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Does follicle excision always result in enlargement of offspring size in lizards?

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Abstract An experimental reduction of offspring number has been reported to result in enlargement of offspring size in lizards. We applied the "follicle excision" technique to a lacertid lizard (*Takydromus septentrionalis*) to examine whether this effect is generalisable to lizards. Of the 82 females that produced 3 successive clutches in the laboratory, 23 females underwent follicle excision after they oviposited the first clutch. Follicle excision reduced clutch size, but did not alter egg size. This result indicates that egg size is not altered during vitellogenesis in *T. septentrionalis*. Females undergoing follicle excision produced a third clutch (a second post-surgical clutch) as normally as did control females. Females switched from producing more but smaller eggs early in the breeding season to fewer but larger eggs later in the season. Our results indicate that female *T. septentrionalis* maximize reproductive success by diverting an optimal, rather than a higher, fraction of the available energy to individual offspring. This optimized allocation of the available energy to offspring production explains why follicle excision does not result in enlargement of egg size in this species. Our study provides evidence that an experimental reduction of offspring number does not always result in enlargement of offspring size in lizards.

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Introduction

One of the central themes of life-history studies over the past decades has been variation in size and number of offspring, because maternal fitness depends on both offspring number and offspring size that are mutually constrained by a trade-off resulting from common dependence on the limited reproductive investment (Roff 1992; Stearns 1992). The optimal offspring size theory predicts that, within a population, offspring size is optimized through natural selection and offspring number is the consequence of the partitioning of total maternal investment into optimally sized offspring (e.g. Smith and Fretwell 1974). Under this prediction, offspring size should be independent of the amount of resources a female allocates to a particular reproductive episode. In contrast to this prediction however, it has increasingly been reported that many organisms show considerable variations in offspring size, and shifts in offspring size that is a function of total reproductive investment or maternal size (e.g. Reznick and Bryga 1987; Sargent et al. 1987; Winkler and Wallin 1987; Reznick et al. 1990; Caley et al. 2001). Studies on lizards by experimental manipulation of clutch size provide further evidence that offspring size can vary as a response to variation in clutch size induced by hormonal manipulation or follicle ablation (Sinervo and Licht 1991a, b; Sinervo and DeNardo 1996; Sinervo and Doughty 1996; Sinervo 1998; Olsson et al. 2002). For example, females undergoing follicle ablation (and thus, decreased clutch size) produce larger offspring in *Uta stansburiana* (Sinervo and Licht 1991b), because yolk, normally allocated to a larger clutch, is subsequently apportioned to the fewer, remaining yolking follicles.

The arguments seem to center on the question of whether females change offspring size as a result of facultative fluctuations in accessible resources or clutch size. Where females do not adjust offspring size according to accessible resources or clutch size due to maternal investment that is partitioned into optimally sized offspring, we hypothesize that offspring size is not altered by manipulating clutch size.

Here, we describe a study applying the follicle excision technique to the northern grass lizard (*Takydromus septentrionalis*) to test our hypothesis. This small [up to 76 mm snout-vent length (SVL)], multiple-clutched oviparous lacertid lizard is endemic to China and ranges in the southern provinces of the country, and northward to Gansu province (Ji et al. 1998). Adults are sexually dimorphic in head size but not in SVL, and large females lay up to 7 clutches per breeding season, which stretches from April to August (Zhang and Ji 2000). The lizard is believed to produce eggs that are well optimized for size (Ji et al. 1998; Du et al. 2005a), and is therefore ideally suited to our investigation.

Materials and methods

Collection and animal care

Adult lizards were collected by hand or noose in late March 2003 from a population in Lishui (28° 46′N, 119° 92′E), eastern China. The lizards were transported to our laboratory in Hangzhou, where females were individually marked by painting. Between 9 and 12 lizards (females/males was *c.* 2/1) were housed together in each 90×65×50 cm^3 (length × width × height) communal cage with 5 cm depth moist soil, pieces of clay tiles and grasses. These cages were placed in a room where the surrounding temperatures were never higher than 28°C, thereby preventing gravid females from being exposed to adversely high temperatures. A 100-W light bulb, suspended at one end of each cage, created a thermal gradient ranging from ambient room temperature to 55°C for 12 h daily. Lizards were exposed to a natural daylight cycle and some direct sunlight, and could regulate their body temperature behaviorally during the photophase. Lizards were fed mealworms (*Tenebrio molitor*) and water enriched with vitamins and minerals (Nekton-Rep, Nekton-product) ad libitum. Females with shelled oviductal eggs were removed from the communal cages, and housed individually in 20×15×20 cm^3 egg-laying cages with 4 cm depth moist soil and a 20-W spotlight mounted in each cage to allow thermoregulation.

