $P = .42$), and their hatchlings were also of similar sizes (mean hatchling masses = 0.59 g from wild females vs. 0.61 g from laboratory-raised animals; unpaired $t = 1.34$, $df = 193$, $P = .18$). However, clutch sizes were larger in the well-fed laboratory lizards than in their wild conspecifics (mean clutch sizes = 8.31 for wild females vs. 9.12 for 43 laboratory-raised animals; unpaired $t = 2.50$, $df = 193$, $P < .013$). Thus, captive lizards produced larger clutch sizes, relative to their SVLs, than did wild lizards (one-factor heterogeneity of slopes test with lab/wild as the factor, female SVL as the covariate, and clutch size as the dependent variable: $F = 6.19$, $df = 1$, 191 , $P < .014$). The clutches of laboratory-raised females, like those of field-caught animals, showed a significant trade-off between clutch size and offspring size ($n = 49$, $r = -0.31$, $P < .031$). That is, mean egg sizes were smaller in large clutches (fig. 1B).

Because hatchling masses decreased with clutch sizes in both field and laboratory lizards, we were able to compare the position of the trade-off lines linking these two traits (i.e., the position of the upper limit on reproductive output). ANCOVA (with wild/lab as the factor, clutch size relative to maternal SVL as the covariate, and hatchling mass as the dependent variable) confirmed that the two groups differed significantly in the elevation of the relationship between offspring size and relative fecundity (slopes: $F = 1.06$, $df = 1$, 175 , $P = .31$; intercepts: $F = 7.90$, $df = 1$, 176 , $P < .014$). That is, because captive-raised females produced larger clutches relative to body size than did wild females, but had similar-sized offspring, the captive lizards produced larger offspring for any given level of size-specific fecundity.

DISCUSSION

Our sand lizards showed a clear trade-off between clutch size and offspring size, both in the field and the laboratory. Larger clutches were consistently associated with smaller offspring (fig. 1). In the field population, for the 5 yr of our study mean values varied for relative fecundity, hatchling mass, and the relationship between these two variables. Hence, the mean offspring size at any level of relative fecundity differed among years (fig. 2). Similarly, when lizards were raised in the laboratory with food available ad libitum, the pattern of reproductive investment changed significantly (i.e., clutch size increased relative to maternal body size), but the trade-off between offspring size and clutch size remained (fig. 1B). This result offers strong support for the notion that the upper limit on total reproductive investment is set by resource availability (which differed among years and differed between the laboratory and the field) rather than abdominal space (which did not differ). Much of the variation in patterns of reproductive

investment among individual females in this population, and among years in individual females, is thus the result of a proximate factor—apparently, one related to energy availability.

Without field manipulations, it is difficult to be certain of the exact causal relationships influencing reproductive output in free-living lizards. However, our data show that weather conditions, feeding rates, female body condition, and reproductive output all vary among years; that offspring size was larger from females in better condition; that cloudy weather resulted in the production of smaller offspring, in comparisons among females and among years; and that lizards fed ad libitum in captivity produced larger clutch sizes relative to maternal SVL than did wild-caught lizards from the same population. All of these data are consistent with the hypothesis that the proximate determinant of reproductive output is prey availability, which in turn is influenced by weather conditions (and, hence, the amount of time that a lizard can forage prior to ovulation). Postponing ovulation is unlikely to be favored in the cold climate of Asketunna, because the period for egg incubation prior to winter is relatively brief, and there may be substantial mortality of eggs laid too late in the season (Olsson et al. 1996).

Although our analysis identifies food availability as the likely limiting factor that induces a trade-off between offspring size and clutch size in the field (fig. 1A), the food-limitation hypothesis cannot explain the persistence of the trade-off in lizards raised in the laboratory and provided with surplus food (fig. 1B). We infer that the trade-off in the laboratory lizards reflects physical constraints, such as the amount of space available to hold the clutch. Such a constraint may be relative (i.e., the upper limit is some proportion of the total available abdominal volume) rather than absolute (i.e., the upper limit is the total available abdominal volume). For example, females may allocate resources to reproduction under some “rule” that keeps the RCM below the level at which it would seriously compromise locomotory performance (Shine 1980; Garland and Losos 1994). The resultant limit under this scenario would presumably be proportional to available abdominal volume, even if the female were physically capable of carrying an even larger clutch volume (Shine 1992).

The two “limiting factors” (energy and space) on reproductive output may thus explain variance at different scales. Temporal variation in reproductive output among individuals in a single population (all of which have similar body shapes) is likely to reflect upper limits imposed by energy availability. These limits will usually fall far enough below the maximal abdominal space availability that this latter factor will be unimportant as an immediate constraint on reproductive output, except in times of unusually great food availability (as in our laboratory-raised sand lizards). Nonetheless, the overall level of reproductive output is set relative to this ultimate constraint, because natural selection should result in a coevolution of body shape and reproductive output such that females of most species are relatively close to “filling themselves up” with eggs when they are gravid (Vitt and Congdon 1978). Hence, differences in body shape (abdominal volume relative to maternal mass) may be highly correlated with interspecific

variance in reproductive output (Shine 1992), even though this factor rarely functions as a proximate constraint. Teasing apart the relative importance of these different factors, and the ways in which they interact, will be an important task for future research.

Our analysis suggests some promising lines for such work. For example, one surprising aspect of our results is that the correlation coefficient between offspring mass and relative clutch size was no higher in the laboratory females than in the field animals (fig. 2). We expected that the captive females, fed ad libitum, might have shown less variation in this relationship. However, recent studies on Australian skinks suggest that variation in body shape among females in a single population may generate significant variance in reproductive output (S. Hudson, personal communication), and future work could usefully attempt to tease apart the determinants of this residual variation in reproductive output. Another topic not addressed by our work concerns the adaptive significance of the temporal variation we have documented. Year-to-year variation in mean offspring sizes may be interpreted either as adaptive (if optimal offspring size varies among years) or as a simple consequence of mechanisms by which female lizards partition energy into the clutch. Either explanation is plausible, and extensive field studies would be needed to distinguish between the two.

Last, we note that our analyses and discussion have been framed in terms of the position of the trade-off line linking fecundity to offspring size (fig. 2) rather than other more commonly used measures of overall reproductive investment such as relative clutch mass (RCM). In fact, RCM remained remarkably constant in our population. For example, RCM did not shift with maternal body size or vary among years. How can this be true, if the mean offspring mass for females at any given level of relative fecundity showed strong temporal variation (fig. 2)? The answer to this apparent paradox depends on the fact that females in better condition (i.e., with higher ratio of mass to body length after oviposition) produced larger (heavier) offspring. Thus, in years when offspring were "unusually" heavy, so were their mothers. The end result was a temporal consistency in RCMs, masking significant among-year variation in the sizes of offspring and the position of the trade-off line between offspring size and clutch size. Investigators should thus be cautious about relying on RCM as a measure of reptilian reproductive investment, because constancy in this ratio may mask important underlying variation in reproductive tactics.

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