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## Seasonal Shifts in Reproductive Investment of Female Northern Grass Lizards (*Takydromus septentrionalis*) from a Field Population on Beiji Island, China

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**ABSTRACT.**—Understanding seasonal variation in reproductive investment is crucial to explore life-history strategies of a given species. We measured the timing of reproduction and the reproductive output of recently captured females to test whether the conclusions on seasonal shifts of reproductive investment made in the laboratory can apply to field populations in a lacertid lizard *Takydromus septentrionalis*. Female *T. septentrionalis* started producing eggs in April and ceased reproduction in August. During the midreproductive season (May to July), more than 60% of field-captured females were gravid. The females produced more eggs and thus had heavier total egg mass in May than in July, although egg mass and hatchling size did not vary seasonally. The seasonal variation in clutch size did not induce seasonal shifts in trade-offs between clutch size and egg size. In general, these results are largely consistent with data collected in the laboratory and support the conclusion of previous studies in this species that seasonal shifts in reproductive output are determined ultimately by natural selection. However, Relative Clutch Mass (RCM) of recently captured females was greater than that of females in captivity, which suggests that RCMs could be affected noticeably by proximate factors. Therefore, when explaining the evolutionary strategies of an organism based on intraspecific comparisons of RCMs, investigators should measure them in a common-garden environment so as to control the potential influence of environmental factors.

Reproductive investment, reflecting optimal allocation of available energy among maintenance, storage, growth, and reproduction, has been a focus of life-history theory (Stearns, 1992). In ectotherms like lizards, reproductive investment may be significantly affected by both genetic and environmental factors (Shine, 1992). Egg size provides a simple and robust measure of reproductive investment because most oviparous lizards lack posthatching parental care. Therefore, lizards are ideal models and have been the focus of studies on variation and its underlying causes in reproductive investment (e.g., Shine, 1992; Niewiarowski and Dunham, 1994; Angilletta et al., 2001).

Reproductive investment shows extraordinary diversity among species as well as within a single species (Dunham et al., 1988; Shine, 1992; Du et al., 2005). Seasonal variation in reproductive investment is an important intraspecific variation in response to temporal variation in environmental factors (e.g., temperature, food availability) and has significant ecological consequences. For example, females may produce offspring with faster growth or higher survival rate in earlier clutches than in later clutches (Landa, 1991; Sinervo and Dough-

ty, 1996; Olsson and Shine, 1997); this may profoundly influence the demography and viability of a population. Given that the offspring from earlier reproductive episodes have more time for food intake, growth, and energy storage prior to hibernation, and potentially have more opportunity to survive to maturity, one may expect that (1) in earlier clutches, females will produce more offspring to maximize current reproductive value; and (2) in later clutches, females will distribute more energy to each offspring to compensate for the disadvantages associated with later birth (Nussbaum, 1981; Ferguson et al., 1982).

The Northern Grass Lizard (*Takydromus septentrionalis*), a small oviparous lizard, produces multiple clutches per breeding season and, thus, provides an excellent model system to study temporal variation in reproductive investment. In this species, reproductive traits significantly differed among populations from geographically separated localities (Du et al., 2005, 2006) as well as within a population (Ji et al., 2007). The seasonal shifts in reproductive output and egg size are determined ultimately by natural selection in the Lishui population (see Ji et al., 2007). These former studies measured reproductive output of females maintained in laboratory throughout the breeding season. How-

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ever, the current study collected data on reproductive investment from a field population of this species. Our aim is to determine whether the conclusions drawn by previous laboratory studies can apply to populations studied in the field. In particular, (1) does the field population show a similar pattern of temporal variation in reproductive output to that observed in the laboratory? and (2) is the seasonal shift in reproductive investment determined ultimately by natural selection in this species?

#### MATERIALS AND METHODS

In 2004 and 2005, we captured *T. septentrionalis* by hand or by noose monthly from April to August and in October from Beiji Island (27°35'N, 120°10'E, and about 69 km away from the continent) in Zhejiang Province of eastern China. Immediately after the lizards were captured, we measured their snout-vent length (SVL), tail length, and mass using a plastic ruler ( $\pm 1$  mm) and a portable electronic balance ( $\pm 0.01$  g). The reproductive condition of mature females (SVL > 58 mm; Du et al., 2006) was determined by palpating their abdomen. In April ( $N = 23$ ), May ( $N = 30$ ), July ( $N = 18$ ), and August ( $N = 7$ ), gravid females with large yolke follicles or oviductal shelled eggs were transported to our laboratory at Hangzhou Normal College to gather data on reproductive output.