Eggs were always collected, measured and weighed within 2h after being laid, thereby avoiding any uncertainty about the egg mass due to loss or gain of water (Lin and Ji 1998). The viability of freshly laid eggs was judged by the presence of an embryonic disc using a spotlight. Post-oviposition females were measured and weighed before they were returned to the communal cages where they remained until they again carried shelled oviductal eggs, at which time they were once again transferred to the egg-laying cages. We ended our experiment after the first three sequential clutches were collected. Lizards were then released to the sites where they were originally collected.

Follicle excision

Because the first clutch potentially reflects field conditions for female recently collected from the field (Ballinger 1983; Ji and Braña 2000; Du et al. 2005b), we conducted the experiment of follicle excision after females produced this clutch. Females having laid the first clutch were individual palpated to determine their reproductive conditions. A total of 60 females in the early stage of vitellogenesis were assigned equally to manipulated and sham-manipulated groups for surgical operations. Prior to surgery, we anaesthetized females with absolute ether. We taped the anaesthetized female to a sterile board and prepared for aseptic surgery by cleaning the abdominal area with 75% alcohol. Thereafter, a 5–8 mm incision was made 2–3 mm to the left of the mid-vent line with a sterile scalpel. The left-sided ovary of the manipulated female was lifted out of the incision and then removed using a pair of forceps. Yolking follicles in the ovary of females were counted and measured before the surgical excision of follicles and their thecae; between 1 and 2 yolking follicles, measuring 2.0–2.5 mm diameter, were excised. The incision was closed using a fine surgical thread and cleaned daily with 75% alcohol. After a 5-day recovery period, females were moved back into the communal cages. Lizards in the sham-manipulated group underwent the same surgical protocol without any follicle excision. Our surgical operations were successful, not only because there was no mortality following surgery but also because the majority (83.3%) of experimental females produced a second post-surgical clutch, their third since start of egg laying, before they were released to the field.

Data analysis

Females that laid fewer than three clutches were excluded from analyses. Prior to using parametric tests, data were tested for homogeneity of variances using Bartlett's test, and for normality using the Kolmogorov–Smirnov test. We used linear regression analysis, one-way analysis of variance (ANOVA) (for female SVL) and repeated-measures ANOVA (for egg size) or ANCOVAs (for post-oviposition body mass, clutch size and clutch mass) with female SVL as the covariate, clutch order as the within subject factor, and surgical treatment (hereafter treatment) as the between subject factor to analyze data. Tukey's post hoc comparison was performed on the traits that differed significantly among treatments. Descriptive statistics were presented as mean ± standard error, and the significance level was set at $\alpha = 0.05$.

Results

Clutches reported in the present study were laid by 32 control (never undergoing surgery, 64.2 ± 0.6 mm SVL), 27 sham-manipulated (63.8 ± 0.7 mm SVL), and 23 manipulated females (64.0 ± 0.8 mm SVL). Control, sham-manipulated, and manipulated females did not differ from each other in SVL ($F_{2,\ 79} = 0.10$, $P = 0.908$). The first clutch was laid between early April and mid-May, the second clutch between early May and late June, and the third between late May and early July. The second clutch was produced an average of 23 days after the first clutch, and control (21.8 ± 0.9 days), sham-manipulated (23.2 ± 0.8 days), and manipulated (23.2 ± 1.0 days) females did not differ from each other in the clutch interval ($F_{2,\ 79} = 0.83$, $P = 0.438$).

Manipulated females laid an average of 1.4 (±0.1) eggs in the first post-surgical clutch (the females' second clutch since start of laying), but their original clutch sizes, after adding the excised yolking follicles, did not differ from those in the other two groups (Fig. 1, Table 1). Follicle excision did not result in enlargement of egg size (Fig. 1, Table 1). Manipulated females produced a second post-surgical clutch (the third clutch since start of laying) as normally as did control and sham-manipulated females (Fig. 1). Within each treatment, egg size was independent of female SVL (all $P > 0.092$). Clutch size, clutch mass and egg size differed significantly among the three successive clutches but not among the three treatments (Table 1). Post-oviposition body mass remained remarkably constant, both among the three treatments, and among the three successive clutches (Fig. 1, Table 1). A seasonal shift in reproductive output was very pronounced, with clutch size and clutch mass being both greater in the first clutch than in the two later clutches; a seasonal shift in egg size was also very pronounced, with eggs laid early in the breeding season (the first clutch) being smaller than eggs laid later in the season (the subsequent clutches) (Fig. 1, Table 1). Within each of the three sequential clutches, egg size was independent of relative fecundity [expressed as the residual derived from the regression of $\log_e$ (clutch size) on $\log_e$ (female SVL)] (all $P > 0.117$). This result provides further evidence that variation in clutch size induced proximately has no influence on egg size in *T. septentrionalis*.

