

Experimentally reducing clutch size reveals a fixed upper limit to egg size in snakes, evidence from the king ratsnake, *Elaphe carinata*

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Abstract

Snakes are free of the pelvic girdle's constraint on maximum offspring size, and therefore present an opportunity to investigate the upper limit to offspring size without the limit imposed by the pelvic girdle dimension. We used the king ratsnake (*Elaphe carinata*) as a model animal to examine whether follicle ablation may result in enlargement of egg size in snakes and, if so, whether there is a fixed upper limit to egg size. Females with small sized yolking follicles were assigned to three manipulated, one sham-manipulated and one control treatments in mid-May, and two, four or six yolking follicles in the manipulated females were then ablated. Females undergoing follicle ablation produced fewer, but larger as well as more elongated, eggs than control females primarily by increasing egg length. This finding suggests that follicle ablation may result in enlargement of egg size in *E. carinata*. Mean values for egg width remained almost unchanged across the five treatments, suggesting that egg width is more likely to be shaped by the morphological feature of the oviduct. Clutch mass dropped dramatically in four- and six-follicle ablated females. The function describing the relationship between size and number of eggs reveals that egg size increases with decreasing clutch size at an ever-decreasing rate, with the tangent slope of the function for the six-follicle ablation treatment being -0.04 . According to the function describing instantaneous variation in tangent slope, the maximum value of tangent slope should converge towards zero. This result provides evidence that there is a fixed upper limit to egg size in *E. carinata*.

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1. Introduction

Offspring size is assumed to be fitness related for many organisms, because larger offspring potentially have better performances and hence greater chances to survive (Ferguson and Fox, 1984; McGinley et al., 1987; Sargent et al., 1987; Sinervo and Adolph, 1989; Sinervo, 1990; Reznick, 1991; Enum and Fleming, 2000; Roff, 2002). This assumption implies that a female should, whenever possible, produce large offspring to increase offspring's survival probability which, in turn, enhances her reproductive success. However, as total reproductive resources available to any female are finite, she cannot increase the

size of individual offspring without concomitant reduction in the number of offspring produced (Sinervo and Licht, 1991a,b; Bernardo, 1996; Downhower and Charnov, 1998; Enum and Fleming, 1999, 2000; Agrawal et al., 2001). Thus, a female's decision to produce few large or many small offspring reflects a strategy adopted by her to maximize the number of surviving young.

The optimal offspring size theory predicts that offspring size should be optimized through natural selection and offspring number is a consequence of the partitioning of total maternal investment into optimally sized offspring (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. However, contrary to this prediction, it has increasingly been reported that offspring size can vary as a function of total reproductive investment or maternal size in many organisms (Congdon and Gibbons, 1987; Reznick and Bryga, 1987; Sargent et al., 1987; Winkler and Wallin, 1987;

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Reznick et al., 1990; Caley et al., 2001). Studies on lizards provide further evidence that offspring size can vary as a response to variation in offspring number 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, reduced offspring number) produce larger offspring in *Uta stansburiana* (Sinervo and Licht, 1991b) and *Lacerta vivipara* (Olsson et al., 2002), because yolk, normally allocated to a larger clutch, is subsequently apportioned to the fewer, remaining yolking follicles. Experimental manipulation of offspring number provides a powerful method which one may use to examine whether a female can adjust offspring size according to facultative fluctuations in reproductive resources available to her and whether there is a fixed upper limit to offspring size for a given species.

Here, we describe a study applying the “follicle ablation” technique to snakes to examine whether follicle ablation may result in enlargement of egg size in this reptilian taxon and, if so, whether there is a fixed upper limit to egg size. Unlike lizards and turtles, snakes are free of the pelvic girdle’s constraint on maximum offspring size (Congdon and Gibbons, 1987; Sinervo and Licht, 1991b), and therefore present an opportunity to investigate the upper limit to offspring size without the limit imposed by the pelvic girdle dimension. We used the king ratsnake (*Elaphe carinata*) as a model animal for two reasons. First, *E. carinata* is among species where egg size (and thus, hatchling size; Ji et al., 1997; Ji and Du, 2001) varies considerably among females differing in body size and clutch size (Ji et al., 2000). Second, the snake can be easily maintained under laboratory conditions, and is therefore ideally suited to the investigation examining egg size-number strategies.

2. Materials and methods

2.1. Study species

Elaphe carinata is a large-sized [to 170 cm SVL (snout-vent length)] colubrid snake that can be found in a variety of habitats in the hilly countryside in southeastern China including Taiwan, northward to the provinces of Henan, Shaanxi and Gansu; it is also found in northern Vietnam and Japan (Ryukyu Island, including the Senkaku Group) (Huang, 1998). In Zhoushan where we collected snakes, females larger than 108 cm SVL can lay a single clutch of 5–17 pliable-shelled eggs per breeding season stretching from July to August (Ji et al., 2000). Eggs can be incubated successfully at temperatures ranging from 24 °C to 32 °C, with the mean incubation length ranging from 74.0 days at 24 °C to 42.5 days at 32 °C (Ji and Du, 2001).