In the laboratory, the animals were individually housed in a small glass terrarium (20 × 15 × 20 cm) filled with 2 cm of moist sand, which was kept in a room with a constant temperature of  $23 \pm 1^\circ\text{C}$  and a light cycle of 12 : 12; light : dark (0630 h on and 1830 h off). A 60-W light bulb was suspended 5 cm above the terrarium as a supplementary heating source from 0800–1600 h. Food (larvae of *Tenebrio molitor*) and water (containing mixed vitamins and minerals) were provided ad libitum. Each small terrarium was checked at least six times a day for freshly laid eggs. All eggs produced by the females were weighed ( $\pm 0.001$  g) promptly so as to minimize initial mass change caused by water exchange. After being weighed, the eggs were placed in containers filled with moist vermiculite (water potential of  $-12$  kPa, dry vermiculite: water = 1 : 2) and then incubated at  $24^\circ\text{C}$ . Every other day, we added water to the substrate to keep its water content constant and moved containers among shelves to minimize any effects of thermal gradients inside the incubator. Toward the end of incubation, containers were checked for hatchlings at least five times a day. Once hatched, each hatchling was weighed ( $\pm 0.01$  g) and measured SVL ( $\pm 0.01$  mm). Of 78 clutches

of eggs incubated, at least one egg hatched successfully from 68 clutches. All females and their offspring were then released where they were captured.

Maternal body condition was quantified by using residual scores from log-transformed mass relative to log-transformed SVL. Relative clutch mass (RCM) was calculated as the ratio of clutch mass to maternal postlaying mass. Initial analyses revealed no between-year differences in reproductive output and hatchling traits (all  $P > 0.05$ ); thus, we pooled the data from the two successive years for subsequent analyses. We also pooled the data on reproductive output and hatchling traits collected in August with those collected in July, because of small sample sizes in August and no significant differences between these two samples. We used *t*-tests to detect differences in body size between gravid and nongravid females. Seasonal variations in maternal body condition, clutch size, egg size, and clutch mass were evaluated with MANCOVA or ANCOVA, in which maternal SVL was used as a covariate. We conducted an ANCOVA on clutch mass using maternal mass as a covariate to evaluate the seasonal variation in RCM. For hatchling traits, we pooled the data on SVL and mass of hatchlings from the same clutch to avoid pseudoreplication. Analyses of covariance were used to detect seasonal differences in these hatchling traits with a covariate of egg mass. Tukey's post hoc multiple-comparisons tests were used to distinguish among means of significantly affected traits.

#### RESULTS

*Timing of Female Reproduction in Field.*—Of all females captured in April (the beginning of reproductive season for this species), only 33% were gravid. The proportion of reproductive females increased dramatically in May, June, and July (up to 84%) and then decreased in August. In October, no gravid females were found in field-caught lizards (Fig. 1).

*Seasonal Variation in Maternal Body Condition and Reproductive Investment.*—In April, gravid females (SVL:  $69.69 \pm 3.61$  mm,  $N = 13$ ) were larger than nongravid females (SVL:  $65.44 \pm 4.43$  mm,  $N = 27$ ) ( $t = 3.002$ ,  $df = 38$ ,  $P < 0.005$ ). In contrast, no significant difference was found in body size between gravid and nongravid females captured in later months (all  $P > 0.05$ ). The body condition of nongravid females in nature did not show seasonal shifts ( $F_{5,58} = 1.71$ ,  $P = 0.14$ ; Fig. 2A).

Gravid females produced eggs in the laboratory a mean of  $12.5 \pm 0.8$  days ( $N = 23$ ) after capture in April,  $7.6 \pm 0.7$  days ( $N = 30$ ) after capture in May, and  $6.0 \pm 0.8$  days ( $N = 25$ )

