

# An Experimental Test of Body Volume Constraint on Female Reproductive Output

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## ABSTRACT

The body volume constraint hypothesis is a widely accepted notion that explains proximate limitation of female reproductive output, but lacks empirical support. To examine how body volume determines reproductive output, we experimentally manipulated the body volume of female lacertid lizard (*Takydromus septentrionalis*), to examine whether a reduction in body volume caused a reduction in reproductive output. Clutch size and mass decreased with a reduction in body volume. Reproductive output (residual clutch mass,  $R_{CM}$ ) was negatively related to body volume reduction (BVR) ( $R_{CM} = -0.008BVR + 0.225$ ). In contrast, neither egg size nor offspring size was affected by the manipulation. Relative clutch mass was lower in manipulated females than in control females, but the actual physical burden (egg plus pearls) carried by manipulated females was much bigger than that of the control females. A normal clutch of eggs only filled 55% of maternal body volume, whereas manipulated females used 75% of body volume to contain eggs and pearls. Therefore, females are capable of carrying more eggs than a normal clutch. Our observations provide unequivocal empirical support for the maternal body volume constraint hypothesis. In addition, these results suggest that female *T. septentrionalis* may produce a clutch of eggs that fill the available abdomen space to an optimal level rather than the physical limit. *J. Exp. Zool.* 313A:123–128, 2010. © 2009 Wiley-Liss, Inc.

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Reproductive output, which may directly influence recruitment and viability of a population, has attracted great scientific attention for several decades (Tinkle, '69; Stearns, '92; Roff, 2002). Whilst tremendous variation in reproductive output has been revealed among and within species (Denno and Dingle, '81; Western and Ssemakula, '82; Dunham et al., '88; Stearns, '92), less is known about how a female determines her reproductive output. Ultimately, female reproductive output may be determined by reproductive strategies and trade-offs, such as reproductive output vs. growth (Stearns, '92). Proximately, a female may determine reproductive output based on available resources, including body volume and energy income. Body volume can determine the upper physical limit up to which a female can fill with a clutch of eggs (offspring), whereas the energy available to an organism may constrain reproductive investment (Shine, '92; Olsson and Shine, '97; Du et al., 2005a). The upper limit for female reproductive output could thus be set by either optimizing selection or proximate constraints; the optimal reproductive output can be below the upper limit imposed by constraints and vice versa (Shine, '92).

Maternal body volume is obviously one of the most important constraints that may limit reproductive output. In most, if not all animals, female body size is an important determinant of reproductive output, with a positive relationship between body size and reproductive output. Maternal body volume constraint hypothesis, suggested by Vitt and Congdon ('78) and Shine ('92), predicts that females are "full" of eggs and thus body volume constrains reproductive output. Certainly, females are not equally "full," given the diverse relative clutch mass (RCM) among

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species (Shine, '92; Goodman et al., 2009). In species with high RCM, females likely produce a clutch of eggs that completely fills the available abdominal volume, defined as “absolute body volume” (Shine, '92), and presumably constrains their reproductive output. In species with low RCM, by contrast, females probably fill the space available to some optimal level, regarded as “functional reproductive volume” (Vitt and Congdon, '78; Shine, '92).

Although maternal body volume has been thought to play an important role in determining reproductive output, experimental evidence for the maternal body volume constraint hypothesis is scarce (Qualls and Andrews, '99). Recently, using body volume manipulation, we demonstrated that body volume reduction can reduce clutch size as well as clutch mass in a lacertid lizard (*Takydromus septentrionalis*), thereby suggesting that body volume can constrain reproductive output in lizards (Du et al., 2005a). However, in an earlier study, reproductive output was only compared between control females and a single group of manipulated females, whose body cavity had been reduced to a similar level by implanting two pearls into the abdomen of each lizard. More powerful evidence to justify the maternal body volume constraint hypothesis, for example, whether reproductive output decreases with a reduction in body volume, would come from a manipulation experiment with an incremental reduction in body volume.

