

STORAGE AND UTILIZATION OF ENERGY AND MATERIAL IN EGGS OF TWO LIZARD SPECIES, *GEKKO JAPONICUS* AND *TAKYDROMUS SEPTENTRIONALIS*

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Abstract—1. During incubation, rigid-shelled eggs of *Gekko japonicus* declined in both wet mass and dry mass, and pliable-shelled eggs of *Takydromus septentrionalis* increased in wet mass but decreased in dry mass.

2. Rapid increase of percentage of lipids in developing eggs occurred during the early period of vitellogenesis, percentage of lipids in medium and large sized follicles of both species remained approximately constant. Caloric value was correlated with lipid content and for ash, uncorrected caloric value was correlated with ash content.

3. Total ash present in developing eggs increased as the eggs developed. Embryos of both species did not rely exclusively on ash supplied in their yolks, and withdrew ash from their eggshells.

INTRODUCTION

An analysis on egg and hatchling components is of interest because it can reveal different storage and utilization patterns of energy and material in eggs of different lizard species. Reptiles, unlike endotherms, rarely exhibit parental care in the form of feeding offsprings (Troyer, 1983, 1987; Wilhoft, 1986; Congdon and Gibbons, 1989). Most energy and material are stored in yolk of developing eggs during vitellogenesis. While developing within the eggs, embryos utilize this stored energy and material. A portion of yolk, however, may remain unutilized until the time of hatching. This remaining yolk sac represents a supply of energy and material for the hatchlings and their early posthatching activity (Kraemer and Bennett, 1981; Troyer, 1983, 1987; Wilhoft, 1986; Congdon and Gibbons, 1989). Although authors have reported caloric values of eggs of several lizard species (Ballinger and Clark, 1973; Tinkle and Hadley, 1975), studies on storage and utilization of energy and material in lizard eggs have been limited. In contrast, abundant data on ash content, lipid content and energy content of eggs and hatchlings of other reptile species have been reported (Congdon *et al.*, 1983; Congdon and Gibbons, 1985; Packard *et al.*, 1984a,b; Lamb and Congdon, 1985; Wilhoft, 1986; Packard and Packard, 1989).

The two lizard species studied here are both insectivores, but quite different. The wall lizard *Gekko japonicus* is a sit-and-wait nocturnal lizard. Females can lay eggs at the mean snout-vent length (SVL) of 62.0 mm. One to three (average 1.8) clutches, with two rigid-shelled eggs each, were oviposited per breeding season (Liu and Hu, 1940; Ji and Wang, 1991). The grass lizard *Takydromus septentrionalis* is a widely foraging diurnal lizard. Females can lay eggs at the mean SVL of 61.0 mm. One to three (average 2.0) clutches, with 2–6 pliable-shelled eggs, were oviposited per breeding season (Liu, 1939; Wang,

1966; Ji *et al.*, in press). Although extensive data on reproductive biology and embryonic metabolism of the two species have been reported (Liu, 1939; Liu and Hu, 1940; Wang, 1966; Ji and Wang, 1991; Ji *et al.*, 1991), previous work uncovered few data sufficient to demonstrate the storage and utilization of energy and material in eggs of the two lizard species. This study investigates the degree of variations present in egg (including yolked follicles, oviductal eggs and newly-laid eggs) and hatchling components in the two species.

MATERIALS AND METHODS

This study was conducted in Hangzhou, China. Adult female *G. japonicus* with SVLs larger than 56.0 mm (Tokunaga, 1984; Ji and Wang, 1990) were collected during 1987–1989 on buildings in Hangzhou (30°16'N, 120°9'E), and adult female *T. septentrionalis* with SVLs larger than 55.0 mm (Wang, 1966; Ji *et al.*, 1989) were collected during 1987–1990 from a field on Xiushan Island (30°11'N, 122°9'E).

