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Measuring temporal variation in reproductive output reveals optimal resource allocation to reproduction in the northern grass lizard, *Takydromus septentrionalis*

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We measured the reproductive output of *Takydromus septentrionalis* collected over 5 years between 1997 and 2005 to test the hypothesis that reproductive females should allocate an optimal fraction of accessible resources in a particular clutch and to individual eggs. Females laid 1–7 clutches per breeding season, with large females producing more, as well as larger clutches, than did small females. Clutch size, clutch mass, annual fecundity, and annual reproductive output were all positively related to female size (snout–vent length). Females switched from producing more, but smaller eggs in the first clutch to fewer, but larger eggs in the subsequent clutches. The mass-specific clutch mass was greater in the first clutch than in the subsequent clutches, but it did not differ among the subsequent clutches. Post-oviposition body mass, clutch size, and egg size showed differing degrees of annual variation, but clutch mass of either the first or the second clutch remained unchanged across the sampling years. The regression line describing the size–number trade-off was higher in the subsequent clutch than in the first clutch, but neither the line for first clutch, nor the line for the second clutch varied among years. Reproduction retarded growth more markedly in small females than in large ones. Our data show that: (1) trade-offs between size and number of eggs and between reproduction and growth (and thus, future reproduction) are evident in *T. septentrionalis*; (2) females allocate an optimal fraction of accessible resources in current reproduction and to individual eggs; and (3) seasonal shifts in reproductive output and egg size are determined ultimately by natural selection.

ADDITIONAL KEYWORDS: clutch size – egg size – Lacertidae – life history – reproductive investment – trade-offs.

INTRODUCTION

Life-history theory predicts that an organism's available resources should be optimally allocated among the conflicting demands of maintenance, growth, and reproduction, such that lifetime reproductive success and other life-history traits can be compromised (Fisher, 1930; Williams, 1966; Shine, 1980; Roff, 1992; Stearns, 1992). For example, increasing allocation of resources to current reproduction may result in a reduction in future reproduction because of reduced growth rate or survival probability (Perrin & Sibly, 1993; Kozlowski, 1996; Heino & Kaitala, 1999; Lardner & Loman, 2003), whereas increased allocation of resources to one offspring in a single reproductive event may result in a reduction in either the size or number of other offspring because the total reproductive resources (space or energy) available to any female are finite (Williams, 1966; Smith & Fretwell, 1974; Roff, 1992; Shine, 1992; Bernardo, 1996; Downhower & Charnov, 1998; Einum & Fleming, 2000;

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Agrawal, Brodie & Brown, 2001; Satoki & Harada, 2004). Life-history trade-offs are the major constraint that conditions the resolution of the conflicting demands, but are difficult to detect without measuring the traits in question over time. This is especially true for the species in which life-history traits show marked phenotypic variation resulting from the effects of proximate or ultimate factors. For example, year-to-year variation in resource availability results in among-year variation in the mean offspring size and reproductive output in the sand lizard *Lacerta agilis* (Olsson & Shine, 1997), whereas natural selection acting on females ultimately determines seasonal shifts in the mean offspring size and the amount of reproductive investment in the common wall lizard *Podarcis muralis* (Ji & Braña, 2000). Therefore, we suggest using an approach focusing on temporal variation in life-history traits, which allows us to examine trade-offs between size and number of offspring, and between current reproduction and other life-history traits such as growth and future reproduction.

In the present study, we use this approach to examine life-history trade-offs by measuring temporal variation in reproductive output of female northern grass lizards (*Takydromus septentrionalis*) collected over 5 years between 1997 and 2005. This lacertid lizard is endemic to China and lives mainly in the southern provinces of the country (Ji *et al*., 1998). Adults are sexually dimorphic in head size but not in snout–vent length (SVL), and the breeding season often stretches from April to August (Zhang & Ji, 2000). The lizard has been reported to produce multiple clutches of eggs and can be easily maintained in the laboratory (Ji *et al*., 1998; Du, Ji & Shine, 2005a; Du *et al*., 2005b), and therefore offers an excellent model organism to address the following questions related to life-history trade-offs: (1) do females maximize lifetime reproductive success by diverting an optimal fraction of accessible resources to current reproduction; (2) do females optimize the relationship between size and number of eggs in single reproductive events by diverting an optimal fraction of resources to individual eggs; and (3) is there a detectable trade-off between size and number of eggs? If so, do females shift the level of the size–number trade-off according to temporal variation in clutch size?