In this study, the earlier work is extended to reduce the female body volume of *T. septentrionalis* to different levels, and thereby to quantify the relationship between body volume reduction and reproductive output. Our objectives are (1) to provide unequivocal empirical evidence to test the maternal body volume constraint hypothesis and (2) to assess whether reproductive output is determined by “absolute body volume,” or instead represents the use of available space, and thus supports the idea of “functional reproductive volume.” In light of the maternal body volume constraint hypothesis, we predict that reproductive output, including clutch size and clutch mass, would decrease as body volume declines. Given the low RCM in *T. septentrionalis* (Du et al., 2005b), we predict that females will produce a clutch of eggs that fills the available abdomen space to a “functional reproductive volume” rather than the “absolute body volume.”

## MATERIALS AND METHODS

### Animal Collection and Husbandry

The northern grass lizard, *T. septentrionalis*, is a small oviparous lizard (up to 80 mm snout–vent length, SVL) distributed in China. Detailed information on reproductive traits has been reported on several populations of this species (Du et al., 2005b, 2006; Ji et al., 2007). In late March 2005, we collected female *T. septentrionalis* that had mated in the field from Quzhou, Zhejiang, eastern China (28°52'N, 118°55'E). All lizards were weighed ( $\pm 0.01$  g) and measured (SVL,  $\pm 0.01$  mm), before being

housed in several terraria (600 × 400 × 300 mm). Each terrarium had sand and grass on its bottom to mimic the natural habitats of the lizard. A 60 W light bulb over the terraria provided thermoregulation opportunities for lizards from 0700 to 1700 hr. The animals were fed larvae of *Tenebrio molitor*, and water containing mixed vitamins and minerals was always available.

### Body Volume Manipulation

After one week, the females were randomly assigned to one of two groups: control (SVL:  $70.89 \pm 0.56$  mm,  $n = 27$ ) or treatment (SVL:  $70.70 \pm 0.50$  mm,  $n = 41$ ). All animals were anesthetized and an 8 mm incision was made on the right side of the abdominal midline. Care was taken to avoid a large vein that lies in the midline. For the experimental treatment, one or two egg-sized real pearls were inserted into the abdomen of females belonging to the pearl-implant group, whereas no pearl was inserted into the body cavity of control females. All pearls were measured and weighed individually, so that we were able to quantify the exact volume and mass of pearls implanted for each female (i.e., reduced volume of body cavity). The implanted pearls ranged from 225.7 to 683.4 mm<sup>3</sup> in volume and from 0.307 to 1.016 g in weight. After that, we sutured the incision and kept the lizards in clean cages for two weeks before returning them to their original terraria. The surgical incision of all lizards was sterilized everyday until fully healed.

### Egg Collection and Incubation

Females started to lay eggs after they had been kept in the laboratory for about 4 weeks. Every 5 days, we palpated the abdomen of females, and any lizard with oviductal eggs was transferred to a small glass terrarium (200 × 150 × 200 mm) filled with 2 cm-depth moist sand. These small terraria were checked at least four times a day for freshly laid eggs. All eggs were weighed ( $\pm 0.001$  g) immediately, so as to minimize the egg mass change owing to water exchange. The postpartum females were measured (SVL,  $\pm 0.01$  mm) and weighed ( $\pm 0.001$  g).

The eggs were incubated in containers filled with moist vermiculite (water potential of  $-12$  kPa, dry vermiculite: water = 1:2). The containers were then kept in an incubator in which the temperature was set at 27°C. During incubation, all containers were weighed every other day and, if necessary, water was added to keep the substrate moisture constant. To minimize any effect of thermal gradients inside the incubator, we moved the containers among shelves daily according to a predetermined schedule. The containers were checked several times a day to collect hatchlings after the first hatchling pipped its eggshell. Each hatchling was weighed, and measured SVL and tail length.

### Female Body Volume Estimation

An additional 11 dead specimens collected from the same population were used to evaluate female body volume by

injecting water into the abdomen of the specimen until it was full. We determined the body volume of the females, in this study, using a regression equation between the volume of injected water (the proxy of body volume, PBV) and female size (SVL):  $PBV = 35.9SVL - 979.8$  ( $r^2 = 0.42$ ,  $F_{1,9} = 6.56$ ,  $P = 0.03$ ). The body volume reduction was then calculated as the percentage of total pearl volume relative to maternal body volume.