All of the collected lizards were taken to the laboratory at Hangzhou Normal College. Some of the lizards were autopsied, size and mass of yolked follicles and oviductal eggs being noted. The remaining lizards were allowed to feed freely on larvae of *Tenebrio molitor* and lay their eggs in the laboratory. Newly-laid eggs were collected within 6 hr of being laid. Each egg was numbered and size (nearest 0.02 mm) and mass (nearest 0.01 g) were noted. Half of the newly-laid eggs were then frozen for lipid extraction and caloric determination, and the remaining half of the eggs were incubated at a temperature of $32 \pm 0.5^\circ\text{C}$. All of the incubating eggs were half-buried on a water-saturated sand substrate and the inside of the incubation chamber was periodically misted with water to keep the moisture. We measured and weighed the incubating eggs every 5 days. After the hatchlings pipped the eggs, they were immediately measured and weighed, and then frozen. All of the materials for lipid extraction and caloric determination were dried to constant mass in an oven at 65°C and dry mass was recorded. Samples of dried eggs (shell was removed) and hatchlings were then ground in a mortar with a pestle.

Table 1. Lipid contents (%) of yolked follicles, oviductal eggs, newly-laid eggs and hatchlings of the two lizard species, *G. japonicus* and *T. septentrionalis*

Species	Hatchling	Newly-laid egg	Oviductal egg	Yolked follicle
<i>G. japonicus</i>	28.1 ± 2.9 (11) 18.7–34.4	43.0 ± 1.3 (10) 39.7–44.8	44.3 ± 3.2 (4) 34.7–45.2	46.2 ± 3.7 (13) 29.5–59.4
<i>T. septentrionalis</i>	15.5 ± 3.2 (8) 12.5–19.4	27.6 ± 1.6 (6) 25.2–29.9	31.9 ± 2.3 (13) 23.0–39.6	28.8 ± 2.0 (9) 22.8–32.3

Values are expressed as mean ± 2SE and range. Samples sizes are indicated in parentheses.

Caloric value was determined with a LR-2800 adiabatic bomb calorimeter manufactured by Changsha Instruments Factory. Ash was defined as the residue remaining after combustion in the bomb calorimeter.

Lipids stored in yolked follicles, oviductal eggs, yolk of newly-laid eggs and hatchlings were extracted with absolute ether in Soxhlet apparatus at 55°C for 5.5 hr. After the extraction, the samples were dried to constant mass in an oven at 65°C and weighed. Lipid content was calculated as (extracted lipid mass/samples dry mass) × 100.

RESULTS

Because almost all adult females of the two species had yolked follicles and oviductal eggs during spring and summer, oviposition occurred annually from mid-May to early August. In both species, not only did litter mass increase but total lipids and caloric values of developing eggs also increased during vitellogenesis. Most energy and material stored in the eggs were utilized during incubation for embryonic development. At the time of hatching, mean wet mass of the yolk sacs of *G. japonicus* and *T. septentrionalis* were 0.034 g and 0.030 g, respectively.

G. japonicus

Newly-laid eggs averaged 74% water by total mass, and wet mass of whole newly-laid egg averaged 0.67 g ($N = 54$). New hatchlings averaged 72% water by the hatchling wet mass (yolk sac was removed), and wet mass of new hatchlings averaged 0.45 g ($N = 32$). Hatchling wet mass was 67% of the total newly-laid egg wet mass, and hatchling dry mass was 72% of the newly-laid egg dry mass ($N = 9$). Eggshell averaged 13% of the total newly-laid egg wet mass. Rigid-shelled eggs of the wall lizard declined in mass (both wet mass and dry mass) during incubation.

Lipid content (per cent lipids) of newly-laid eggs was significantly higher than that of new hatchlings (Mann-Whitney test: $U = 110$, $P < 0.01$), but there was no significant difference in lipid content among eggs at different developing stages [$F(2,24) = 1.201$, $P > 0.05$] (Table 1). Lipid content per g of hatchling dry mass averaged 65% of that of newly-laid egg dry mass per g.