MATERIAL AND METHODS

COLLECTION AND ANIMAL CARE

Adult lizards were collected by hand or noose in late March in 1997, 1998, 1999, 2003, and 2005 from Lishui (28°46′-N, 119°92′-E), eastern China, where annual variation in monthly mean air temperature and rainfall is very pronounced: monthly mean air temperatures varying from 8 °C (January) to 30 °C (July), and monthly mean rainfall varying from 20 mm (October) to 360 mm (May). Lizards collected in each sampling year were transported to our laboratory in Hangzhou, where they were sexed and marked via unique combinations of clipped toes. We housed 9–12 lizards (the female : male ratio was approximately 2 : 1) in each (length × width × height: 90 × 65 × 50 cm) communal cage with moist soil (5 cm in depth), pieces of clay tile, and grasses. These cages were placed in a room where temperatures were never outside the range of 20–28 °C. A 100-W light bulb, suspended at one end of each cage, created a thermal gradient from room temperature to 55 °C for 12 h daily. Lizards were exposed to a natural daylight cycle and some direct sunlight, and could regulate body temperature behaviourally during the photophase. Lizards were fed mealworms (*Tenebrio molitor*) and water enriched with vitamins and minerals (Nekton-Rep, Nekton Products, Germany) *ad libitum*. Females with shelled oviductal eggs were removed from the communal cages, and housed individually in egg-laying cages (20 × 15 × 20 cm) with moist soil (4 cm in depth) and a 20-W spotlight mounted in each cage to allow thermoregulation. The time that females remained in the egg-laying cages was never more than 7 days and often less than 3 days.

All eggs were collected, measured for length and width (to the nearest 0.01 mm), and weighed (to the nearest 1 mg) immediately after being laid. The viability of freshly-laid eggs was judged by the presence of an embryonic disc using a spot light. 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. Females collected in 1997 were allowed to produce as many clutches as they could in the laboratory; females collected in the other 4 years were released to the sites where they were originally captured, soon after they laid the first two clutches.

DATA AND STATISTICAL ANALYSES

Females occasionally laid abnormal eggs with condensed yolk or were retained for an unusually long period. For these females, we recorded their reproductive events but excluded the clutch data from statistical analyses. Clutch mass was calculated as the total mass of eggs in a clutch. Relative clutch mass (RCM) was calculated by dividing clutch mass by the postoviposition mass. Variances in clutch size and egg size (clutch mean egg mass) were calculated as the coefficient of variation (= standard deviation divided by the mean). To remove the influence of variation in female SVL on fecundity, we calculated relative fecundity by using the residuals derived from the regression of $\log_e$ (clutch size) on $\log_e$ (female SVL) (Olsson & Shine, 1997).

All data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett's test). We used one-way analysis of variance (ANOVA), one- and two-way analysis of coviance (ANCOVA), repeated measures ANOVA, repeated measures ANCOVA, partial correlation analysis and Tukey's post-hoc comparison to analyse the corresponding data. Log_e transformation was performed when necessary to achieve the assumptions for parametric tests. We used the Kruskal–Wallis test for differences in clutch interval (the time interval between two successive clutches) among females that differed in clutch frequency because the normality assumption was not met. Values are presented as the mean ± SE and $\alpha = 0.05$ was considered statistically significant.

RESULTS

CLUTCH FREQUENCY AND CLUTCH INTERVAL

The egg-laying season stretched from early April to late August in 1997. Whereas the smallest reproductive female was 52.6 mm SVL, only females larger than 56 mm SVL could unexceptionally produce at least one clutch per breeding season (Table 1). Females differing in clutch frequency differed in mean SVL ($F_{6,100} = 20.23$, $P < 0.0001$), with large females producing more clutches overall than did small ones. Single-clutched females were smaller on average than those producing three or more clutches (Tukey's test, all $P < 0.04$), females producing three or fewer clutches were smaller on average than those laying more clutches (Tukey's test, all $P < 0.004$), and females producing 4–7 clutches did not differ in mean SVL (Tukey's test, all $P > 0.490$).

In each sampling year, large females laid eggs earlier than small ones. Whereas one single-clutched female (55.9 mm SVL) laid the first clutch in mid-July, none of the females larger than 63 mm SVL laid eggs later than April. The mean clutch interval differed among females that differed in clutch frequency (Kruskal–Wallis test, $H_5 = 46.30$, $N = 96$, $P < 0.0001$), with 'three-clutch' females taking on average the longest time to prepare a clutch of eggs (Table 2).

SEASONAL VARIATION IN CLUTCH SIZE, CLUTCH MASS, AND EGG SIZE

Clutch size varied from 2–5 eggs for the first clutch, 1–4 eggs for the second to sixth clutches, and 1–3 eggs for the seventh clutch (Table 1). The mean coefficient of variation in clutch size was 28.0% for the first clutch, and 30.8% for the subsequent clutches. Clutch size was positively related to female SVL in the first

Table 1. Descriptive statistics of reproductive traits of female *Takydromus septentrionalis* that were collected in 1997