#### Data Analysis

RCM was calculated as the ratio of clutch mass to maternal postpartum mass. Maternal postpartum body condition was quantified as residual scores from log-transformed body mass relative to log-transformed SVL. We used  $\chi^2$  test to compare the percentage of reproducing females between treatments. One-way analysis of variance (ANOVA) was used to detect between-treatment differences in SVL, body mass, RCM, and postpartum body condition of females. One-way analysis of covariance (ANCOVA) with maternal SVL as the covariate was employed to test for the effect of treatments on clutch size, clutch mass, and egg size. To quantify the relationship between reproductive output and body volume reduction, we calculated residual scores from the regression of clutch mass on maternal SVL as size-independent reproductive output, and did linear regression of the size-independent clutch mass against body volume reduction.

## RESULTS

#### The Effect of Body Volume Reduction on Reproductive Traits

During our experiment, 21 out of 27 individuals in control, 31 out of 41 individuals in pearl-implant treatment laid one normal clutch of eggs. Accordingly, the percentage of females that successfully produced eggs did not differ between control and treatment ( $\chi^2 = 0.01$ ,  $df = 1$ ,  $P = 0.94$ ).

Females from the two treatments did not differ in SVL ( $F_{1,50} = 0.06$ ,  $P = 0.80$ ), body mass ( $F_{1,50} = 0.35$ ,  $P = 0.56$ ), or postpartum body condition ( $F_{1,50} = 0.61$ ,  $P = 0.44$ ). Clutch size and mass of manipulated females were significantly smaller than those of control females, whereas egg size and mass did not differ between the treatments (Table 1). Although RCM was lower for manipulated females than for control ones ( $F_{1,50} = 5.27$ ,

$P = 0.03$ ), the actual physical burden carried by reproducing females was much bigger in manipulated females than in controls, if both eggs and pearls were considered ( $F_{1,50} = 36.06$ ,  $P < 0.0001$ ) (Fig. 1a). Similarly, after the pearl volume had been taken into account, the percentage of egg volume relative to body volume for manipulated females was significantly higher than that for control females ( $F_{1,50} = 22.64$ ,  $P < 0.0001$ ) (Fig. 1b).

Clutch mass was positively related to female body size (SVL). The slope of regression line in control females was similar to that in manipulated females ( $F_{1,49} = 0.10$ ,  $P = 0.76$ ), whereas the intercept was higher for control females than manipulated females ( $F_{1,49} = 8.35$ ,  $P = 0.006$ ) (Fig. 2). Looking at the relationship between body volume reduction and the reproductive output of pearl-implanted females, we found that clutch size and clutch mass were negatively related to the volume of implanted pearls after controlling maternal SVL (partial correlation: clutch size,  $r = -0.50$ ,  $t = -3.02$ ,  $df = 28$ ,  $P = 0.005$ ; clutch mass,  $r = -0.48$ ,  $t = -2.91$ ,  $df = 28$ ,  $P = 0.007$ ). However, such correlation did not occur to egg mass (partial correlation:  $r = -0.04$ ,  $t = -0.23$ ,  $df = 28$ ,  $P = 0.82$ ). The relationship between reproductive output, namely residual clutch mass ( $R_{CM}$ ), and body volume reduction (BVR) could be predicted by the following equation:  $R_{CM} = -0.008BVR + 0.225$ ,  $r^2 = 0.20$  (Fig. 3).

#### The Effect of Body Volume Reduction on Hatchling Traits

Seventeen out of twenty-one clutches in control and 21 out of 31 clutches in pearl-implant treatment hatched successfully; hatching success did not differ between treatments ( $\chi^2 = 0.17$ ,  $df = 1$ ,  $P = 0.68$ ). To avoid pseudoreplication, mean values for incubation duration and phenotypic traits of hatchlings from each clutch were used in subsequent analyses. The manipulation did not affect the incubation period of eggs and the SVL, body mass, and tail length of hatchlings (Table 2).

