

## Income breeding and temperature-induced plasticity in reproductive traits in lizards

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### SUMMARY

We used the northern grass lizard *Takydromus septentrionalis* as a model animal to examine the energy allocation strategy and whether body temperature can affect maternal reproductive traits in lizards. We maintained adult females collected from the field soon after winter dormancy under three thermal conditions ( $24.0 \pm 1.0^\circ\text{C}$ ,  $28.0 \pm 1.0^\circ\text{C}$  and  $32.0 \pm 1.0^\circ\text{C}$ ) throughout the subsequent reproductive season, and then recorded their energy intake, digestive efficiency, body size and mass changes, and reproductive parameters. Manipulation of body temperature in reproducing females affected energy intake and several maternal reproductive characteristics. Females allocated energy to reproduction following the fulfillment of energetic requirements for metabolism and growth. The reduced energy availability for reproduction constrained females at low or high body temperatures to produce fewer and smaller eggs per season but had no role in influencing the number of eggs produced in single reproductive bouts. Females used currently acquired energy to fuel reproduction, and this strategy did not shift seasonally from the first to subsequent clutches. Our study supports the idea that the use of currently acquired energy for reproduction may be more widespread in ectotherms than thought before, and shows for the first time that egg size is thermally plastic in *T. septentrionalis*, where eggs produced in single reproductive bouts are believed to be well optimized for size.

Key words: reptile, lizard, *Takydromus septentrionalis*, income breeding, energy allocation, body temperature, reproduction, phenotypic plasticity.

### INTRODUCTION

During the reproductive season, animals devote time and energy to the production of offspring. The energy requirement for producing offspring may constrain current reproduction through limited availability of energy and future reproduction through deleterious effects of energy expenditure on parental somatic condition (or growth) and survival. Life-history theory predicts that maximization of lifetime reproductive success should be achieved in animals by balancing energetic expenditure between current reproductive investment and the future probability of reproduction (Williams, 1966; Stearns, 1992; Nilsson and Svensson, 1996; Roff, 2002). For example, if an individual allocates energy to reproduction rather than body growth within a given reproductive season or episode, then more or larger offspring might be produced at that time. However, if fecundity or mating success is highly dependent on body size, then the individual might achieve higher lifetime reproductive success by reducing current reproductive investment in favor of body growth. In the field, it is often difficult to measure how energy is allocated to different functions of an animal, because temporal and spatial variations in environmental attributes such as food supply and temperature can have profound repercussions on energy allocation (Ballinger, 1983; van Noordwijk and de Jong, 1986; Stearns, 1992; Gregory and Skebo, 1998; Lourdaïs et al., 2002). However, with suitable animals, it is possible to do so in the laboratory where carefully controlled measurements are possible (Seigel and Ford, 1991; Ford and Seigel, 1994; Du, 2006; Lourdaïs et al., 2003; Kubička and Kratochvíl, 2009).

Most studies in this field have focused upon the temporal relationship between the acquisition of energy and its expenditure for reproduction. One distinction that has emerged in life-history theory is that of capital *versus* income breeders. In capital breeders current reproductive output is largely a function of energy

accumulated over long periods prior to reproduction, while in income breeders reproductive output is closely linked to current energy availability (Jönsson, 1997; Bonnet et al., 1998). These alternative breeding tactics are actually two ends of a continuum of all possibilities, and in many animals the energy allocation strategy for reproduction is well beyond a simple capital/income breeding dichotomy (Gregory and Skebo, 1998; Warner et al., 2008). For example, females of the side-blotched lizard *Uta stansburiana* (Hahn and Tinkle, 1965), the oriental garden lizard *Calotes versicolor* (Shanbhag and Prasad, 1992), the Jacky dragon *Amphibolurus muricatus* (Warner et al., 2008), the asp viper *Vipera aspis* (Bonnet et al., 1999; Bonnet et al., 2001; Lourdaïs et al., 2002) and the Weddell seal *Leptonychotes weddellii* (Wheatley et al., 2008) use a mixed capital–income breeding strategy to reproduce. Capital breeding is thought to be very common in ectotherms (Bonnet et al., 1998; Girish and Saidapur, 2000; Glazier, 2000; Arrington et al., 2006). Nonetheless, recent studies of lizards show that the use of recently acquired energy for reproduction is more widespread in ectotherms than thought before (Ji and Braña, 2000; Du, 2006; Warner et al., 2008; Kubička and Kratochvíl, 2009).