There were significant differences in ash uncorrected caloric values ($U = 84$, $P < 0.01$) and ash-free caloric values ($U = 69$, $P < 0.05$) between the newly-

laid eggs and the hatchlings, the caloric values of the hatchlings were much less than those of the newly-laid eggs. Although there were no significant differences in ash uncorrected caloric values among the eggs at different developing stages [$F(2,22) = 3.081$, $P > 0.05$], the differences in ash-free caloric values were statistically significant [$F(2,22) = 11.840$, $P < 0.01$]. Newly-laid eggs had significantly greater ash-free caloric values than oviductal eggs (Duncan's new multiple range test: $LSR_{0.05} = 193.6$) and yolked follicles ($LSR_{0.01} = 275.5$), and oviductal eggs had significantly greater ash-free caloric values than yolked follicles ($LSR_{0.05} = 193.6$) (Table 2).

Total ash in yolk increased as the eggs developed. There were significant differences in the levels of ash in yolk among eggs at different developing stages [$F(2,17) = 56.101$, $P < 0.01$]. Yolk of newly-laid eggs had significantly higher ash content than oviductal eggs ($LSR_{0.01} = 0.9\%$) and yolked follicles ($LSR_{0.01} = 0.9\%$), and oviductal eggs had significantly higher ash content than yolked follicles ($LSR_{0.01} = 0.9\%$). Hatchlings had significantly higher ash content than newly-laid eggs ($U = 56$, $P < 0.01$) (Table 3).

T. septentrionalis

Newly-laid eggs averaged 75% water by total mass, and wet mass of whole newly-laid eggs averaged 0.27 g ($N = 31$). Hatchlings averaged 86% water by the hatchling wet mass (yolk sac was removed), and wet mass of hatchlings averaged 0.31 g ($N = 15$). Hatchling wet mass was 115% of the total newly-laid egg wet mass, and the hatchling dry mass was 73% of the newly-laid egg dry mass ($N = 8$). Eggshell averaged 19% of the total newly-laid egg wet mass. Pliable-shelled eggs of the grass lizard increased in size and wet mass, but decreased in dry mass during incubation.

Lipid content of newly-laid eggs was significantly higher than that of hatchlings ($U = 24$, $P < 0.05$). In contrast to *G. japonicus*, lipid contents of eggs at different developing stages were statistically different [$F(2,25) = 3.924$, $P < 0.05$]. Oviductal eggs had the highest levels of lipid in their yolks, which were significantly higher than those in yolked follicles ($LSR_{0.05} = 3.4$) and those in yolks of newly-laid eggs ($LSR_{0.05} = 3.6$), but there was no significant difference between yolked follicles and yolks of newly-laid eggs

Table 2. Caloric values (cal/g dry mass) of yolked follicles, oviductal eggs, newly-laid eggs and hatchlings of the two lizard species, *G. japonicus* and *T. septentrionalis*

	<i>G. japonicus</i>				<i>T. septentrionalis</i>	
	Hatchling	Newly-laid egg	Oviductal egg	Yolked follicle	Hatchling	Newly-laid egg
Ash uncorrected caloric value	5916.6 ± 175.7 (7) 5504.8–6295.0	6916.5 ± 101.7 (12) 6733.7–7217.8	6784.9 ± 102.8 (6) 6555.5–6908.8	6662.6 ± 246.0 (7) 6285.9–7049.3	5613.1 ± 158.7 (5) 5313.5–5722.1	6522.1 ± 320.1 (7) 5950.2–7043.9
Ash free caloric value	6726.0 ± 328.6 (7) 6053.0–7296.9	7179.5 ± 98.8 (12) 6904.2–7364.3	6968.6 ± 77.5 (6) 6846.8–7086.7	6752.4 ± 196.8 (7) 6339.5–7124.1	6642.9 ± 356.9 (5) 6167.1–7058.4	6981.3 ± 352.5 (7) 6320.4–7546.8

Values are expressed as mean ± 2SE and range. Sample sizes are indicated in parentheses.