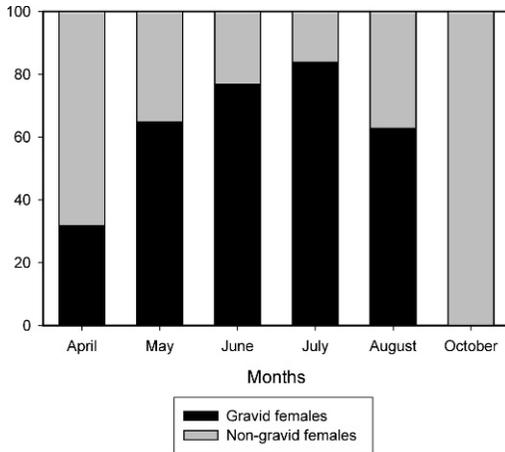


FIG. 1. Monthly variation in the proportion of gravid females in field-captured female Northern Grass Lizards (*Takydromus septentrionalis*). Females with large yolked follicles or oviductal shelled eggs were regarded as gravid.

after capture in July. For these recently field-captured females, maternal postlaying body condition changed temporally during the reproductive season ( $F_{2,74} = 11.44$ ,  $P < 0.0001$ ), with lizards having better body conditions in April than in May and July (Fig. 2B).

A MANCOVA on clutch size, egg mass, and clutch mass with maternal SVL as a covariate revealed significant seasonal differences in reproductive output (Wilk's- $F_{6,144} = 2.50$ ,  $P < 0.05$ ). Further analysis indicated that the patterns of seasonal variation were different among these traits. After the effect of maternal body size was statistically removed, clutch size and clutch mass varied seasonally (clutch size:  $F_{2,74} = 7.50$ ,  $P = 0.001$ ; clutch mass:  $F_{2,74} = 3.16$ ,  $P < 0.05$ ), but egg mass did not ( $F_{2,74} = 1.82$ ,  $P = 0.17$ ). The females produced more eggs and, thus, had a heavier clutch mass in May than in July (Fig. 3A–C). In contrast, the RCM of lizards in April was lower than in July ( $F_{2,74} = 5.22$ ,  $P < 0.01$ ; Fig. 3D).

**Hatching Success and Hatchling Traits.**—When incubated at a constant temperature of 24°C, eggs produced by field-captured females did not show seasonal shifts in hatching success (April, 91%; May, 83%; July and August, 88%;  $G = 0.05$ ,  $df = 2$ ,  $P > 0.75$ ). To verify whether there are seasonal variations in hatchling size, we applied ANCOVAs on hatchling SVL and mass with maternal SVL as a covariate. Neither hatchling SVL nor mass showed significant seasonal changes (SVL:  $F_{2,64} = 1.71$ ,  $P = 0.19$ ; mass:  $F_{2,64} = 0.97$ ,  $P = 0.38$ ). When the variation in hatchling traits induced by different initial egg masses was removed using an analysis of covariance, hatchling SVL and mass also did not

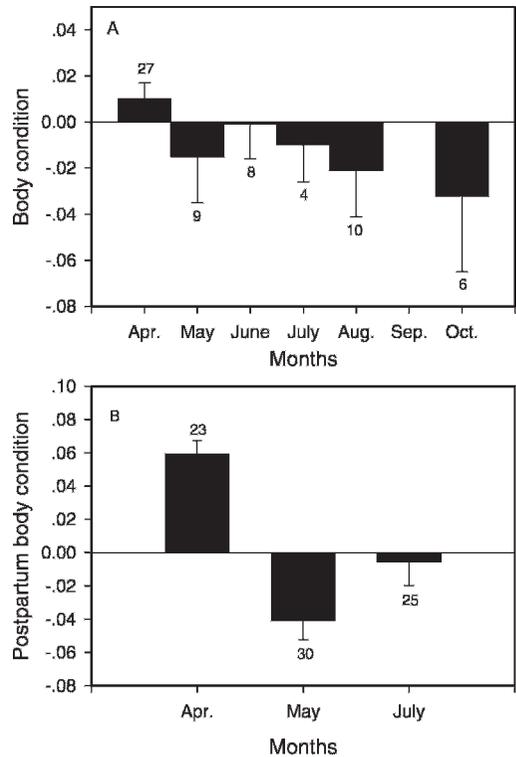


FIG. 2. Seasonal variation in maternal body condition of the Northern Grass Lizard (*Takydromus septentrionalis*). (A) Body condition of nongravid females in the field; (B) body condition of recently captured females after they produced eggs in the laboratory. Maternal body condition was quantified by using residual scores from log-transformed mass relative to log-transformed SVL. An analysis of covariance with maternal SVL as the covariate was used to detect the seasonal variation in maternal body condition.

vary seasonally (SVL:  $F_{2,64} = 0.58$ ,  $P = 0.57$ ; mass:  $F_{2,64} = 0.69$ ,  $P = 0.50$ ).