	First clutch ($N = 143$)	Second clutch ($N = 120$)	Third clutch ($N = 93$)	Forth clutch ($N = 76$)	Fifth clutch ($N = 47$)	Sixth clutch ($N = 19$)	Seventh clutch ($N = 9$)
Snout–vent length (mm)	64.2 ± 0.3 52.6–70.8	64.0 ± 0.3 56.4–77.1	64.9 ± 0.3 57.7–77.1	65.4 ± 0.3 58.7–77.1	66.1 ± 0.4 60.9–77.1	66.4 ± 0.4 63.6–69.5	66.3 ± 0.6 63.5–69.5
Tail length (mm)	143.8 ± 4.5 14.6–232.0	147.0 ± 4.6 13.4–232.0	152.3 ± 4.8 38.8–227.5	148.6 ± 4.8 65.5–219.5	147.4 ± 6.1 66.0–227.5	156.6 ± 9.3 99.0–225.0	150.4 ± 12.9 105.0–222.0
Body mass (g)	5.0 ± 0.1 2.9–7.2	4.9 ± 0.1 2.9–8.3	5.0 ± 0.1 3.0–7.6	5.0 ± 0.1 2.7–8.4	5.0 ± 0.1 3.4–8.4	4.8 ± 0.2 3.8–6.6	4.6 ± 0.2 3.5–5.5
Clutch size (eggs)	3.1 ± 0.1 2–5	2.3 ± 0.1 1–4	2.4 ± 0.1 1–4	2.4 ± 0.1 1–4	2.4 ± 0.1 1–4	2.6 ± 0.2 1–4	2.1 ± 0.2 1–3
Clutch mean egg mass (g)	0.24 ± 0.003 0.15–0.36	0.26 ± 0.004 0.16–0.43	0.26 ± 0.004 0.18–0.40	0.26 ± 0.005 0.16–0.41	0.25 ± 0.005 0.18–0.35	0.25 ± 0.007 0.18–0.32	0.25 ± 0.012 0.21–0.33
Clutch mass (g)	0.75 ± 0.02 0.33–1.29	0.58 ± 0.02 0.16–1.41	0.61 ± 0.02 0.18–1.37	0.61 ± 0.02 0.30–1.26	0.61 ± 0.03 0.28–1.29	0.63 ± 0.04 0.44–1.03	0.50 ± 0.05 0.25–0.69
Relative clutch mass	0.15 ± 0.004 0.07–0.27	0.12 ± 0.004 0.03–0.24	0.12 ± 0.004 0.05–0.30	0.12 ± 0.004 0.06–0.23	0.12 ± 0.005 0.07–0.25	0.13 ± 0.010 0.09–0.24	0.11 ± 0.014 0.05–0.19

Data are expressed as the mean ± standard error and range.

Table 2. Annual fecundity, annual mean egg mass, and annual reproductive output of female *Takydromus septentrionalis* that were collected in 1997

	Single-clutched ($N = 9$)	Two-clutched ($N = 14$)	Three-clutched ($N = 14$)	Four-clutched ($N = 29$)	Five-clutched ($N = 18$)	Six-clutched ($N = 14$)	Seven-clutched ($N = 9$)
Snout–vent length (mm)	59.1 ± 0.4 52.6–60.1	60.5 ± 0.5 57.5–63.6	62.0 ± 0.7 57.7–66.5	64.9 ± 0.5 58.7–70.8	65.5 ± 0.7 60.9–70.3	66.3 ± 0.4 63.6–68.5	66.3 ± 0.6 63.5–69.5
Clutch interval (days)	–	23.0 ± 1.3 17.0–32.0	30.4 ± 1.8 20.5–39.5	24.4 ± 0.8 17.7–34.3	22.6 ± 0.4 19.8–26.3	19.6 ± 0.4 16.6–22.0	17.5 ± 0.7 14.0–21.0
Annual fecundity (eggs)	2.7 ± 0.2 2–4	4.5 ± 0.3 3–6	7.2 ± 0.5 5–12	10.5 ± 0.5 6–19	12.8 ± 0.6 9–17	16.6 ± 0.7 12–20	17.8 ± 0.7 14–20
Annual mean egg mass (g)	0.24 ± 0.009 0.21–0.29	0.25 ± 0.01 0.15–0.36	0.23 ± 0.007 0.20–0.29	0.25 ± 0.004 0.20–0.30	0.25 ± 0.006 0.20–0.28	0.25 ± 0.004 0.22–0.28	0.25 ± 0.01 0.23–0.32
Annual reproductive output (g)	0.65 ± 0.06 0.43–1.04	1.08 ± 0.07 0.60–1.53	1.70 ± 0.14 1.13–3.06	2.63 ± 0.13 1.58–4.89	3.15 ± 0.16 2.15–4.21	4.07 ± 0.16 3.14–5.12	4.48 ± 0.22 3.18–5.68

Data are expressed as the mean ± standard error and range.

five clutches (all $P < 0.001$), but not in the sixth ($r^2 = 0.0007$, $F_{1,17} = 0.01$, $P = 0.917$) and seventh ($r^2 = 0.003$, $F_{1,7} = 0.02$, $P = 0.881$) clutches because of the limited sample sizes (Table 1). An ANCOVA on the linear relationship between clutch size and female SVL revealed homogeneous slopes ($F_{4,469} = 1.34$, $P = 0.252$) but different intercepts ($F_{4,473} = 33.11$, $P < 0.0001$) among the first five clutches. When setting female SVL at 65 mm, the adjusted mean clutch size of the first, second, third, fourth, and fifth clutches was 3.2, 2.3, 2.4, 2.4 and 2.3 eggs, respectively. The SVL-specific clutch size was larger in the first clutch than in the subsequent four clutches (Tukey's test, all $P < 0.0001$), but the trait did not differ among the four subsequent clutches (Tukey's test, all $P > 0.937$).