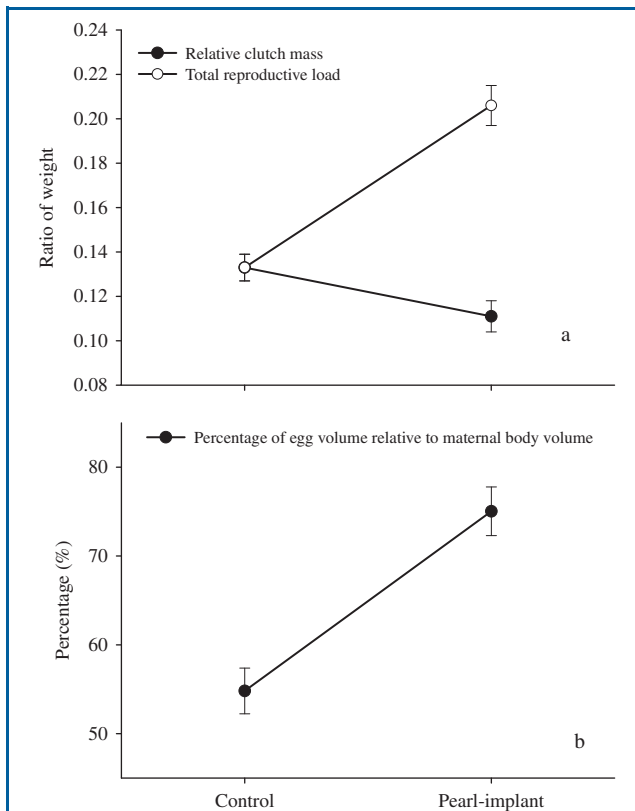
## DISCUSSION

The maternal body volume constraint hypothesis has been widely accepted to explain the proximate limitation of reproductive output, but lacks direct empirical support. Our earlier study found that body volume reduction may decrease reproductive output in *T. septentrionalis* (Du et al., 2005a). This study further

**Table 1.** The effect of body volume reduction on reproductive traits in the northern grass lizards (*T. septentrionalis*).

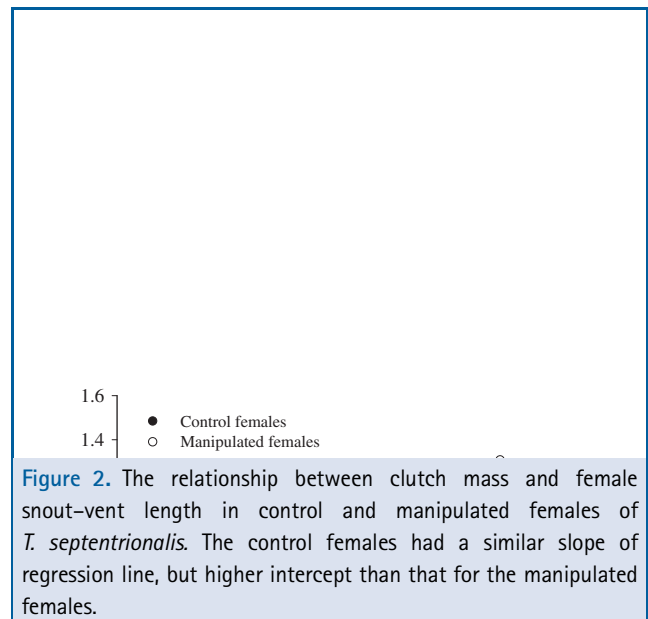
	Sham control (n = 21)	Pearl-implant treatment (n = 31)	
Clutch size	3.0 ± 0.2	2.5 ± 0.1	$F_{1,49} = 6.86$ , $P = 0.01$
Clutch mass (g)	0.821 ± 0.044	0.707 ± 0.036	$F_{1,49} = 5.63$ , $P = 0.02$
Egg mass (g)	0.282 ± 0.007	0.284 ± 0.006	$F_{1,49} = 0.06$ , $P = 0.80$
Egg length (mm)	10.69 ± 0.12	10.71 ± 0.10	$F_{1,49} = 0.02$ , $P = 0.88$
Egg width (mm)	6.75 ± 0.07	6.84 ± 0.06	$F_{1,49} = 0.91$ , $P = 0.35$

Data are expressed as adjusted mean ± SE. One-way ANCOVA with maternal snout-vent length (SVL) as the covariate was used to analyze the differences in clutch size and mass, and egg size.