Discussion

Intuitively, offspring size should be inversely correlated with offspring number because the total maternal

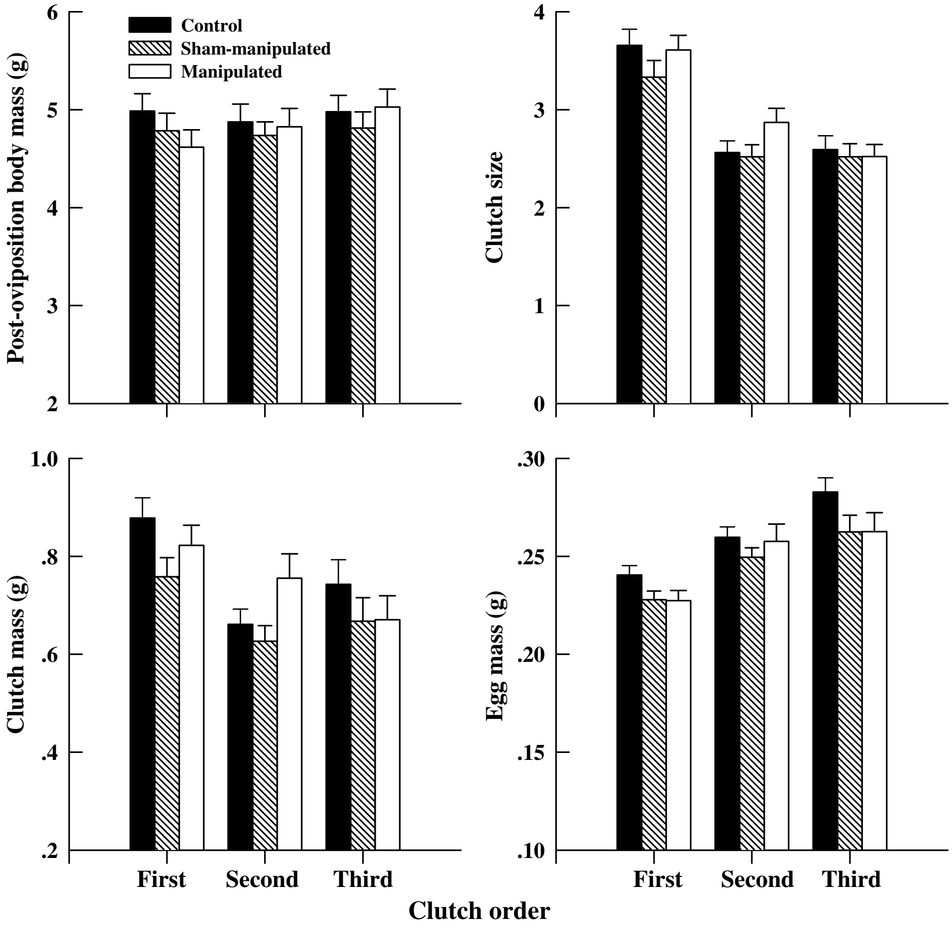

Fig. 1 Mean values (+ standard error) for post-oviposition body mass, clutch size, clutch mass and egg mass. Clutch size for the manipulated female was calculated as the sum of yolking follicles removed and eggs produced, and the clutch mass as the product of clutch mean egg mass and clutch size. *Black bars* control females, *diagonal bars* sham-manipulated females, *open bars* manipulated females

Table 1 Results of repeated-measures ANOVA (for egg size) or ANCOVAs (for post-oviposition body mass, clutch size and clutch mass), with female SVL as the covariate, clutch order as the within subject factor, and surgical treatment as the between subject factor. Tukey's post hoc comparison was performed on the traits that differed significantly among treatments

	Clutch order	Surgical treatment	Interaction
Post-oviposition body mass	$F_{2,\ 158} = 1.51 P = 0.223$	$F_{2,\ 78} = 0.18 P = 0.832$	$F_{4,\ 158} = 1.00 P = 0.407$
Clutch size	$F_{2,\ 158} = 59.48 P < 0.0001$; 1st > 2nd = 3rd	$F_{2,\ 78} = 1.28 P = 0.283$	$F_{4,\ 158} = 1.05 P = 0.384$
Clutch mass	$F_{2,\ 158} = 17.88 P < 0.0001$; 1st > 2nd = 3rd	$F_{2,\ 78} = 1.83 P = 0.168$	$F_{4,\ 158} = 1.70 P = 0.152$
Egg mass	$F_{2,\ 158} = 37.88 P < 0.0001$; 1st < 2nd < 3rd	$F_{2,\ 79} = 2.93 P = 0.059$	$F_{4,\ 158} = 0.67 P = 0.614$

investment is limited due to physical, energetic or physiological constraints (e.g. Smith and Fretwell 1974; Sinervo and Licht 1991b; Roff 1992; Doughty and Shine 1997). In the present study, however, the size-number trade-off could not be detected within each particular clutch. In all three successive clutches, egg size was independent of clutch size when removing the influence of variation in female SVL. Moreover, unlike female *U. stansburiana* (Sinervo and Licht 1991b) that produce larger offspring when they undergo follicle ablation, female *T. septentrionalis* undergoing follicle excision did not produce larger eggs.