Clutch mass was positively related to post-oviposition body mass in the first five clutches (log$_e$-transformed data, all $P < 0.009$), but not in the sixth ($r^2 = 0.03$, $F_{1,17} = 0.62$, $P = 0.444$) and seventh ($r^2 = 0.13$, $F_{1,7} = 0.44$, $P = 0.672$) clutches. We examined differences in RCM among the first five clutches using an ANCOVA with clutch mass as the dependent variable and postoviposition body mass as the covariate. This analysis again revealed homogeneous slopes ($F_{4,469} = 0.93$, $P = 0.444$) but different intercepts ($F_{4,473} = 16.01$, $P < 0.0001$). When setting postoviposition body mass at 4.9 g, the adjusted mean clutch mass of the first, second, third, forth, and fifth clutches was 0.75, 0.58, 0.61, 0.61, and 0.60 g, respectively. The mass-specific clutch mass (and thus RCM) was larger in the first clutch than in the subsequent four clutches (Tukey's test, all $P < 0.0001$), but it did not differ among the four subsequent clutches (Tukey's test, all $P > 0.762$).

The coefficient of variation in egg size was 14.1% for the first clutch, and 16.0% for the subsequent clutches. When the influence of variation in the female SVL was removed using one-way ANCOVA, the mean egg size was smaller in the first clutch than in the subsequent clutches (all $P < 0.027$), but it did not differ among the six subsequent clutches ($F_{5,357} = 1.72$, $P = 0.130$).

ANNUAL FECUNDITY AND REPRODUCTIVE OUTPUT

Annual fecundity (the total number of eggs produced per season) varied from 2–20 eggs (Table 2), and was positively related to female SVL ($r^2 = 0.65$, $F_{1,105} = 195.55$, $P < 0.0001$) (Fig. 1). Annual reproductive output (the total mass of eggs produced per season) varied from 0.43–5.68 g (Table 2), and was also positively related to female SVL ($r^2 = 0.65$, $F_{1,105} = 194.34$, $P < 0.0001$) (Fig. 1). When holding female SVL constant using a partial correlation analysis, the annual mean egg size was independent of annual fecundity ($r = -0.06$, $t = 0.65$, d.f. = 104, $P = 0.518$).

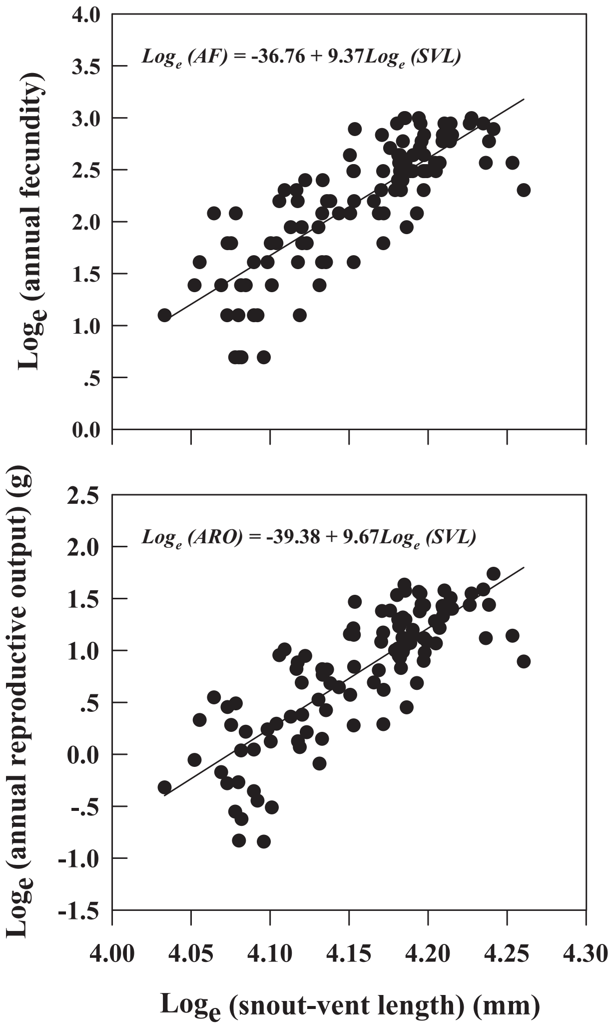

Figure 1. The relationships between annul fecundity (total number of eggs produced per breeding season) and annual reproductive output (total mass of eggs produced per breeding season) and female snout–vent length. All data are $\log_e$ transformed, and the regression equations are indicated in the figure.