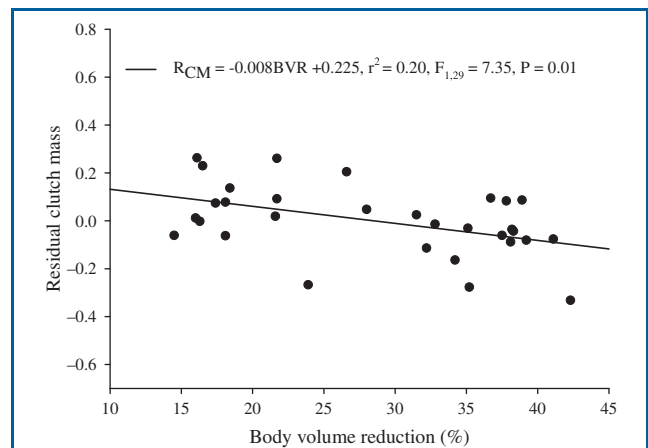


**Figure 1.** Relative clutch mass, total reproductive load (a), and relative egg volume (b) of female *T. septentrionalis* from control ( $n = 21$ ) and pearl-implant treatment ( $n = 31$ ). Relative clutch mass was calculated as the ratio of clutch mass to maternal postoviposition mass. Total reproductive load was calculated as the ratio of clutch mass and pearl mass to maternal postoviposition mass. Relative egg volume was calculated as the percentage of egg volume (including pearl volume for manipulated females) relative to maternal body volume. Data are expressed as mean  $\pm$  SE.

demonstrated a negative relationship between body volume reduction and female reproductive output (Fig. 3), which clarifies that the decrease in the reproductive output is a result of different levels of body volume reduction rather than the artifact of experimental manipulation (inserting pearls). These results provide unequivocal support for the maternal body volume hypothesis, which suggests female body volume can constrain reproductive output. Apart from the experiments of body volume manipulation, some other studies have provided indirect evidence to support this hypothesis. For example, compared with their oviparous conspecifics, the viviparous lizards (*Lerista bougainvillii*) carry heavier clutch mass with a corresponding increase in body size and a predicted change in body shape (Qualls and Shine, '95); maternal body volume can constrain the amount of water taken up by eggs in utero (Qualls and Andrews, '99).



**Figure 2.** The relationship between clutch mass and female snout-vent length in control and manipulated females of *T. septentrionalis*. The control females had a similar slope of regression line, but higher intercept than that for the manipulated females.



**Figure 3.** The relationship between residual clutch mass ( $R_{CM}$ ) and body volume reduction (BVR) in manipulated females of *T. septentrionalis*. Residual clutch mass was calculated as residual scores from regression analysis of clutch mass on maternal snout-vent length. Body volume reduction was expressed as the percentage of pearl volume relative to total body volume.

Female reptiles may adjust their reproductive output to physical maximum limit, or an optimal level of body volume (Vitt and Congdon, '78; Shine, '92). Although female *T. septentrionalis* produced a clutch of eggs filling about 55% of their total body volume (Fig. 1b), the manipulated females could carry 1.5 times more weight (eggs plus pearls) than control (Fig. 1a). Females proportionally decreased reproductive output when body volume was reduced (Fig. 2). These results suggest that the females are capable of carrying more eggs than a normal clutch,

**Table 2.** Incubation duration and the size of hatchlings from eggs produced by control and body-volume manipulated females in *T. septentrionalis*.