**Trade-Off between Egg Size and Clutch Size.**—We statistically removed the effect of maternal SVL on clutch size by calculating residual scores of log<sub>e</sub>-transformed clutch size relative to log<sub>e</sub>-transformed female SVL. Egg mass decreased with increasing residual clutch size (April:  $R^2 = 0.22$ ,  $N = 22$ ,  $P = 0.02$ ; May:  $R^2 = 0.15$ ,  $N = 29$ ,  $P = 0.03$ ; July:  $R^2 = 0.17$ ,  $N = 24$ ,  $P = 0.04$ ) but did not vary seasonally when residual clutch size was kept constant ( $F_{2,74} = 0.13$ ,  $P = 0.88$ ; Fig. 4). Therefore, there is no seasonal shift in the trade-off between clutch size and egg mass in this population.

## DISCUSSION

Reproductive data in this study came from wild or recently field-captured females and, thus,

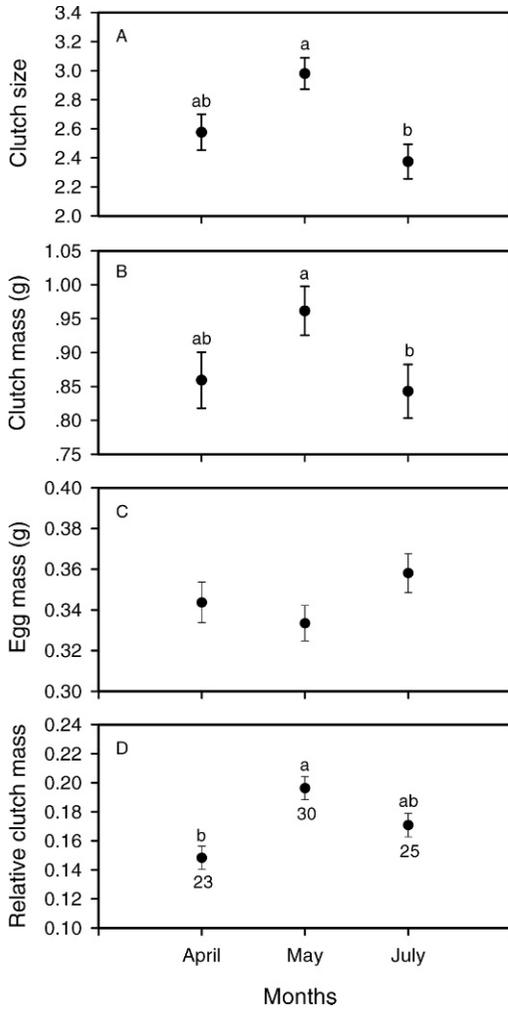


FIG. 3. Seasonal variation in clutch size (A), clutch mass (B), egg mass (C), and relative clutch mass (RCM) (D) of the Northern Grass Lizard, *Takydromus septentrionalis*. Relative clutch mass was calculated as the ratio of clutch mass to maternal postlaying mass. Analyses of covariance were performed to detect the seasonal variation. Maternal snout-vent length was used as the covariate, which was set at 68 mm. Graphs show adjusted mean values and associated standard errors. Means with different alphabets above the error bars are statistically different (Tukey's test). Numbers under the error bars in the lowest graph are sample sizes and apply to all graphs within this figure.

largely reflects the reproductive investment under natural conditions for *T. septentrionalis*. Our data on reproductive output are largely consistent with those from previous studies that kept females in the laboratory throughout the breeding season and give support to the conclusions drawn by these laboratory studies; that natural selection is the ultimate determinant of seasonal change in reproductive output (Du et al.,

