- 13. Mitsuhara, I. et al., Efficient promoter cassettes for enhanced expression of foreign genes in dicotyledonous and monocotyledonous plants. Plant Cell Physiol., 1996, 37, 49-59.
- 14. Murashige, T. and Skoog, F., A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant., 1962, 15, 473-497.
- 15. Jefferson, R. A., Kavanagh, T. A. and Bevan, M. W., GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J., 1987, 6, 3901-3907.
- 16. Aldemita, R. R. and Hodges, T. K., Agrobacterium tumefaciensmediated transformation of japonica and indica rice varieties. Planta, 1996, 199, 612-617.
- 17. Tingay, S., McElroy, D., Kalla, R., Fieg, S., Wang, M., Thornton, S. and Brettell, R., Agrobacterium tumefaciens-mediated barley transformation. Plant J., 1997, 11, 1369-1376.
- 18. Chateau, S., Sangwan, R. S. and Sangwan-Norreel, B. S., Competence of Arabidopsis thaliana genotypes and mutants for Agrobacterium tumefaciens-mediated gene transfer: Role of phytohormones. J. Exp. Bot., 2000, 51, 1961-1968.
- 19. Park, S. and Facchini, P. J., Agrobacterium rhizogenes-mediated transformation of opium poppy, Papaver somniferum L. and California poppy, Eschscholzia californica Cham., root cultures. J. Exp. Bot., 2000, 51, 1005-1016.

ACKNOWLEDGEMENTS. K.K.N. and K.S. are grateful to CSIR, New Delhi for providing Junior Research Fellowship. Funds provided by the Department of Biotechnology, New Delhi to Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram are duly acknowledged. We also thank I. Mitsuhara, National Institute of Agrobiological Resources, Japan for providing pBE2113 construct.

Received 20 August 2002; revised accepted 1 April 2003

## Interrelationships among reproductive traits of female lizard, Sitana ponticeriana (Cuvier)

Rajkumar S. Radder and Bhagyashri A. Shanbhag\*

Department of Zoology, Karnatak University, Dharwad 580 003, India

Interrelationships among maternal body size, clutch size and egg size were studied in the ground lizard, Sitana ponticeriana. In these lizards, clutch size varied from 7 to 19. Initial clutches were larger than the potential next clutch. Clutch size and mass were positively correlated with maternal body size (Snout-vent length, SVL), but not with maternal body condition. Maternal SVL or clutch size had no influence on egg mass and egg volume. No trade-off was evident between clutch and egg sizes. Thus, in S. ponticeriana egg size is essentially invariant despite a great variation in clutch size across the females of different sizes.

REPRODUCTION is an important event in the life history of all organisms. Female body size, clutch/egg and off-

\*For correspondence. (e-mail: bhagyashri\_shanbhag@hotmail.com)

spring body sizes are the basic components of life-history traits. Oviparous reptiles show complex interrelationships among maternal body size, clutch and egg sizes. In lizards, clutch size varies with proximate climatic factors, food availability and fat body reserves 1-5. However, among the 456 species of reptiles inhabiting India<sup>4</sup>, factors influencing clutch, egg and offspring sizes are studied in detail in only one species, the garden lizard, Calotes versicolor<sup>6</sup>. Such information on reproductive traits in different species of lizards is needed to understand the diversity in reproductive trade-offs, if any. Sitana ponticeriana (family Agamidae) is a small lizard restricted to moderately moist scrub, sandy and rocky areas with plenty of bushes and other such vegetation in India. But for some scattered information on clutch size, incubation duration and size of hatchling<sup>7</sup>, no information is available with respect to the interrelationships among reproductive traits in this species. Hence, the present study was undertaken to investigate interrelationships among traits such as maternal size, clutch size and mass, and egg mass and size in S. ponticeriana.

Females of adult S. ponticeriana (n = 29) were collected during May-August 1998 from the surrounding areas of Dharwad (n = 4, 14, 6 and 5 in May, June, July and August, respectively). They were brought to the laboratory on the day of collection. Snout-vent length (SVL, cm) and mass (g) of these lizards were recorded. At autopsy, ovarian condition (presence/absence of vitellogenic follicles and their number), number of oviductal eggs (clutch size), if any, individual egg mass (mg), total clutch mass (g), egg length (mm) and egg width (mm) were recorded. Diameter of the largest ovarian follicles (mm) was measured using a micrometer. Follicles measuring > 2.5 mm were considered as vitellogenic<sup>8</sup>. An estimate of egg volume was taken as an overall measure of egg size. Egg volume was derived using mean egg length and width by a formula for prolate spheroid  $4/3\pi$ (length/2) (width/2)<sup>2</sup>. The number of vitellogenic follicles (diameter > 4 mm) in females having oviductal eggs was considered as their potential next clutch of the season8.