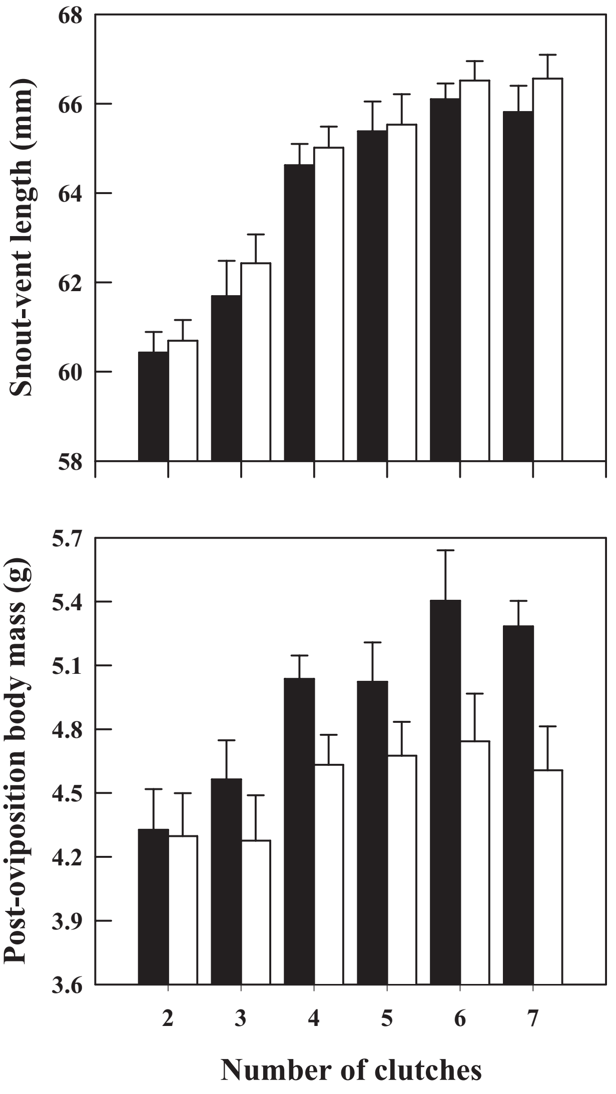

Figure 2. Changes in size (snout–vent length) and mass during the breeding season for females that produced different numbers of clutches in 1997. Data are expressed as the mean ± standard error. Solid bars, measurements were taken when the first clutch was laid; open bars, measurements were taken when the last clutch was laid.

CHANGES IN FEMALE SIZE AND MASS DURING THE BREEDING SEASON

Females overall increased size (SVL) but lost mass during the breeding season (Fig. 2). The mass loss was significant for the females producing four or more clutches, but not for the females producing fewer than four clutches (Table 3). When holding the length of breeding season constant using a partial correlation analysis, the SVL increase was negatively correlated with the initial female SVL measured when the first clutch was laid ($r = -0.21$, $t = 2.52$, d.f. = 95, $P = 0.013$). This result indicates that reproduction retards growth more pronouncedly in small females than in large ones.

AMONG-YEAR VARIATION IN REPRODUCTIVE TRAITS

Females differed in SVL among the five sampling years (first clutch: $F_{4,302} = 17.62$, $P < 0.0001$; second clutch: $F_{4,274} = 17.54$, $P < 0.0001$). The SVL-specific postoviposition body mass did not differ between the first and the second clutches, but it was greater in 1997 and 2005 than in the remaining 3 years (Fig. 3, Table 4). Females switched from producing more but smaller eggs in the first clutch to fewer but larger eggs in the second clutch in all five sampling years (Fig. 3). The SVL-specific clutch size was slightly