Just as what occurs throughout the life of an animal, energy acquired within a reproductive season should be allocated towards two main competing demands, maintenance and production. Maintenance costs include both the energy costs for basic or standard metabolism and the energy necessary for other physiological, biochemical and behavioral activities essential for the continuity of life whereas energy allocated towards production supports growth and reproduction (Congdon et al., 1982; McNab, 2002). For ectotherms, although they can withstand a wide range of body temperatures, variation in body temperature can have a key role in influencing the rates of processes such as digestion, metabolism and reproduction (Huey, 1982). In the field, ectotherms often encounter

thermally challenging environments where they often have difficulty in acquiring and maintaining body temperatures that allow them to maximize reproductive performance. For example, low body temperatures reduce the rates of many processes including reproduction whereas high body temperatures increase the metabolic costs and, thus, reduce the energy availability for reproduction. Usually it is assumed that parental ectotherms should elevate time or energy requirement to reproduce in the environment where they are unable to maintain appropriate body temperatures. The thermal sensitivity of reproduction and the scarcity of studies about how multi-clutching ectotherms allocate energy to different functions within a reproductive season raise several questions that form the basis of this study: (1) do females shift the energy allocation strategy in successive clutches within a reproductive season? (2) How do females adjust particular reproductive characteristics in a given set of thermal conditions? (3) Are females constrained to lay fewer and/or smaller eggs in single reproductive bouts when they are exposed to low (resulting in a reduction of feeding rates) or high (resulting in an increase of metabolic costs) thermal environments? (4) Do females hierarchically allocate energy for production to somatic growth and reproduction?

In the present study, we maintained adult females of the northern grass lizard *Takydromus septentrionalis* Günther collected from the field soon after winter dormancy under three thermal conditions throughout the subsequent reproductive season, and then recorded their food (energy) intake, digestive efficiency, size (snout-vent length, SVL) and mass changes, and reproductive parameters. We used this small-sized (up to 76 mm SVL), oviparous lacertid lizard as a model system to address the above questions for three reasons: (1) females consistently lay eggs under laboratory conditions; thus, energy requirements and reproductive parameters can be easily measured; (2) females have been known to lay up to seven clutches per season stretching from April to August, and this extended reproductive season provides an ample opportunity to examine how (or if) females adjust the energy allocation strategy and reproductive investment in successive clutches within a season; and (3) copulation can easily occur between individuals of both sexes in the laboratory (Du et al., 2005a; Du et al., 2005b; Du, 2006; Ji and Diong, 2006; Ji et al., 2007; Du and Shou, 2008).

## MATERIALS AND METHODS

A total of 90 adults (60 females and 30 males) were collected by hand or noose in late March 2006 from a previously studied population in Lishui (28°46'N, 119°92'E), Zhejiang, East China. Lizards were brought to our laboratory in Hangzhou, where they were weighed (to the nearest 0.1 g) on a Mettler top loading balance (Zurich, Switzerland) and measured for SVL (to the nearest 1 mm) with Mitutoyo digital callipers (Kawasaki, Kanagawa, Japan). We housed 10 males in each of three communal cages (length × width × height: 90 cm × 65 cm × 50 cm) with moist soil (5 cm in depth), pieces of clay tile and grasses. These cages were placed in a room where temperatures were controlled within the range of 20–28°C. A 200 W light bulb, suspended at one end of each cage, created a thermal gradient from the room temperature to about 55°C for 14 h daily. Males were fed a combination of mealworms (*Tenebrio molitor*) and house crickets (*Achetus domesticus*), and water enriched with vitamins and minerals was provided *ad libitum*.

Females were equally assigned to three 3 m × 4 m AAPS (artificial atmospheric phenomena simulator) rooms, where temperatures were controlled at 24.0±1.0°C [hereafter the LT (low temperature) treatment], 28.0±1.0°C [hereafter the MT (middle temperature) treatment] and 32.0±1.0°C [hereafter the HT (high