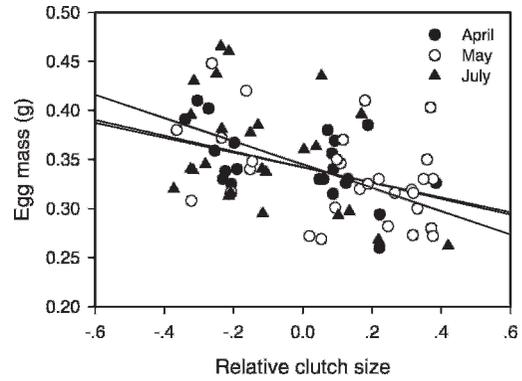


FIG. 4. Trade-off between clutch size and egg mass in the Northern Grass Lizard, *Takydromus septentrionalis*. Egg mass is negatively correlated to residual clutch size (April:  $R^2 = 0.22$ ,  $F_{1,21} = 5.96$ ,  $P = 0.02$ ; May:  $R^2 = 0.15$ ,  $F_{1,28} = 4.96$ ,  $P = 0.03$ ; July:  $R^2 = 0.17$ ,  $F_{1,23} = 4.85$ ,  $P = 0.04$ ). The residual clutch size was calculated as residual scores from the regression of  $\log_e$ -transformed clutch size on  $\log_e$ -transformed female SVL.

2005, 2006; Ji et al., 2007). Nonetheless, we find that relative clutch mass may differ between individuals in the field and laboratory, and seasonal shifts in egg size may differ among populations in this species.

In the field, large females on Beiji Island began reproduction earlier than did small females and, thus, could produce more clutches per breeding season. Similarly, large females produced more clutches than did small females in captivity (Ji et al., 2007). The clutch size and clutch mass of wild female *T. septentrionalis* was larger in May than in July (Fig. 3A,B). This seasonal shift in reproductive output could be a result of evolutionary adaptation or simply caused by seasonal variation in resource availability. Our laboratory studies on this population as well as other populations indicate that clutch size in earlier clutches is larger than in later clutches, even when resources are unlimited (Ji et al., 1998, 2007; Du et al., 2005, 2006), suggesting that the seasonal decrease in reproductive output for wild females is likely determined ultimately by natural selection (Ji et al., 2007). Such patterns of seasonal decreases in reproductive investment fits well with the prediction that females would produce more offspring to maximize current reproductive value in earlier clutches, and these patterns have also been found in some other multiple-clutched lizards (Nussbaum, 1981; Ji and Brana, 2000; Shangha et al., 2000; Angilletta et al., 2001). Nonetheless, environmental factors may influence reproductive output to some extent. For instance, energy available for reproduction

may affect clutch size of the first clutch, which is mainly fueled by stored energy in this species (Du et al., 2003; Ji et al., 2007). This may explain the lack of a difference in reproductive output between April and July in this study. The effect of environmental factors on temporal variation in energy available to females and their reproductive investment has also been demonstrated in some other species (Ballinger, 1983; Adolph and Porter, 1993; Olsson and Shine, 1997).

As we found in the laboratory (Du, 2003; Du et al., 2006), egg size did not vary temporally for the Beiji population in the field. This result does not follow the prediction that females would distribute more energy to offspring in later clutches to compensate for the disadvantage associated with later birth. In contrast, the Lishui population of *T. septentrionalis* shows significant seasonal shifts in egg size, with the average egg mass or offspring size being greater for the later clutches than for the first clutch (Ji et al., 2007). This interpopulation discrepancy of temporal variation in egg size may reflect various reproductive strategies among populations, or may simply be caused by the different sample sizes used in these studies. The small difference of egg mass between the first and subsequent clutches (0.02 g, Fig. 3C) could be statistically significant only with an extremely large sample (Ji et al., 2007) rather than a more moderately sized sample (Du et al., 2006; this study).

In lizards, RCMs may show temporal and spatial changes, namely significant interclutch variation in some species (Jones et al., 1987; Angilletta et al., 2001), and among-population differences in other species (Du et al., 2005, 2006). These differences in RCMs could arise from both ultimate and proximate factors. Our data indicate the RCMs are higher in wild females than in captive females, although the majority of reproductive traits, including clutch size, egg size and clutch mass, are consistent between the field and laboratory populations (Du et al., 2006; Fig. 3). This suggests that RCM could be noticeably affected by proximate factors. Sufficient food availability in captivity may lead to better body condition and in turn lower RCMs for females in the laboratory. This also accounts partly, if not completely, for the higher RCM in April than in May in this study because of females in April being maintained in the laboratory for longer periods (Figs. 2B, 3D). Therefore, when addressing intraspecific differences in RCM to explain the evolutionary strategies of an organism, investigators need to bear the influence of proximate factors in mind and, if possible, keep females in a common-garden environment so as to control for the potential influence of environmental factors.

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