2.2. Methods

Seventy females (111–149 cm SVL) and ten males (142–167 cm SVL) were collected in early May 2003 from an island population in Dinghai (30° 02' N, 122° 10' E), Zhoushan Islands (eastern China), and transported to our laboratory in Hangzhou, where females were marked via unique combinations of clipped

ventral scales for future identification. In mid-May, females with small sized yolking follicles (c. 15–20 mm length) were equally assigned to five (three manipulated, one sham-manipulated and one control) groups (treatments). Two, four or six yolking follicles in the manipulated females were ablated by removing ~0.5 mL yolk from each ablated follicle with sterile syringes. Females in the sham-manipulated group underwent the same protocol without any yolk extraction. Females of different treatments were all maintained in a 24 × 14 × 2.5 m enclosure in the backyard of our laboratory. Food (commercially sold eggs of *Coturnix coturnix* and *Gallus gallus domesticus*) was provided ad libitum.

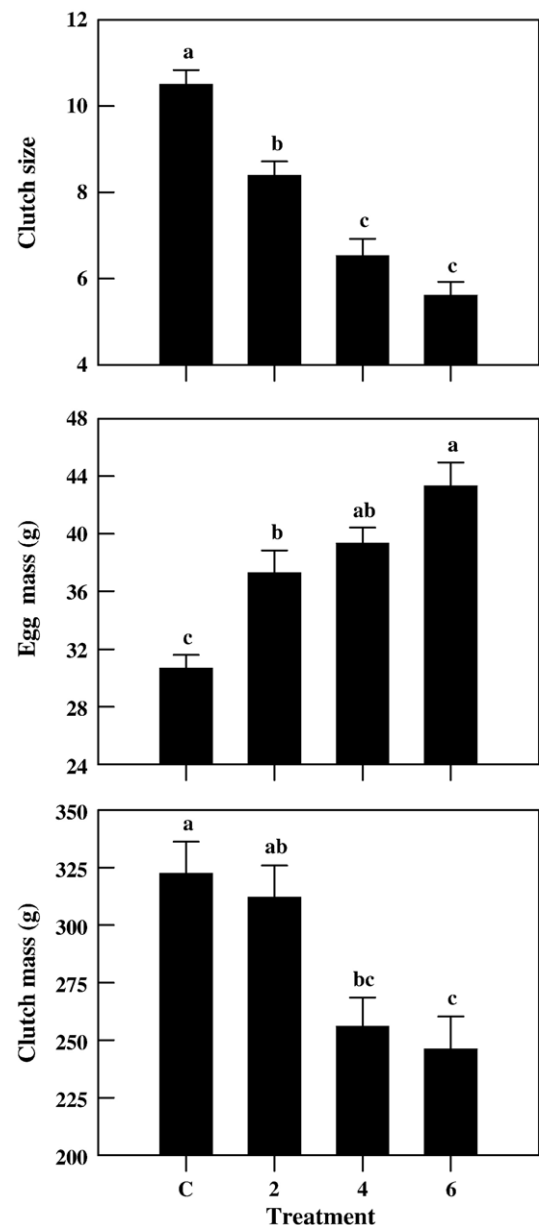


Fig. 1. Adjusted means (and SE) for clutch size, egg mass and clutch mass of females undergoing different treatments, with female SVL being set at 130 cm. Adjusted means with different lettered superscripts differ significantly (Tukey’s post hoc test, $\alpha=0.05$). C: control ($N=23$); 2: two-follicle ablation treatment ($N=11$); 4: four-follicle ablation treatment ($N=10$); and 6: six-follicle ablation treatment ($N=11$).

Females with shelled oviductal eggs were housed individually in $50 \times 45 \times 35$ cm wire cages placed in a room where the surrounding temperature varied from 26°C to 30°C . Eggs were collected, measured and weighed within 3 h after being laid, thereby avoiding any uncertainty about the initial egg mass due to loss of water. The viability of freshly laid eggs was judged by the presence of an embryonic disc using a spotlight. SVL, tail length and body mass were taken for each post-oviposition female.