Table 3. Ash contents (%) of yolked follicles, oviductal eggs, newly-laid eggs and hatchlings of the two lizard species, *G. japonicus* and *T. septentrionalis*

Species	Hatchling	Newly-laid egg	Oviductal egg	Yolked follicle
<i>G. japonicus</i>	12.8 ± 1.4 (7)	4.5 ± 1.7 (8)	2.6 ± 0.4 (6)	1.5 ± 0.4 (6)
	9.1–14.8	3.0–5.8	1.3–3.1	0.8–2.1
<i>T. septentrionalis</i>	15.4 ± 2.7 (5)	6.8 ± 0.2 (7)		
	12.0–18.9	6.4–7.2		

Values are expressed as mean ± 2SE and range. Samples sizes are indicated in parentheses

($LSR_{0.05} = 3.4$) (Table 1). Lipid content per g of new hatchling dry mass averaged 56% of that per g of newly-laid egg dry mass.

Although caloric values of newly-laid eggs were greater than those of hatchlings, only the difference in ash uncorrected caloric values was statistically significant ($U = 35$, $P < 0.01$) (Table 2).

Hatchlings had a significantly higher level of ash than newly-laid eggs ($U = 35$, $P < 0.01$) (Table 3).

DISCUSSION

Temporal changes in mass of incubating eggs reflect largely on exchanges of water between eggs and their surroundings. Liquid water can flow across substrate-contacting aspects of eggshell in response to gradients in water potential, whereas water vapour simultaneously diffuses across air-contacting aspects of eggshell in response to gradients in vapour pressure (Packard *et al.*, 1981; Packard and Packard, 1989). Hatching success for the rigid-shelled eggs of *G. japonicus* was very high, and the eggs declined in both wet mass and dry mass during incubation owing to (1) the continuous escape of water vapour to air and (2) ever-increasing utilization of material stored in the incubating eggs (Ji and Wang, 1991). Eggs of *G. japonicus* appear to contain sufficient water at oviposition to allow embryos to use energy reserves of yolk in growth, for the variations of water exchanges on different substrates had no apparent influences of size and water content of hatchlings and on the successful completion of embryonic development (Ji and Wang, 1991). Pliable-shelled eggs of *T. septentrionalis*, in contrast, increased in wet mass but decreased in dry mass during incubation, because the quantity of liquid water absorbed by incubating eggs from the substrates exceeded the quantity of water vapour that is lost. On average, the eggs weighed 0.65 g more (about 242%) at the end of incubation than at oviposition (Wang *et al.*, 1989). Eggs of *T. septentrionalis* do not contain sufficient water necessary for embryonic development, and water absorbed from substrates provide the incubating eggs with an auxiliary supply of water to support embryonic development. Indeed, water potential of substrates had an apparent influence on hatching success for eggs of the grass lizard.

Although not comparable data for changes in dry mass of embryos and yolks of the two species were available, we can here estimate the growth rates of embryos of the two species according to changes in oxygen consumption of embryos during incubation. In both species, growth was slow during the first half of incubation, because the embryos during this period consumed much less oxygen. During the second half of incubation, the growth, however, was essentially

linear and gave no indication of a plateau late in development (Wang *et al.*, 1989, in press). There was an apparent difference in embryonic metabolic intensity between *G. japonicus* and *T. septentrionalis* (Wang *et al.*, in press). Embryos of the grass lizard had a significantly higher metabolic rate throughout incubation. Although the duration of incubation of the wall lizard at 32°C (42 days) was 16 days longer than that of the grass lizard at the same incubation temperature (26 days), total oxygen consumed by *G. japonicus* embryos (243.0 ml O₂/egg) was 36 ml O₂/egg less than that consumed by *T. septentrionalis* embryos (279.0 ml O₂/egg). We could detect the amount of oxygen consumed by *T. septentrionalis* embryos but could not detect the amount of oxygen consumed by *G. japonicus* embryos at oviposition. We could also demonstrate the difference in metabolic intensity between the embryos of the two species at the time of hatching. Although body mass of hatchlings of the wall lizard was apparently greater than that of the grass lizard, oxygen consumption of the embryos of the two species was almost the same (0.214 ml O₂/hr for *G. japonicus* and 0.203 ml O₂/hr for *T. septentrionalis*). Thus, it is reasonable to conclude that the much lower per cent lipids remaining in *T. septentrionalis* hatchlings (56% in *T. septentrionalis* vs 65% in *G. japonicus*) may primarily result from the differences in metabolic intensity between the embryos of the two species, since lipids are an ideal source of energy utilized for maintenance and growth (Congdon and Tinkle, 1982; Wilhoft, 1986; Ji and Wang, 1990).