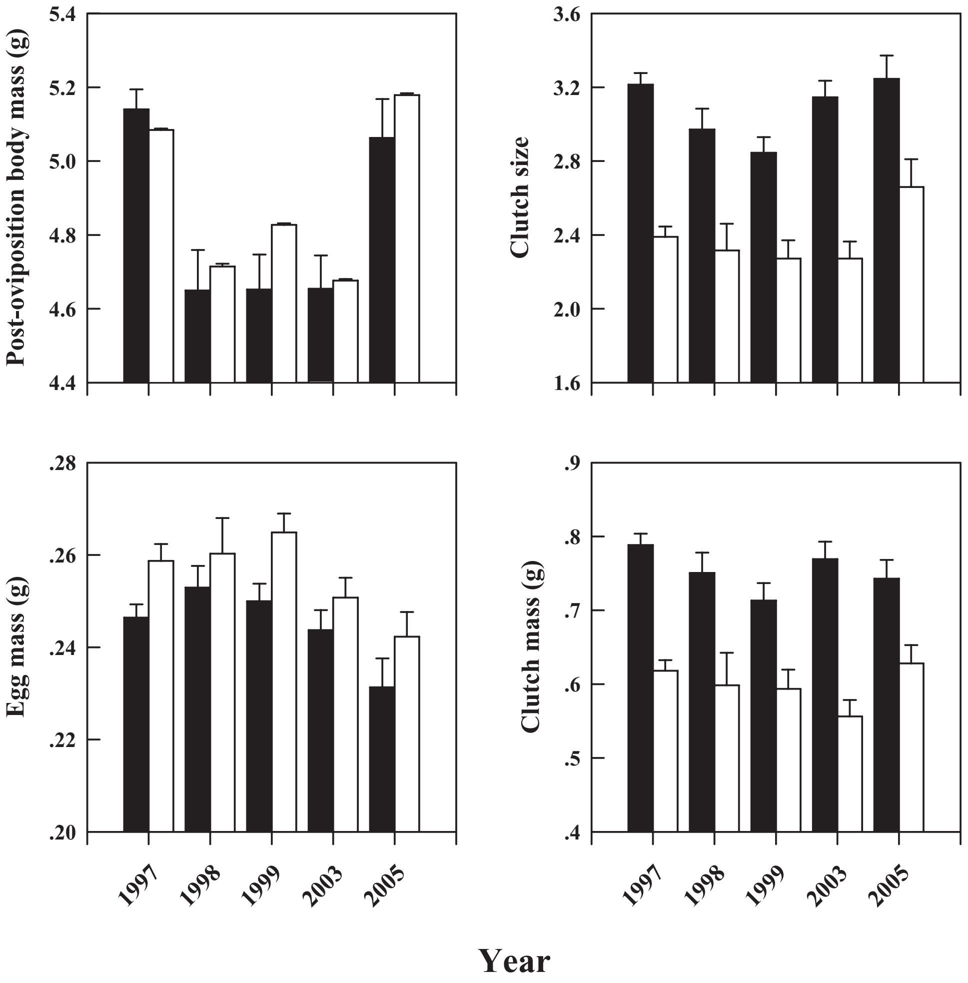

Figure 3. Among-year variation in postoviposition body mass, clutch size, egg size, and clutch mass. Data are expressed as adjusted mean ± standard error, with female snout–vent length (set at 65 mm) as the covariate. Sample sizes are indicated in Table 4. Solid bars represent the first clutch, and open bars represent the second clutch.

greater in females collected in 1997 and 2005, and the SVL-specific egg mass was slightly greater in females collected in 1997, 1998, and 1999 (Table 4). The SVL-specific clutch mass was significantly greater in the first clutch than in the second clutch, but it did not differ among the five sampling years (Table 4).

THE TRADE-OFF BETWEEN SIZE AND NUMBER OF EGGS

One-way ANCOVA with year as the factor, egg size as the dependent variable, and relative fecundity as the covariate revealed that both slopes and intercepts of the regression models did not differ among the five sampling years in either the first or the second clutch (all $P > 0.05$). When data for the first two clutches collected in different years were pooled separately, the size-number trade-off line was significantly higher in the second clutch than in the first clutch (ANCOVA: $F_{1,583} = 18.41$, $P < 0.0001$; Fig. 4). The result adds evidence that female *T. septentrionalis* lay larger eggs in later clutches.

DISCUSSION

The present study was carried out over several years and, in 1997, covered the whole breeding season. In each sampling year females were collected from the field approximately 2 weeks prior to the onset of the breeding season. Thus, year-to-year environmental variability, if present, would exert more direct effects on the first clutch than on the subsequent clutches because the energy for the production of those subsequent clutches was artificially maintained under laboratory conditions. The result that females produced significantly more eggs in the first clutch in 1997

Table 3. Results of repeated measures analysis of variance on changes in size and mass of female *Takydromus septentrionalis* that produced clutches in 1997

	Snout–vent length	Post-oviposition body mass
Two-clutched females	$F_{1,13} = 5.43$, $P = 0.037$	$F_{1,13} = 0.09$, $P = 0.766$
Three-clutched females	$F_{1,13} = 5.52$, $P = 0.035$	$F_{1,13} = 2.94$, $P = 0.110$
Four-clutched females	$F_{1,28} = 15.43$, $P < 0.001$	$F_{1,28} = 9.36$, $P < 0.005$
Five-clutched females	$F_{1,17} = 2.15$, $P = 0.161$	$F_{1,17} = 6.60$, $P = 0.020$
Six-clutched females	$F_{1,13} = 2.41$, $P = 0.144$	$F_{1,13} = 16.10$, $P < 0.002$
Seven-clutched females	$F_{1,8} = 3.26$, $P = 0.109$	$F_{1,8} = 25.56$, $P < 0.002$