temperature) treatment], respectively. The fluorescent tubes in these three rooms were on a 14h:10h light:dark cycle, with the photophase starting at 07:00h. Heat sources necessary for behavioral thermoregulation were not available in the three rooms; thus, variation in body temperature should track variation in room temperature (Wang and Xu, 1987). Females individually housed in 30 cm × 25 cm × 30 cm (length × width × height) glass cages were provided with mealworms and water enriched with vitamins and minerals *ad libitum*. Feces, urates and subsamples of food gathered for each female prior to each egg-laying episode were separately dried to constant mass at 65°C and weighed. Dried samples were burnt in a WGR-1 adiabatic calorimeter (Changsha Instrument, Changsha, China), and data on energy density were automatically downloaded to a computer. The assimilation efficiency (AE) was calculated as  $100 \times (I - F - U) / I$ , where  $I$  = energy ingested,  $F$  = energy in feces and  $U$  = energy in urates; the apparent digestive coefficient (ADC) was calculated as  $100 \times (I - F) / I$  (Xu and Ji, 2006).

The cages holding females were checked at least five times daily for eggs as soon as the first female laid eggs, such that eggs could be always collected, measured and weighed within a few hours after being laid. SVL and body mass were taken for each post-oviposition female before she was sired by a male in her own cage soon after oviposition. Females were allowed to produce as many clutches as they could under the conditions described above. The experiment was terminated for each female two weeks (a time interval longer enough to know whether a female would get gravid again) after the last clutch was laid.

Statistical analyses were performed with Statistica software (version 6.0 for PC, Tulsa, OK, USA). We tested data for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using the Bartlett's test (univariate level) or the Box's M test (multivariate level). We used  $G$ -test, one-way analysis of variance (ANOVA), repeated-measures ANOVA, one-way analysis of covariance (ANCOVA), multivariate analysis of variance (MANOVA) and Tukey's *post hoc* test to analyze the corresponding data. The homogeneity of slopes was checked prior to examining differences in the adjusted means. Throughout this paper, values are presented as mean ± s.e.m., and the significance level is set at  $\alpha=0.05$ .

## RESULTS

Of the 60 females used in this study, 19 LT, 15 MT and 14 HT females normally laid clutches throughout the experimental period (Table 1). The remaining females were excluded from statistical analyses because they either laid abnormal clutches with eggs containing condensed yolk at least once (one MT female and three HT females) or died during the course of the experiment; the number of these females differed among the three treatments ( $G=6.62$ , d.f.=2,  $P<0.05$ ). Overall, females laid the first clutch between early April and early July, and the subsequent clutches between mid-April and late July (Fig. 1). The time intervals (hereafter the CF interval) after capture but before the first clutch differed among the three treatments (one-way ANOVA with temperature treatment as the factor;  $F_{2,45}=5.53$ ,  $P<0.008$ ), with the mean value being greater in the LT treatment than in the MT and HT treatments (Tukey's test; both  $P<0.021$ ). The time intervals (hereafter the FL interval) between the first and last clutches also differed among the three treatments (one-way ANOVA with temperature treatment as the factor;  $F_{2,45}=5.86$ ,  $P<0.006$ ), with the mean value was greater in the MT treatment than in the LT and HT treatments (Tukey's test; both  $P<0.035$ ). Within each treatment, females laid successive clutches at almost the same time interval (repeated-measures ANOVA, all  $P>0.191$ ).

Table 1. Results of repeated-measures ANOVA for snout-vent length, body mass, apparent digestive coefficient and assimilation efficiency, with time step as the within subject factor and temperature treatment as the between subject factor

	Time step	Temperature	Interaction
Snout-vent length (SVL)	$F_{2,90}=69.98, P<0.0001; C^c, F^b, L^a$	$F_{2,45}=0.46, P=0.635$	$F_{4,90}=1.69, P=0.159$
Body mass	$F_{2,90}=19.47, P<0.0001; C^b, F^a, L^b$	$F_{2,45}=0.30, P=0.741$	$F_{4,90}=2.67, P=0.037$
Apparent digestive coefficient	$F_{1,45}=42.33, P<0.0001; CF>FL$	$F_{2,45}=2.85, P=0.068$	$F_{2,45}=3.16, P=0.052$
Assimilation efficiency	$F_{1,45}=67.30, P<0.0001; CF>FL$	$F_{2,45}=4.01, P=0.025; LT^b, MT^a, HT^{a,b}$	$F_{2,45}=0.71, P=0.497$

Means with different superscripts differ significantly (Tukey's test,  $\alpha=0.05, a>b>c$ ). C, at capture; F, the time laying the first clutch; L, the time laying the last clutch; CF, the time interval after capture but before the first clutch; FL, the time interval between the first and last clutches; LT, low temperature ( $24.0\pm 1.0^\circ\text{C}$ ); MT, middle temperature ( $28.0\pm 1.0^\circ\text{C}$ ); and HT, high temperature ( $32.0\pm 1.0^\circ\text{C}$ ). The direction of the effects on SVL and body mass can be visualized in Fig. 2A,B.