	Sham control (n = 17)	Pearl-implant treatment (n = 21)	
Incubation duration (day)	45.3 ± 0.2	44.7 ± 0.2	$F_{1,36} = 3.41, P = 0.07$
Hatchling mass (g)	0.339 ± 0.010	0.346 ± 0.007	$F_{1,35} = 0.12, P = 0.73$
Snout-vent length (mm)	26.46 ± 0.28	26.51 ± 0.17	$F_{1,35} = 0.23, P = 0.63$
Tail length (mm)	58.21 ± 0.81	57.66 ± 0.97	$F_{1,35} = 1.06, P = 0.31$

Data are expressed as mean ± SE (incubation duration) or adjusted mean ± SE (hatchling sizes). One-way ANCOVA with initial egg mass as the covariate was used to analyze the between-treatment differences in hatchling sizes, whereas one-way ANOVA was used to detect the difference in incubation duration.

but naturally produce a clutch of eggs to an optimal level instead of the physical maximum of their body cavity. Our earlier studies give additional support to this suggestion. For instance, *T. septentrionalis* produced more clutches rather than increased reproductive output in a single clutch when there was sufficient energy (Ji et al., 2007). Conversely, lizards reduced the number of clutches they produced during the reproductive season, but did not change reproductive output per clutch when faced with low food availability (Du, 2006). Therefore, the reproductive output of *T. septentrionalis*, which is probably shaped by optimizing selection, is below the upper limit imposed by the physical constraints of maternal body volume.

Why do female *T. septentrionalis* produce a clutch of eggs that fill the available abdomen space to an optimal level rather than the maximum body volume? First, this result is likely linked to the ecology of this species. *T. septentrionalis* is a slender-bodied lizard living in open grassy area of mountainous regions and forages actively on invertebrates (Zhao and Adler, '93; Ji et al., '94; Du et al., 2005b). Obviously, low reproductive output may facilitate their movement and foraging activity (Vitt and Congdon, '78). Second, low reproductive output may reduce the cost of reproduction, because relatively small clutches have no measurable impact on maternal locomotor ability, whereas big burden in pregnancy may lead to reduced locomotor performance and thereby high predation risk (Miles et al., 2000; Shine, 2003). It would be of great interest to study the effect of clutch size manipulation on female functional performance and/or survival, to clarify whether females with experimentally high reproductive output compared with unmanipulated females have lower survivorship and therefore lifetime reproductive success.

It is noteworthy mentioning that reproductive investment strategy in females with low RCMs, such as *T. septentrionalis*, may not be applied to those species with high RCMs, because maternal body volume probably imposes more severe constraints on reproductive output in species with high RCMs. Accordingly, high RCM species, such as skinks that produce a single clutch per reproductive season but have a relatively short abdomen (Shine, '92), may make excellent subjects in further studies on identifying the relationship between body volume and reproductive output in lizards.

Reproductive decision regarding energy allocation by a female is usually treated as a two-step process: decision about optimal investment of energy to current reproduction, followed by decision on energy allocation among offspring (Sargent et al., '87; Sinervo et al., '92; Stearns, '92; Roff, 2002). Offspring size may thus be affected by the total reproductive investment as well as the trade-off between clutch size and egg size. Clutch size modification (hormonal manipulation or follicle ablation) may lead to change in egg size of some lizards and snakes (Sinervo and Licht, '91; Sinervo and DeNardo, '96; Sinervo, '99; Olsson et al., 2002; Ji et al., 2006, 2009). In *T. septentrionalis*, however, clutch size manipulation did not result in reduction or enlargement of egg (offspring) size (Du, 2003; Ji and Diong, 2006). This study further indicates that a body volume reduction does not affect egg (offspring) size in spite of the significant impact of body volume manipulation on total reproductive output. Therefore, changes in total reproductive investment, owing to female body volume reduction or clutch size manipulation, do not affect energy allocation to each offspring in this species, supporting the notion that reproductive effort per offspring is optimized independently of total reproductive effort (Smith and Fretwell, '74). However, in some other species, offspring size varies considerably with evolutionary shift in total reproductive effort or as a function of maternal size (Reznick and Bryga, '87; Winkler and Wallin, '87; Caley et al., 2001; Beck and Beck, 2005).

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