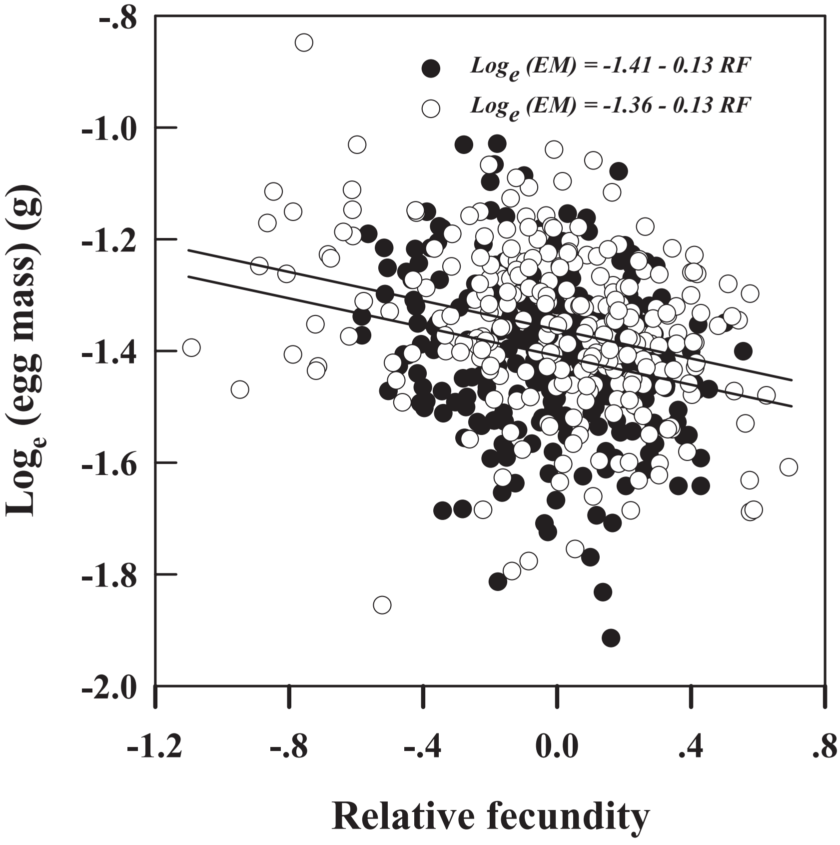

Figure 4. Seasonal variation in the trade-off between egg size and relative fecundity. All data are $\log_e$ transformed. Regression lines are adjusted for the first two successive clutches with a common slope to facilitate comparison. Solid dots and the lower line represent the first clutch, and open dots and the upper line represent the second clutch.

than in 1999 primarily resulted from the difference in the extent of energy stores, as indicated by post-body condition, between females collected in the 2 years (Fig. 3). Thus, our results are consistent with the prediction that differences in energy acquisition among females can influence clutch size (Ford & Seigel, 1989; Seigel & Ford, 1992). Female *T. septentrionalis* fuel the first clutch mainly by stored energy and subsequent clutches by currently ingested energy (Xu, Wu & Ou, 2002; Du, Lu & Ji, 2003). Because females collected in different years were maintained under the identical laboratory conditions without biotic and physical constraints imposed by the natural environment, it is not surprising that annual variation in clutch size, clutch mass, and egg size was absent in the second clutch. Although our

Table 4. Results of two-way analysis of covraiance (with year and clutch order as the factors, and female SVL as the covariate) on postoviposition body mass, clutch size, egg mass, and clutch mass of females that produced the first two successive clutches in 1997 (first clutches/second clutches = 143/120); 1998 (first clutches/second clutches = 33/23); 1999 (first clutches/second clutches = 61/70); 2003 (first clutches/second clutches = 50/45); and 2005 (first clutches/second clutches = 20/21)

	Effects		
	Clutch order	Year	Interaction
Post × oviposition body mass	$F_{1,5775} = 0.42$, $P = 0.519$	$F_{4,575} = 13.59$, $P < 0.0001$ 1997^a, 1998^b, 1999^b, 2003^b, 2005^a	$F_{4,575} = 0.36$, $P = 0.834$
Clutch size	$F_{1,5775} = 95.35$, $P < 0.0001$ First clutch > Second clutch	$F_{4,575} = 3.67$, $P < 0.006$ 1997^a, $1998^{a,b}$, 1999^b, $2003^{a,b}$, 2005^a	$F_{4,575} = 1.27$, $P = 0.281$
Egg mass	$F_{1,5775} = 7.51$, $P < 0.007$ First clutch < Second clutch	$F_{4,575} = 3.67$, $P < 0.006$ 1997^a, 1998^a, 1999^a, $2003^{a,b}$, 2005^b	$F_{4,575} = 0.33$, $P = 0.856$
Clutch mass	$F_{1,5775} = 70.46$, $P < 0.0001$ First clutch > Second clutch	$F_{4,575} = 1.73$, $P = 0.141$	$F_{4,575} = 1.25$, $P = 0.290$

Means corresponding to the years with different superscripts differ significantly (Tukey's test, $\alpha = 0.05$, a > b).

study was conducted in the laboratory, it is straightforward to extend the results to reveal the following trends.

FEMALES DIVERT AN OPTIMAL FRACTION OF RESOURCES IN CURRENT REPRODUCTION

Environmental conditions in nature vary temporally and spatially, and the environmentally-induced variation in opportunities for basking and feeding may influence female bodily condition and, thus, reproductive output (Ballinger, 1983; Olsson & Shine, 1997). In this study, however, clutch mass, a measure of reproductive output, remained almost constant across the sampling years in females producing either the first or the second clutch, although their bodily conditions did vary among years (Fig. 3). This result suggests that, for a particular clutch, there is a fixed upper limit of reproductive output that is less sensitive to the proximate influence of environmental variability in *T. septentrionalis*.