The difference in protocol between *U. stansburiana* (Sinervo and Licht 1991b) and *T. septentrionalis* is that the follicle theca, a potential source of estrogen to keep the liver production of vitellogenin elevated, was left intact in the former species. The excess vitellogenin available to the remaining growing follicles enlarged egg size in *U. stansburiana*, but whether the effect occurs in *T. septentrionalis* is presently unclear as the follicle theca was not left intact in the present study. Nonetheless, our data do provide evidence that an experimental reduction of offspring number does not always result in enlargement of offspring size in lizards. It seems that a higher fraction of the available energy can be allocated to the yolking follicles in *U. stansburiana* but not in *T. septentrionalis*. This suggests that there does seem to be a key difference in egg regulation between *T. septentrionalis* and *U. stansburiana*. *T. septentrionalis* egg size is more resistant to perturbation, and clutch size does not change upward, in the face of an early follicle excision. We interpret our results as due to the fact that, within each particular clutch, reproductive investment per offspring is well optimized (and thus, nearly fixed) in *T. septentrionalis*. As high expenditure in current reproduction can reduce "residual reproductive value" by decreasing survival, growth, or the amount of energy available for future reproduction (Schwarzkopf 1994), our results therefore suggest that maximization of reproductive success should be achieved in *T. septentrionalis* by diverting an optimal, rather than a higher, fraction of the available energy to individual offspring in single reproductive episodes.

Lizards in nature are subject to competing energy demands, such as those necessary for mating and reproduction, escape from predators, foraging, and basking activities. Thus, the relationships among clutch size, clutch frequency, and egg size can be more complex than expected. Under laboratory conditions, however, the unlimited food availability, the absence of predators, and the suitable thermal environments allow us to study relationships of life history components without complications arising from the conflicting energetic demands in nature. In the present study, the invariant post-oviposition body mass presumably resulted from reproducing females that did not significantly accumulate energy stores but diverted most of their current surplus energy to production of eggs during the breeding season, or that they were not energy limited and ate in sufficient quantity to maximize energy stores and production of eggs.

Our data show that female *T. septentrionalis* switch from producing a larger number of smaller eggs early in the breeding season to a smaller number of larger eggs later in the season (Fig. 1, Table 1). The seasonal shift in clutch characteristics could not be a laboratory artifact, because such a shift has been reported for several other species of lizards (e.g. Ferguson and Snell 1986; James and Whitford 1994; Ji and Braña 2000), including *T. septentrionalis* (Du et al. 2005b). The initial clutch of *T. septentrionalis* is larger than subsequent clutches, presumably due to the energy reserves in the fat bodies (Xu et al. 2002; Du et al. 2003). Dividing accessible resources into smaller amounts for each egg allows females to increase the total number of eggs produced. Hatchlings from eggs produced early in the breeding season have a longer growth period and, presumably, forage in a relatively less competitive environment than progenies from later clutches (Zhang and Ji 2000). Thus, laying more but smaller eggs early in the breeding season is presumably a reproductive tactic in *T. septentrionalis* that improves female reproductive success.

In multiple-clutched oviparous species, preparing the next clutch involves two factors. First, a female's somatic condition (an indicator of energy reserves) determines the number of oocytes to be incorporated in the clutch. Second, resource acquisition, which is dependent on foraging success, and the energy available for vitellogenesis until the time of ovulation that determines final egg size. In our study, lizards produced subsequent clutches under the laboratory conditions, thus, both clutch mass and egg size were not constrained by energy availability. Accordingly, we conclude that seasonal shifts in total reproductive investment and egg size are determined ultimately by natural selection in *T. septentrionalis*, presumably as a consequence of its adaptation to local environmental conditions at the evolutionary level.

In summary, our data provide evidence that an experimental reduction of offspring number may not always result in enlargement of offspring size in lizards, and confirms that eggs produced in a given reproductive episode are well optimized for size in *T. septentrionalis* (Ji et al. 1998; Du et al. 2005a). This optimized allocation of the available energy to offspring production explains why follicle excision does not result in enlargement of egg size in *T. septentrionalis*. Overall, our data conform to the prediction from the Smith–Fretwell model that offspring size is independent of the amount of resources a female allocates to a particular reproductive event.

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