2.3. Statistical analysis

Fifteen females laid abnormal clutches with various numbers of eggs containing condensed yolk for unknown reasons, and these females were found in each treatment (G -test, $G=0.53$, $P>0.90$). Data generated from females laying abnormal clutches were excluded from analysis, and the remaining data were tested for normality using the Kolmogorov–Smirnov test, and for homogeneity of variances using the Bartlett's test. Log_e transformation was performed when necessary to satisfy the assumptions for using parametric tests. We used linear regression analysis, one-way analysis of variance (ANOVA), one-way analysis of covariance (ANCOVA) and Tukey's post hoc comparison to analyze the corresponding data, when the assumptions for parametric analyses were met. Throughout this paper, values are given as the mean \pm SE, and the significance level is set at $\alpha=0.05$.

3. Results

Clutches reported in the present study were laid by 13 control (130.6 ± 27.9 cm SVL), 10 sham-manipulated (132.5 ± 35.5 cm SVL), 11 two-follicle ablated (131.5 ± 28.3 cm SVL), 10 four-follicle ablated (128.5 ± 30.0 cm SVL) and 11 six-follicle ablated females (124.4 ± 30.2 cm SVL). Females of different treatments did not differ from each other in mean SVL ($F_{4, 50}=1.10$, $P=0.369$).

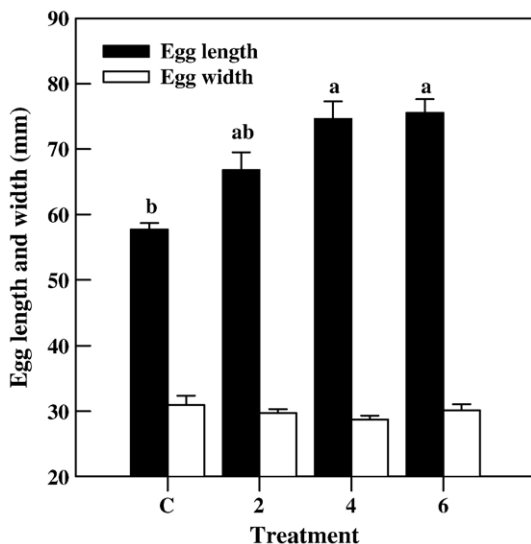


Fig. 2. Means (and SE) for length and width of eggs produced by females undergoing different treatments. Means with different lettered superscripts differ significantly (Tukey's post hoc test, $\alpha=0.05$). See Fig. 1 for key to symbols.

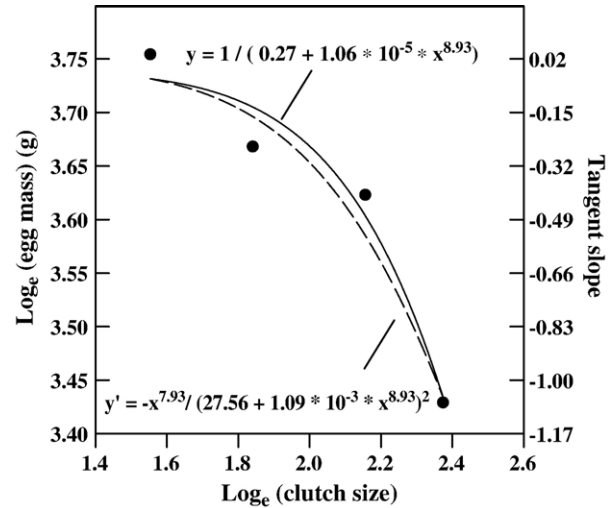


Fig. 3. The relationship between egg size and egg number in *E. carinata*. The solid curve is generated from a fit of standard curve on size-specific log_e transformed data of egg size and clutch size, with female SVL being set at 130 cm. The dash curve describes instantaneous variation in tangent slopes of the solid curve.

Oviposition occurred between 10–26 July. The sham-manipulated females did not differ from the control females in any examined traits (all $P>0.25$); therefore, we pooled data for these females and considered them together as controls. Clutch size, clutch mass, egg mass (clutch mean egg mass) and post-oviposition body mass were all positively correlated with female SVL (all $P<0.05$). ANCOVAs using SVL as the covariate showed that females of different treatments differed in clutch size ($F_{3, 50}=39.14$, $P<0.0001$), egg size (clutch mean egg mass; $F_{3, 50}=21.05$, $P<0.0001$) and clutch mass ($F_{3, 50}=6.37$, $P<0.001$) but not in post-oviposition body mass ($F_{3, 50}=2.15$, $P=0.105$).

Females undergoing follicle ablation produced fewer but larger eggs than did control females (Fig. 1). Clutch mass (and thus, total reproductive investment) did not differ between control and two-follicle ablated females, but was dramatically reduced in four- and six-follicle ablated females (Fig. 1).

Egg mass was positively correlated with egg length (all $P<0.05$) but not with egg width in each treatment (all $P>0.068$). Egg width did not differ among treatments (ANOVA: $F_{3, 51}=0.64$, $P=0.660$), whereas eggs produced by manipulated females were longer than those produced by control females (ANCOVA: $F_{3, 50}=5.60$, $P<0.003$; Fig. 2).