As in other lacertid lizards (Galán, 1996; Castilla & Bauwens, 2000; Ji & Braña, 2000), clutch mass decreases as the breeding season progresses in *T. septentrionalis*. This seasonal shift has been suggested to result from differences in the proximate source of energy allocated to different seasonal clutches because variation in energy reserves or in energy intake makes some individuals better able to allocate more energy to increased reproductive output through increasing clutch size and/or egg size (Braña, González & Barahona, 1992; Castilla & Bauwens, 2000). However, this explanation is unlikely to be applicable to *T. septentrionalis* for two reasons: (1) clutch mass did not vary among the subsequent clutches, although female bodily condition did decline steadily as the breeding season progressed (Fig. 2, Table 3) and (2) food availability was unlikely to be a proximate factor constraining reproductive output because females were provided with surplus food. Accordingly, the seasonal shift in clutch mass must have a genetic basis in *T. septentrionalis*. Because clutch mass did not vary among years in either the first or the second clutch, and because the seasonal shift in clutch mass did not result from the effects of proximate factors, we conclude that the fraction of accessible resources diverted in current reproduction is optimized in *T. septentrionalis*. This pattern actually gives an example of evolutionary optimization of reproductive output.

FEMALES DIVERT AN OPTIMAL FRACTION OF RESOURCES TO INDIVIDUAL EGGS

Offspring size is a crucial element of life history because of its direct consequences for both parental and offspring fitness (Sinervo *et al.*, 1993; Bernardo, 1996; Einum & Fleming, 2000; Agrawal *et al.*, 2001; Satoki & Harada, 2004). The optimal offspring size theory predicts that offspring size should be optimized through natural selection, and that offspring number is the consequence of partitioning the total maternal investment into optimally-sized offspring (Smith & Fretwell, 1974). By 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 are a function of total reproductive investment or maternal size (Sargent, Taylor & Gross, 1987; Winkler & Wallin, 1987; Reznick, Bryga & Endler, 1990; Caley, Schwarzkopf &

Shine, 2001). In this study, larger females generally laid larger eggs but, in any particular clutch, female SVL only explained less that 20% of variation in egg size. Moreover, although egg size was negatively related to relative fecundity in either the first or the second clutch (Fig. 4), data were so scattered that only a very small proportion of variation in egg size could be explained by relative fecundity (approximately 5% for the first clutch, and 8% for the second clutch). We interpret these results as being due to the fact that, in each particular clutch, reproductive investment per offspring is well optimized and, thus, less plastic in *T. septentrionalis*.

It is noteworthy that females switched from producing more but smaller eggs in the first clutch to fewer but larger eggs in the subsequent clutches. Dividing accessible resources into smaller amounts for each egg allows females to increase the total number of eggs produced. Hatchling *T. septentrionalis* from eggs produced early in the season have a longer growth period and, presumably, forage in a relatively less competitive environment than progenies from later clutches (Zhang & Ji, 2000). Female *T. septentrionalis* lay larger eggs in later clutches, presumably as a consequence of the shortened time for hatchling growth, higher intraspecific competition from earlier progenies, and energy repletion before the onset of the winter dormancy. Thus, the seasonal shift in the relationship between size and number of eggs is a reproductive tactic in *T. septentrionalis* that maximizes female reproductive fitness. In *T. septentrionalis*, a female's body volume is not a limiting factor that influences egg size because clutches rarely completely fill the available space (Du *et al*., 2005a). Resource availability is also unlikely to be a factor affecting egg size because females were provided surplus food. Accordingly, we conclude that the seasonal shift in egg size is determined ultimately by natural selection in *T. septentrionalis*.

REPRODUCTION IS RELATIVELY MORE COSTLY IN SMALL FEMALES

Reproducing females increased in SVL but decreased in mass during the breeding season (Fig. 2). This result indicates that *T. septentrionalis* is among the species confronted with the problem of resolving the conflicting demands of reproduction and growth. Because high expenditure in current reproduction can reduce 'residual reproductive value' by decreasing survival, growth, or the amount of energy available for future reproduction (Perrin & Sibly, 1993; Schwarzkopf, 1994; Kozlowski, 1996; Heino & Kaitala, 1999; Lardner & Loman, 2003), maximizing reproductive fitness can therefore be expected to be achieved in *T. septentrionalis* by diverting an optimal, rather than a higher, fraction of the available energy to current reproduction. This expectation is especially true for small females because reproduction retards growth more evidently and, thus, is relatively more costly in small females. Reducing total reproductive investment in current reproduction coupled with the presumed increase in growth rate may enhance lifetime reproductive success. Thus, the results of the present study conform to the repeatedly cited idea that the trade-off between current reproduction and future reproduction may place constraints on reproductive output in a particular reproductive episode (Williams, 1966; Tinkle, Wilbur & Tilley, 1970; Schwarzkopf, 1994; Kozlowski, 1996; Heino & Kaitala, 1999).

Collectively, our data show that trade-offs between size and number of eggs and between current reproduction and growth (and thus future reproduction) are evident in *T. septentrionalis*. Life-history trade-offs not only lead females to invest an optimal fraction of accessible resources in any particular clutch, but also lead them to optimize the trade-off between size and number of eggs. Although females switch from producing more but smaller eggs in the first clutch to fewer but larger eggs in the subsequent clutches, eggs are well optimized for size in any particular reproductive episode. Our data generally conform to the prediction from the Smith–Fretwell model that offspring size is relatively canalized compared with offspring number.

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