Mean  $\pm$  SEm was calculated from the untransformed data for all recorded variables. For the study of interrelationships among various reproductive traits, data were log transformed to meet the assumptions of parametric statistics and to facilitate the biological interpretation<sup>9</sup>. Maternal condition for each lizard was obtained by generating residuals of body mass. Residuals for body mass were generated by regressing log body mass on log SVL. Linear regression analysis was used to study the interrelationships among various reproductive traits. Variation in the number of eggs between the clutches (oviductal eggs and vitellogenic follicles) of an individual was analysed by paired sample t test. Significance level was accepted at P < 0.05 level. All statistical analyses were performed using SPSS software.

All the adult lizards (n = 29) collected during May–August were in a reproductively active condition as evident by the presence of either vitellogenic follicles or oviductal eggs, and in some cases both (4, 8 and 3 in May, June and July, respectively; n = 15). All the recorded traits are summarized and represented in Table 1. The SVL of the smallest female exhibiting oviductal eggs was 5.2 cm and that of the largest was 7.5 cm (Table 1). Clutch size varied from 7 to 19 and had a mean value of 15.47 (Table 1).

Maternal SVL and clutch size were positively correlated (linear regression  $r^2 = 0.28$ , P < 0.05, Figure 1). Similarly, clutch mass was positively correlated with the SVL (linear regression  $r^2 = 0.25$ , P < 0.05, Figure 1). But there was no relationship between SVL and egg mass (linear regression  $r^2 = 0.02$ , P > 0.05) as well as between SVL and egg volume (linear regression  $r^2 = 0.08$ , P > 0.05).

Interestingly, there was no correlation between body condition and clutch size (linear regression  $r^2 = 0.03$ , P > 0.05), clutch mass (linear regression  $r^2 = 0.05$ , P > 0.05) and egg mass (linear regression  $r^2 = 0.002$ , P > 0.05). Further, clutch size and egg volume showed no correlation with each other (linear regression  $r^2 = 0.05$ , P > 0.05, Figure 2). Similarly, no significant relationship was evident between egg mass and clutch size (linear regression  $r^2 = 0.10$ , P > 0.05, Figure 2). Initial clutches (oviductal eggs) were larger than the potential future (vitellogenic follicles) clutches (paired t test: df 14, t = 4.80, P < 0.001, n = 15, Figure 3).

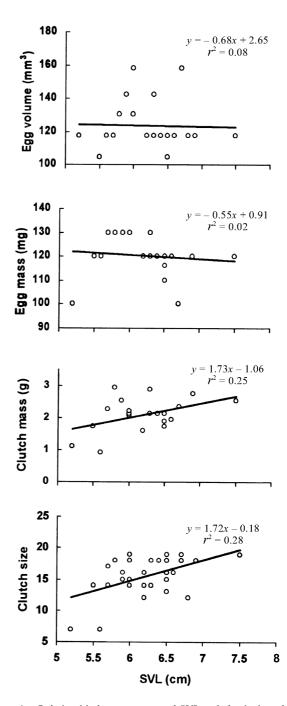
In the present study, *S. ponticeriana* exhibited greater reproductive output than that reported in previous studies on the same species. Subba Rao and Rajabai<sup>7</sup> reported that these lizards produce 8-13 eggs with a mean value of 11.2 eggs/clutch (n=10 clutches, SVL range 36-52 mm), while Tikader and Sharma<sup>10</sup> reported 11-14 eggs/clutch. But, in the present study a large range for clutch size (7-19 eggs, n=29 clutches) with a mean value of 15.47 was observed. The smaller clutch sizes reported in the previous studies may be due to the smaller-sized females sampled.

The manner in which energy is allocated to reproduction has received considerable attention in the discussion of life-history tactics in several organisms. Several paren-

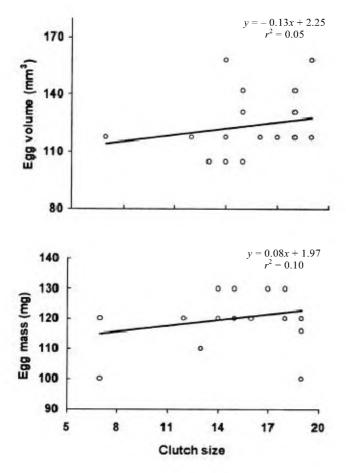
**Table 1.** Summary of the recorded traits in *Sitana ponticeriana* (n = 29)

Variable	Mean ± SEm	Range
SVL (cm)	$6.22 \pm 0.48$	5.2-7.50
Body mass (g)	$6.84 \pm 2.29$	5-17
Clutch size	$15.47 \pm 3.13$	7–19
Clutch mass (g)	$2.09 \pm 0.52$	0.91 - 2.94
Egg mass (g)	$0.12 \pm 0.01$	0.10 - 0.13
Egg length (mm)	$9.10 \pm 0.64$	8.0 - 10.00
Egg width (mm)	$5.18 \pm 0.34$	5.0-6.0
Egg volume (mm <sup>3</sup> )	$123.39 \pm 3.50$	104.47 - 158.01