Fig. 2 shows mean values (+ s.e.m.) for body size and mass, ADC and AE of females measured in the CF and FL intervals. Females of different treatments did not differ from each other in SVL, body mass and ADC (Table 1). The overall mean AE was greatest in the MT treatment (82.4%) and smallest in the LT treatment (80.4%), with the HT treatment (81.8%) in between (Table 1). Females gained mass within the CF interval but lost mass to the level at capture by the end of the experiment; females gained linear size (SVL) during the reproductive season but the final SVL did not differ among the three treatments (Table 1). Mean values for ADC and AE were both greater in the CF interval than in the FL interval (Table 1).

Clutch frequency (the number of clutches produced per season), clutch interval (the time interval between two successive clutches), annual fecundity (the number of eggs produced per season), annual reproductive output (the total mass of eggs produced per season), annual mean egg mass (the overall mean mass of eggs produced per season) and energy ingested during the experiment differed among the three treatments (MANOVA with temperature treatment as the factor; Wilks' Lambda=0.311, d.f.=12, 80,  $P<0.0001$ ; Table 2). Clutch frequency, annual fecundity, annual reproductive output and annual mean egg mass were greater but clutch interval was shorter in the MT treatment than in the LT and HT treatments (Tukey's test, all  $P<0.031$ ); these variables did not differ between the LT and HT treatments (Tukey's test, all  $P>0.771$ ). Energy ingested during the experiment was greatest in the MT treatment and smallest in the LT treatment, with the HT treatment in between (Table 2).

Females switched from laying a larger number of smaller eggs in the first clutch to a smaller number of larger eggs in the subsequent

clutches (repeated-measures ANOVA; clutch size,  $F_{2,45}=7.21, P<0.011$ ; egg mass,  $F_{2,45}=31.85, P<0.0001$ ). The mass but not the number of eggs produced in single reproductive bouts differed among the three treatments (repeated-measures ANOVA; egg mass,  $F_{2,45}=6.08, P<0.005$ ; egg number,  $F_{2,45}=1.32, P=0.278$ ), with the MT females overall laying larger eggs than did the LT and HT females (Tukey's test, both  $P>0.021$ ). Energy ingested within the CF interval did not differ among the three treatments (one-way ANOVA with temperature treatment as the factor;  $F_{2,45}=2.33, P=0.109$ ). The number and mass of eggs produced in the FL interval differed among the three treatments, so did the energy ingested within the interval (one-way ANOVA with temperature treatment as the factor; all  $P<0.005$ ) (Fig. 3). The number and mass of eggs produced within the FL interval were greater in the MT treatment than in the LT and HT treatments (Tukey's test, both  $P<0.0007$ ) but did not differ between the latter two treatments (Tukey's test, both  $P>0.890$ ). Energy ingested within the FL interval was smaller in the LT treatment than in the MT and HT treatments (Tukey's test, both  $P<0.033$ ) but did not differ between the latter two treatments (Tukey's test,  $P=0.077$ ).