A rapid increase in the percentage of lipids in developing eggs of the two species occurred during the early period of vitellogenesis, because the percentage of lipids in small-sized yolked follicles (diameter shorter than 5.0 mm) increased as the eggs developed during this period. Percentages of lipids in medium- and large-sized yolked follicles remained approximately constant (43–58% in *G. japonicus* and 28–39% in *T. septentrionalis*), which indicated that the nutrients were stored at almost the same rate in these larger yolked follicles in both species.

Embryos of the grass lizard do not develop past blastulas within maternal oviducts (Wang, 1966). Thus, all eggs were assumed to be at the same stage of development and consumed a negligible quantity of oxygen at oviposition. To explain the difference in lipid content between oviductal eggs and newly-laid egg yolks and the detectable oxygen consumption of embryos at oviposition in *T. septentrionalis*, we need further experimental data. We have even fewer data from embryology to demonstrate why the amount of oxygen consumed by embryos of the wall lizard at oviposition cannot be detected.

Caloric values of eggs and hatchlings were correlated with lipid content and/or ash content of samples. The maximum caloric value occurred in the samples having the highest level of lipids and, for ash uncorrected caloric value, the lowest level of ash.

As we can see in *G. japonicus*, total ash present in developing eggs increased as the eggs developed, and reached the peak in newly-laid egg yolks. Ash stored in eggs can be utilized for embryonic development. Embryos of both species do not rely exclusively on ash supplied in their yolks for support of growth and development, and withdrew ash from their eggshells, because hatchlings of both species contained much more total ash in their bodies than was present in egg yolk at oviposition. In this study, we only provided data on ash contents of eggs and hatchlings. The data provided here do not demonstrate where the embryos of the two species obtain a specific mineral from. Sources of a specific mineral are quite different for different reptilian embryos. According to previous work, embryos of some oviparous reptiles obtain Ca from their eggshells and yolks but all of their P from their yolks (Packard *et al.*, 1984a,b, 1985; Packard and Packard, 1988, 1989).