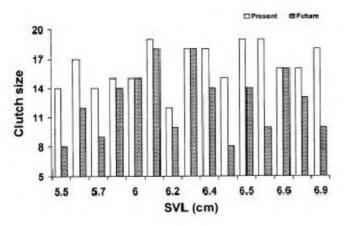
tal investment models that have been developed primarily for organisms with variable clutch size suggest that under a given set of environmental conditions, there are 'optimal' values for egg/offspring size to maximize parental fitness<sup>11,12</sup>. According to these models, when females have more energy available for reproduction, they produce more number of eggs/offspring of optimal size, rather than increasing the size of the egg or offspring. In such cases, egg or offspring size is independent of maternal size. These models are mainly based on organisms that



**Figure 1.** Relationship between maternal SVL and clutch size, clutch mass, egg mass and egg volume in *Sitana ponticeriana* (n = 29).



**Figure 2.** Relationship between clutch size and egg mass and egg volume in S. ponticeriana (n = 29).



**Figure 3.** Variation between the present clutch and future clutch (vitellogenic follicles) with respect to maternal SVL in *S. ponticeriana* (n = 15).

reproduce in one bout or produce few offspring/eggs in each bout. In *S. ponticeriana*, the clutch size increased with an increase in maternal SVL, but there was no evidence for any relationship between maternal SVL and egg size. Further, lack of inverse correlation between clutch size and egg size suggests that there is no trade-off

between these two reproductive traits, implying that there is optimization of egg size. S. ponticeriana produces at least two clutches in a breeding season (May-August), the early clutches being larger than the later ones as reported in other species of lizards<sup>1-5</sup>. In several species of lizards, a decrease in clutch size is attributed to tradeoff between egg size and clutch size (larger eggs, but few in number), food availability and modes of energy allocation (switching from capital to income breeding), etc.<sup>4,5</sup>. A previous study on a multiclutched Agamid lizard, C. versicolor has reported a trade-off between clutch size and egg size<sup>6</sup>. In this species, the early clutches are larger with smaller eggs and late clutches are smaller with larger eggs. Thus, no optimization of egg size is found in C. versicolor. However, in S. ponticeriana there is no tradeoff between egg size and clutch size, since eggs of invariant size are produced throughout the breeding season. A variation in reproductive trade-offs between clutch and egg sizes of these two Agamids inhabiting the same area may be due to the differences in their microhabitat and locomotory modes.

- Vitt, L. J., Diversity of reproductive strategies among Brazilian lizards and snakes: The significance of lineage and adaptation. Reproductive Biology of South American Vertebrates (ed. Hamlett, W. C.), Springer-Verlag, New York, 1992, pp. 135-149.
- Dunham, A. E., Reproductive ecology. Lizard Ecology: Historical and Experimental Perspective (eds Vitt, L. J. and Pianka, E. R.), Princeton Univ. Press, Princeton, NJ, 1994, pp. 3–5.
- Schwarzkopf, L., Measuring trade-offs. Lizard Ecology: Historical and Experimental Perspective (eds Vitt, L. J. and Pianka, E. R.), Princeton Univ. Press, Princeton, NJ, 1994, pp. 7–29.
- Shanbhag, B. A., Reproductive biology of Indian reptiles. Proc. Indian Natl. Sci. Acad., 2002, B68, 497–528.
- Shanbhag, B. A., Reproductive strategies in the lizard Calotes versicolor. Curr. Sci., 2003, 84, 646-652.
- Shanbhag, B. A., Radder, R. S. and Saidapur, S. K., Maternal size determines clutch mass, whereas breeding timing influences clutch and egg sizes in the tropical lizard *Calotes versicolor* (Agamidae). *Copeia*, 2000, 2000, 1062–1067.
- Subba Rao, M. V. and Rajabai, B. S., Reproduction in the ground lizard Sitana ponticeriana and the garden lizard Calotes nemoricola. Br. J. Herpetol., 1972, 4, 245–251.
- 8. Shanbhag, B. A. and Prasad, B. S. K., Follicular dynamics and germinal bed activity during annual ovarian cycle in the lizard *Calotes versicolor* (Daud.). *J. Morphol.*, 1993, **215**, 1–7.
- King, R. B., Analyzing the relationship between clutch size and female body size in reptiles. J. Herpetol., 2000, 34, 148–150.
- Tikader, B. K. and Sharma, B. K., Indian fan throated lizard Sitana ponticeriana Cuvier 1884. Handbook of Indian Lizards, Zoological Survey of India, Kolkata, 1992, pp. 83–84.
- 11. Brockelman, W. Y., Competition, the fitness of offspring and optimal clutch size. *Am. Nat.*, 1975, **109**, 677-699.
- 12. Smith, C. C. and Fretwell, S. D., The optimal balance between size and number of offspring. *Am. Nat.*, 1974, **108**, 499–506.

ACKNOWLEDGEMENTS. This work is partly supported by a grant from UGC SAP-II, New Delhi. R.S.R. thanks CSIR, New Delhi for financial support.

Received 18 January 2003; revised accepted 15 April 2003