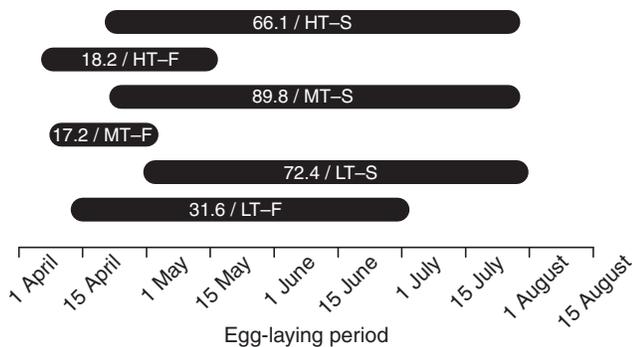


Fig. 1. Egg-laying dates of females maintained in three thermal environments. The black horizontal bars represent the range of dates of when clutches were laid, and numbers in the bars indicate mean days since the first day of April. LT-F, MT-F and HT-F – first clutches laid by female at  $24.0\pm 1.0^\circ\text{C}$ ,  $28.0\pm 1.0^\circ\text{C}$  and  $32.0\pm 1.0^\circ\text{C}$ , respectively; LT-S, MT-S and HT-S – subsequent clutches laid by female at  $24.0\pm 1.0^\circ\text{C}$ ,  $28.0\pm 1.0^\circ\text{C}$  and  $32.0\pm 1.0^\circ\text{C}$ , respectively. LT, low temperature ( $24.0\pm 1.0^\circ\text{C}$ ); MT, middle temperature ( $28.0\pm 1.0^\circ\text{C}$ ); HT, high temperature ( $32.0\pm 1.0^\circ\text{C}$ ); F, first clutch; and S, subsequent clutches.

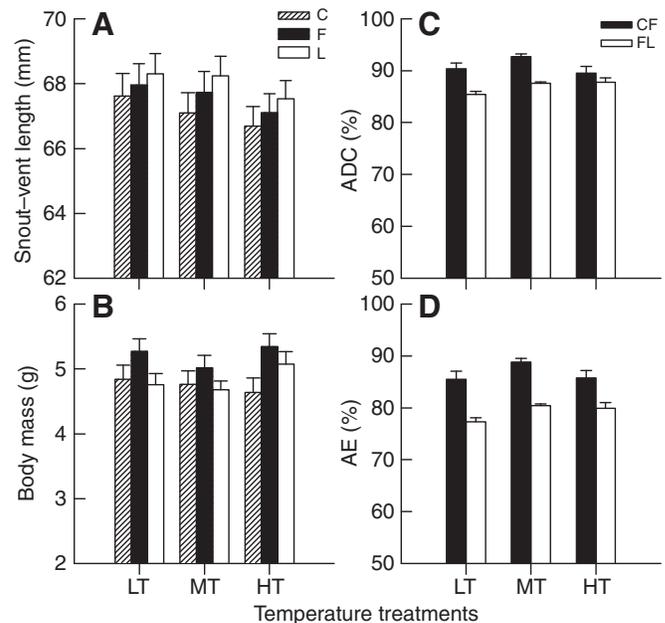


Fig. 2. Mean values (+ s.e.m.) for (A) snout-vent length, (B) body mass, (C) apparent digestive coefficients (ADC) and (D) assimilation efficiency (AE) of females measured in the CF and FL intervals. C, at capture; F, the time laying the first clutch; L, the time laying the last clutch; CF, the time interval after capture but before the first clutch; FL, the time interval between the first and last clutches.

Table 2. Descriptive statistics for snout–vent length, clutch frequency, annual fecundity, annual reproductive output, annual mean egg mass and energy intake monitored during the experiment

	Temperature treatments		
	Low temperature	Middle temperature	High temperature
N	19	15	14
Snout–vent length (mm)	68.0±0.7 (63.5–72.7)	67.7±0.6 (63.7–72.1)	67.1±0.6 (63.9–70.4)
Clutch frequency	4.2±0.3 (2–6)	6.5±0.3 (5–9)	4.5±0.5 (2–8)
Clutch interval (days)	24.7±1.7 (16.4–45.7)	16.9±0.7 (12.3–22.5)	22.6±3.6 (11.8–58.1)
Annual fecundity	9.8±0.8 (4–16)	14.9±0.8 (8–20)	9.4±0.9 (4–14)
Annual reproductive output (g)	2.34±0.17 (1.05–3.64)	3.98±0.23 (2.08–5.58)	2.28±0.24 (0.74–3.55)
Annual mean egg mass (mg)	241.3±4.2 (203.5–286.3)	266.8±5.9 (219.6–300.1)	238.7±4.6 (204.4–282.9)
Energy intake (kJ)	152.7±6.2 (111.2–209.1)	204.4±7.6 (161.3–271.0)	181.3±12.4 (69.2–230.3)

Data are presented as means ± s.e.m. with range in parentheses.

When performing an ANCOVA with annual reproductive output as the covariate to control the differences in trait, we found once again that energy ingested during the experiment differed among the three treatments ( $F_{2,44}=4.22$ ,  $P=0.021$ ). Energy ingested during the experiment was greatest in the HT treatment and smallest in the LT treatment, with the MT treatment in between (Fig. 4). Daily energy intake differed among the three treatments (repeated-measures ANOVA;  $F_{2,45}=23.05$ ,  $P<0.0001$ ) and between the CF and FL intervals (repeated-measures ANOVA;  $F_{1,45}=102.84$ ,  $P<0.0001$ ). Daily energy intake was greatest in the HT treatment and smallest in the LT treatment, with the MT treatment in between (Tukey's test, all  $P<0.009$ ); females daily ingested more energy in the CF interval than in the FL interval (Fig. 5).