The function describing the relationship between size and number of eggs reveals that egg size increases with decreasing clutch size at an ever-decreasing rate, with the tangent slope of the function for the six-follicle ablation treatment being -0.04 (Fig. 3). According to the function describing instantaneous variation in tangent slope, the maximum value of tangent slope should converge towards zero (Fig. 3). This result indicates that there is a fixed upper limit to egg size in *E. carinata*.

4. Discussion

Our manipulation of clutch size had the desired effect of inducing variation in egg size, and data generated from this

study show clearly that follicle ablation may result in enlargement of egg size in *E. carinata*, and that egg size may vary as a result of facultative fluctuations in clutch size (Fig. 1). Post-oviposition body mass did not differ among treatments, presumably because reproducing females did not accumulate energy but diverted most of their current surplus energy to production of eggs during the breeding season, or because they were not energy limited and ate in sufficient quantity to maximize energy stores and production of eggs.

Females undergoing follicle ablation increased egg size primarily by increasing egg length rather than by increasing egg width, thereby producing more elongated eggs (Fig. 2). Because egg shape is an indicator of crowdedness of eggs in the oviduct and because more rounded eggs are always associated with larger or heavier clutches in species where total reproductive investment is constrained by the maternal body volume (Castilla et al., 1992; Ji and Braña, 2000; Ji et al., 2002a,b; Ji and Wang, 2005), the result that the manipulated females produced more elongated eggs implied that the oviducts of these females were less tightly packed when they were gravid.

The maximum egg size has been proved to be constrained by the pelvic girdle dimension in groups like lizards and turtles (Congdon and Gibbons, 1987; Sinervo and Licht, 1991b). Compared to egg length, egg width is more likely to be constrained by the pelvic girdle dimension in lizards and turtles. Our results show, however, that egg width is more likely to be shaped by the morphological feature (the maximum oviduct diameter in particular) of the oviduct in *E. carinata* that is free of the pelvic girdle's constraint on maximum egg size.

With the six-follicle ablation, the mean clutch size dropped from 10.7 to 4.7, the mean egg mass increased from 30.9 g to 42.7 g and the mean clutch mass dropped from 330.6 g to 200.7 g for females of which body sizes were controlled at 130 cm SVL (Fig. 3). The mean egg mass in the six-follicle ablation treatment was much closer to the upper limit to egg size, because the tangent slope of the function describing the relationship between size and number of eggs for this treatment was -0.04 , which is much closer to zero at which eggs are theoretically maximized for size (Fig. 3). These findings suggest that there is a fixed upper limit to egg size in *E. carinata*.

Females producing largest eggs did not completely fill the available body volume, as indicated by the fact that clutch mass dropped dramatically in females undergoing six-follicle ablation (Fig. 1). This result suggests that the potential constraints of the body volume on total reproductive investment as well as maximum egg size can be mitigated to some extent in female *E. carinata* producing fewer eggs. Moreover, as females preparing clutches in the enclosure had free access to food, both clutch mass and egg size were therefore unlikely to be constrained by energy availability. Thus, as the result reported recently for *Takydromus septentrionalis* (northern grass lizard; Du et al., 2005; Ji and Diong, 2006), the upper limit to egg size may not be determined by morphological factors such as the maternal body volume (Vitt and Congdon, 1978; Shine, 1992), but by natural selection as a consequence of the female's response to long-term local environmental conditions. 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), maximization of life-time reproductive success could be achieved in female *E. carinata* not producing as large eggs as they can.

Egg size increased with decreasing clutch size at an ever-decreasing rate (Fig. 3). This result implies that, within the size-number trade-off continuum involved, the potential gain of offspring fitness due to the increased egg size becomes decreasingly pronounced in females producing fewer eggs. In this study, fecundity selection (favoring many small offspring) and survival selection (favoring few large offspring) are, in fact, balanced to the greatest extent in control females. ANCOVAs using female SVL as the covariate reveal that clutch size ($F_{1, 43}=0.40$, $P=0.532$), clutch mass ($F_{1, 43}=0.16$, $P=0.689$) and egg mass ($F_{1, 43}=1.64$, $P=0.207$) do not differ between control females and females ($N=23$) preparing clutches in nature (Ji et al., 2000). This comparison provides an inference that fecundity selection and survival selection are often balanced to a large extent in nature.

In summary, our data show that follicle ablation may result in enlargement of egg size in *E. carinata*, and confirm that eggs of this species may vary as a result of facultative fluctuations in clutch size (Ji et al., 2000). There is a fixed upper limit to egg size in *E. carinata*, and the limit is not determined by morphological factors but by natural selection at the evolutionary level. Fecundity selection and survival selection are often well balanced in a natural population of *E. carinata*.

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