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REFERENCES

- Ballinger R. E. and Clark D. R. (1973) Energy content of lizard eggs and the measurement of reproductive effort. *J. Herpetology* **7**, 129–132.
- Congdon J. D. and Gibbons J. W. (1985) Egg components and reproductive characteristics of turtles: relationships to body size. *Herpetologica* **41**, 194–205.
- Congdon J. D. and Gibbons J. W. (1989) Posthatching yolk reserves in hatchling American alligators. *Herpetologica* **45**, 305–309.
- Congdon J. D. and Tinkle D. W. (1982) Reproductive energetics of the painted turtle (*Chrysemys picta*). *Herpetologica* **38**, 228–237.
- Congdon J. D., Tinkle D. W. and Rosen P. C. (1983) Egg components and utilization during development in aquatic turtles. *Copeia* **1983**, 264–268.
- Ji X., Ma W. and Wang P. C. (1989) Study on home range of the lizard *Takydromus septentrionalis* during reproduction period. *J. Hangzhou Normal College* **1989**, 60–63.
- Ji X. and Wang P. C. (1990) Annual cycles of lipid contents and caloric values of carcass and some organs of the gecko, *Gekko japonicus*. *Comp. Biochem. Physiol.* **96A**, 267–271.
- Ji X. and Wang P. C. (1991) Incubation character of eggs of the gecko *Gekko japonicus*. *Zool. Res.* **12**, 28–72.
- Ji X., Wang P. C. and Hong W. X. (1991) The reproductive ecology of the gecko *Gekko japonicus*. *Acta Zoologica Sinica* **37**, 185–192.
- Ji X., Tang Y. W. and Hong W. X. (1992) Further ecological observations of the grass lizard *Takydromus septentrionalis* during the breeding season. *J. Herpetology* (in press).
- Kraemer J. E. and Bennett S. H. (1981) Utilization of posthatching yolk in loggerhead sea turtle, *Caretta caretta*. *Copeia* **1981**, 406–411.
- Lamb T. and Congdon J. D. (1985) Ash content: relationships to flexible and rigid eggshell types of turtles. *J. Herpetology* **19**, 527–530.
- Liu C. C. (1939) Notes on the life history and breeding habit of *Takydromus septentrionalis*. *Peking Nat. Hist. Bull.* **14**, 35–42.
- Liu C. C. and Hu S. C. (1940) Notes on the life history and breeding habit of *Gekko japonicus* and *Gekko subpalmaris*. *J. West China Bor. Res. Soc.* **12B**, 63–70.
- Packard G. C., Taigen T. L., Packard M. J. and Boardman T. J. (1981) Changes in mass of eggs of softshell turtles (*Trionyx spiniferus*) incubated under hydric conditions simulating those of natural nests. *J. Zool., Lond.* **193**, 81–90.
- Packard M. J. and Packard G. C. (1988) Sources of calcium and phosphorus during embryogenesis in bullsnakes (*Pituophis melanoleucus*). *J. exp. Zool.* **246**, 132–138.
- Packard M. J. and Packard G. C. (1989) Mobilization of calcium, phosphorus, and magnesium by embryonic alligators (*Alligator mississippiensis*). *Am. J. Physiol.* **257**, R1541–R1547.
- Packard M. J., Packard G. C. and Gutzke W. H. N. (1984a) Calcium metabolism in embryos of the oviparous snake *Coluber constrictor*. *J. exp. Biol.* **110**, 99–112.
- Packard M. J., Packard G. C., Miller J. D., Jones M. E. and Gutzke W. H. N. (1985) Calcium mobilization, water balance, and growth in embryos of the agamid lizard *Amphibolurus barbatus*. *J. exp. Zool.* **235**, 349–357.
- Packard M. J., Short T. M., Packard G. C. and Gorell T. A. (1984b) Sources of calcium for embryonic development in eggs of the snapping turtle *Chelydra serpentina*. *J. exp. Zool.* **230**, 81–87.
- Tinkle D. W. and Hadley N. F. (1975) Lizard reproductive effort: caloric estimated and comments on its evolution. *Ecology* **56**, 427–434.
- Tokunaga S. (1984) Morphological variation and sexual dimorphism in *Gekko japonicus* from Fukuoka, Northern Kyushu, Japan. *Jap. J. Herpetol.* **10**, 80–88.
- Troyer K. (1983) Posthatching yolk energy in a lizard: utilization pattern and interclutch variation. *Oecologia, Berlin* **58**, 34–344.
- Troyer K. (1987) Posthatching yolk in a lizard: internalization and contribution to growth. *J. Herpetology* **21**, 102–106.
- Wang P. C. (1966) Studies on the ecology of four species of lizards in Hangzhou II. Breeding. *Acta Zoologica Sinica* **18**, 170–185.
- Wang P. C., Ji X. and Lu B. (1992) A comparison between the embryonic metabolic rates in two species of lizards. *J. Herpetology* (in press).
- Wang P. C., Ji X. and Ma W. (1989) Oxygen consumption of *Takydromus septentrionalis* eggs and hatchlings. In *Current Herpetology in East Asia* (Edited by Matsui M., Hikida T. and Goris R. C.), pp. 49–53. The Herpetological Society of Japan.
- Wilhoft D. C. (1986) Eggs and hatchling components of the snapping turtle (*Chelydra serpentina*). *Comp. Biochem. Physiol.* **84A**, 483–486.