## DISCUSSION

Our manipulation of body temperature in reproducing females had significant effects on energy intake and several maternal reproductive characteristics. Females at high body temperatures did compensate for the increased metabolic costs by increasing feeding rates but not

by increasing digestive efficiency (Table 1, Fig. 2). The LT and HT females laid clutches less frequently than did the MT females (Table 2). One possible explanation for the reduced clutch frequency in the LT and HT treatments may involve their effects on feeding rates and metabolism. Although our study was conducted in the laboratory, the results allow us to draw the following conclusions.

Firstly, females of *T. septentrionalis* do not shift the energy allocation strategy in successive clutches within a season because they always use an income breeding strategy to reproduce. The northern grass lizard has been hypothesized to fuel the first clutch mainly by stored energy (Xu et al., 2002; Du et al., 2003; Du, 2006). This hypothesis is, however, not supported by the present study because, unlike capital breeders that should show a decrease in body mass due to depletion of stored energy (Warner et al., 2008), our females exhibited an increase in body mass in the CF interval within which the first clutch of the season was prepared and laid. There is no argument against fueling the subsequent clutches by currently acquired energy in *T. septentrionalis* (Du, 2006; Ji and Diong, 2006; Ji et al., 2007; Du and Shou, 2008). Thus, if females used a capital or a mixed capital–income breeding strategy to produce the first clutch, they should eat less or lay clutches over shorter intervals in comparison with those producing the subsequent clutches. Interestingly, however, females daily ingested more food and assimilated it more efficiently in the CF interval than in the FL interval. Moreover, females of the same treatment took almost the same time to lay successive clutches. These observations provide strong evidence that females of *T. septentrionalis* use an income breeding strategy to produce the first clutch. The result that females increased feeding rates in the CF interval could be best explained by the seasonal shift in reproductive output in *T. septentrionalis* where clutches produced early in the breeding season (the first clutch) are heavier than those produced later in the season (the subsequent clutches) (Du et al., 2005b; Du, 2006; Ji and Diong, 2006; Ji et al., 2007; Du and Shou, 2008). Females gained mass in the CF interval, presumably because energy acquired within the interval exceeded the needs for maintenance and production.

Secondly, the investment in reproduction is highly temperature-dependent, while the investment in growth is fixed at our experimental temperature levels. Females at low or high body temperatures were constrained to lay fewer clutches per season when compared with those at moderate body temperatures. Nonetheless, body mass remained remarkably constant among the three treatments and between females weighed at capture and after laying the last clutch (Table 1, Fig. 2). These results provide two inferences regarding maternal decisions on energy allocation in *T. septentrionalis*: (1) reproducing females hierarchically allocate energy to maintenance, growth and reproduction; and (2) they do

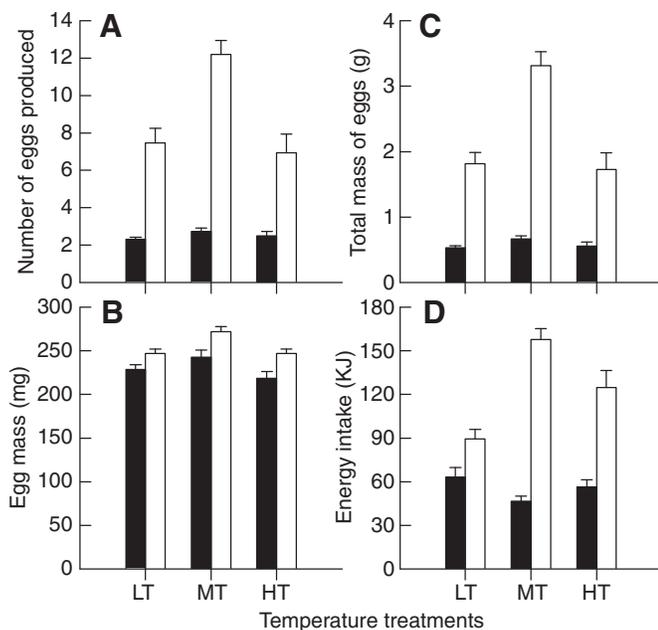


Fig. 3. Mean values (+ s.e.m.) for (A) the number of eggs produced, (B) egg mass, (C) total mass of eggs and (D) energy intake. Solid bars represent the CF interval, and open bars represent the FL interval. CF, the time interval after capture but before the first clutch; FL, the time interval between the first and last clutches.

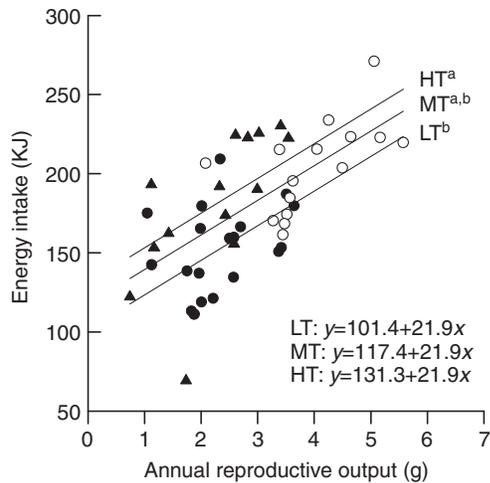


Fig. 4. The relationship between energy intake and annual reproductive output. Regression lines were adjusted for the three treatments with a common slope (21.9) to facilitate comparisons. Solid dots represent the LT females, open dots represent the MT females and solid triangles represent the HT females. The treatments with different superscripts differ significantly (Tukey's test,  $\alpha=0.05$ ,  $a>b$ ). CF, the time interval after capture but before the first clutch; FL, the time interval between the first and last clutches; LT, low temperature ( $24.0\pm 1.0^\circ\text{C}$ ); MT, middle temperature ( $28.0\pm 1.0^\circ\text{C}$ ); HT, high temperature ( $32.0\pm 1.0^\circ\text{C}$ ).

not significantly accumulate energy stores but divert most of their current surplus energy to the production of eggs. In many animals, energetic costs necessary for living firstly should be met and, during times of limited energy availability, energy allocated towards growth and reproduction is often restricted (Congdon et al., 1982; Lucas, 1996; McNab, 2002). For these animals, growth and reproduction have lower priorities in energy allocation, and only are allowed after meeting maintenance costs. In the present study, all females gained linear size during the experiment but the final SVL did not differ among the three treatments. What can be inferred from these results is that reproducing females of *T. septentrionalis* allocate the same amounts of energy to linear size over a wide range of body temperatures. This thermal independency of energy allocation to linear size highlights the importance of SVL for maternal fitness in *T. septentrionalis* where fecundity and reproductive output are dependent on maternal size (Du et al., 2005b; Ji and Diong, 2006; Ji et al., 2007) and, more importantly, it suggests that females allocate energy to reproduction following the fulfillment of energetic requirements for metabolism and growth. The consecutive hierarchical allocation of energy to growth and reproduction has also been described in the Madagascar ground gecko *Paroedura picta* (Kubička and Kratochvíl, 2009).

Thirdly, egg size is temperature-dependent, while the number of eggs produced (clutch size) in single reproductive bouts is fixed at our experimental temperature levels. Consistent with earlier studies of *T. septentrionalis* (Du et al., 2005b; Ji and Diong, 2006; Ji et al., 2007; Du and Shou, 2008), our females switched from producing a larger number of smaller eggs in the first clutch to a smaller number of larger eggs in the subsequent clutches. Such a seasonal shift is not affected by year-to-year environmental variability, and is thus believed to have a genetic basis in the lizard (Ji and Diong, 2006; Ji et al., 2007; Du and Shou, 2008). An experimental reduction of maternal body volume does not affect egg size in *T. septentrionalis* (Du et al., 2005a). Manipulation of food availability in reproducing

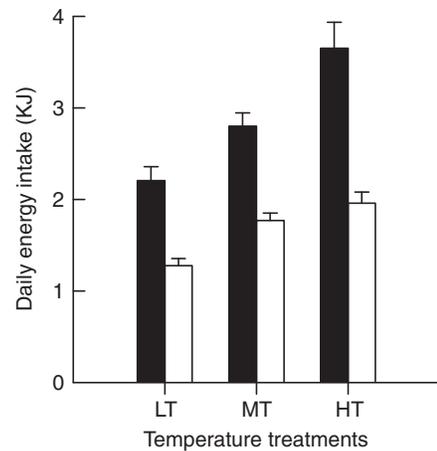


Fig. 5. Mean values (+ s.e.m.) for daily energy intake. Solid bars represent the CF interval and open bars represent the FL interval. CF, the time interval after capture but before the first clutch; FL, the time interval between the first and last clutches.

females influences clutch frequency and clutch interval but not the number and size of eggs produced in single reproductive episodes (Du, 2006). Moreover, inconsistent with the results reported for other lizards such as *U. stansburiana* (Sinervo and Licht, 1991), *Lacerta (Zootoca) vivipara* (Olsson et al., 2002) and *Eremias argus* (Wang et al., 2009) where females undergoing follicle ablation (and, thus, reduced clutch size) produce larger eggs, females of *T. septentrionalis* undergoing a similar manipulation do not change egg size (Ji and Diong, 2006). Previous studies of *T. septentrionalis* by manipulation of maternal body volume, food availability or clutch size were never conducted under multiple thermal regimes, with results generally showing that females divert an optimal fraction of energy to individual eggs in a given reproductive episode and, as such, changes in energy acquisition or in energy availability for reproduction do not change this optimal investment per egg and therefore egg size.

Our study demonstrates that egg size is a thermally plastic life-history trait in *T. septentrionalis*, with females at moderate body temperatures laying larger eggs in comparison with those at low and high body temperatures. The egg size reached in the LT and HT females could be the result of limited energy availability for reproduction or it could be large enough and, thus, its further increase would not bring any significant fitness advantage. In fact, diverting a large enough, rather than an extraordinarily high, fraction of the available energy to individual eggs in single reproductive episodes is a strategy adopted by females of *T. septentrionalis* to maximize their lifetime reproductive success (Ji and Diong, 2006; Ji et al., 2007). Female bodily condition can influence clutch size and, thus, reproductive output in lizards including *T. septentrionalis* (Ballinger, 1983; Olsson and Shine, 1997; Ji et al., 2007). Presumably, the thermal independency of clutch size in single reproductive bouts could be explained by the lack of differences in body mass among the three treatments.

Lastly, the investment in reproduction only accounts for a small proportion of currently acquired energy in *T. septentrionalis*. Assuming that eggs of *T. septentrionalis* contain 7kJ of energy per gram wet mass (Ji, 1992), we estimate that the LT, MT and HT females, respectively, devoted approximately 16kJ, 28kJ and 16kJ (see Table 2 for the total mass of eggs produced during the experiment in each treatment) of energy to reproduction during the experiment.

Based on these values, we further estimate that the LT, MT and HT females, respectively, allocated approximately 10%, 14% and 9% (see Table 2 for energy ingested during the experiment in each treatment) of currently acquired energy to reproduction. These estimates coupled with the finding that in no treatment did our females gain mass during the experiment provide an inference that division of energy among competing needs in *T. septentrionalis* follows the pattern described in other reptiles, where a large proportion of available energy is allocated towards maintenance costs but substantially less is allocated towards production (Congdon et al., 1982).

In summary, our data demonstrate that females of *T. septentrionalis* use an income breeding strategy to produce clutches throughout the reproductive season, and that manipulation of body temperature in reproducing females can affect energy intake and several maternal reproductive characteristics but not the general pattern of energy allocation. Females allocate energy to reproduction following the fulfillment of energetic requirements for metabolism and growth. The reduced energy availability for reproduction constrains females at low or high body temperatures to produce fewer and smaller eggs per season but has no role in influencing the number of eggs produced in single reproductive bouts. Our study supports the idea that the use of currently acquired energy for reproduction may be more widespread in ectotherms than thought before, and shows for the first time that egg size is a thermally plastic life-history trait in *T. septentrionalis*, where females form eggs of optimal size not affected by manipulation of maternal body volume (Du et al., 2005a), food availability (Du, 2006) or clutch size (Ji and Diong, 2006).

#### LIST OF ABBREVIATIONS

AAPS	artificial atmospheric phenomena simulator
ADC	apparent digestive coefficients
AE	assimilation efficiency
CF	time interval after capture but before the first clutch
F	energy in feces
FL	time interval between the first and last clutches
HT	high temperature
I	energy ingested
LT	low temperature
MT	medium temperature
SVL	snout-vent length
U	energy in urates

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