Can snakes use yolk reserves to maximize body size at hatching?

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Abstract

We experimentally miniaturized freshly laid eggs of the Chinese cobra Naja atra (Elapidae) by removing ~10% and ~20% of original yolk. We tested if yolk-reduced eggs would produce 1) normal-sized hatchlings with invariant yolk-free body mass (and thus invariant linear size) but dramatically reduced or even completely depleted residual yolk, 2) smaller hatchlings with normal-sized residual yolk but reduced yolk-free body mass, or 3) smaller hatchlings of which both yolk-free body mass and residual yolk are proportionally reduced. Yolk quantity affected hatchling linear size (both snout-vent length and tail length) and body mass. However, changes in yolk quantity did not affect incubation length or any hatchling trait examined after accounting for egg mass at laying (for control and sham-manipulated eggs) or after yolk removal (for manipulated eggs). Specifically, yolk-reduced eggs produced hatchlings of which all major components (carcass, residual yolk, and fat bodies) were scaled down proportionally. We show that snakes cannot use yolk reserves to maximize their body size at hatching. Furthermore, our data also suggest that the partitioning of yolk in embryonic snakes is species-specific.

Key words: hatchling phenotype, Naja atra, residual yolk, snake, yolk partitioning, yolk removal

One of the central goals of life history studies in invertebrates and non-mammalian vertebrates is to assess maternal investment in offspring via yolk quantity and egg size. It is common and perhaps ubiquitous among these animals that yolk deposited by a mother in individual eggs exceeds the requirements to produce a fully developed young. Therefore, prior to hatching (for oviparous species) or birth (for viviparous species), a fraction of unutilized yolk (i.e., residual yolk) is internalized into the abdominal cavity of the young for later use (Kaplan 1980; Goudien et al. 1987; Congdon and Gibbons 1989; Kamler et al. 1998; Koláčková et al. 2015). Residual yolk has multiple functions and its size varies within and among taxa, among populations of the same species, among clutches of the same population or family, and even between sexes of the same clutch (Allsteadt and Lang 1995; Ji et al. 2002a; Spencer and Janzen 2014; Koláčková et al. 2015; Wu et al. 2017). One function of residual yolk is to support post-embryonic growth in the same way as yolk does during the embryonic stage, a function that has been reported for a diverse array of ectothermic vertebrates including salamanders (Orr and Maple 1978), lizards (Troyer 1987; Pandav et al. 2006), snakes (Ji et al. 1997, 1999; Ji and Sun 2000; Wu et al. 2017), and turtles (Filoramo and Janzen 1999; Lance and Morafka 2001; Alava et al. 2006; Lee et al. 2007; Van Dyke et al. 2011; Carpenter et al. 2015). However, 1) the coupling mechanism between these 2 stages, and 2) whether stochastic fluctuations in egg size and thus per capita maternal investment alter the partitioning of yolk between these 2 stages of growth remain poorly understood. Previous studies on these aspects have produced mixed conclusions. For instance, a study of the oriental garden lizard Calotes versicolor...
shows that selection favors the maximization of yolk reserves for post-hatching needs, and the miniaturization of egg size through experimental removal of yolk results in small hatchlings with a normal-sized residual yolk (Radder et al. 2004). Another study on the green iguana (Iguana iguana) indicates that the inter-clutch variation in yolk size at hatching is much greater than variation in either body mass or length (Troyer 1983). Overall, this suggests that selection for maximization of body size at hatching and thus post-embryonic growth rate is favored over residual yolk (Troyer 1983).

Here, we test if stochastic fluctuations in egg size, and thus per capita maternal investment, alter yolk partitioning between the embryonic and post-hatching stages in the Chinese cobra Naja atra. We applied a technique of yolk removal that was created for 2 species of sea urchins (Strongylocentrotus droebachiensis and S. purpuratus; Sinervo and McEdward 1988) and subsequently applied to the western fence lizard Sceloporus occidentalis; as the model system (Sinervo 1990), the eastern fence lizard Sceloporus undulatus (Storm and Angilletta 2007; Niewiarowski and Angilletta 2008) and the jacky dragon Amphibolurus muricatus (Warner and Shine 2007). Specifically, we first miniaturized freshly laid eggs of N. atra by surgically removing ~10% and ~20% of original yolk (see below for details) and then tested if yolk-reduced eggs would produce 1) normal-sized hatchlings with invariant yolk-free body mass (and thus invariant linear size) but dramatically reduced or even completely depleted residual yolk (Figure 1A), 2) smaller hatchlings with reduced yolk-free body mass but normal-sized residual yolk (Figure 1B), or 3) smaller hatchlings of which both yolk-free body mass and residual yolk are proportionally reduced (Figure 1C). An earlier study incubating eggs of N. atra at multiple constant temperatures within the range of 24°C–32°C shows that the dry mass of residual yolk accounts for 20–33% of the total hatching dry mass (Ji and Du 2001). Thus, if selection for maximization of body size at hatching is favored over yolk reserves for post-hatching use in this species, we predict that eggs of N. atra would have the potential to produce normal-sized hatchlings even undergoing the 20% reduction in yolk quantity.

Materials and Methods

Eggs (N = 165) used in this study were laid by 11 females collected in late June 2014 from Dinghai (30°02’N, 122°10’E), Zhoushan Islands, East China. Females laid eggs in the laboratory within 2 weeks after capture under the conditions described previously for snakes (Lin et al. 2012). Eggs from individual clutches were assigned among 4 treatments: manipulated (2), sham-manipulated (1) and control (1). Eighty-eight eggs underwent yolk removal on the day of oviposition, which was accomplished by aspirating yolk with a sterile syringe (#16 needle gauge) inserted into the yolk sac on the side opposite to the embryonic disk. The injection was sealed by a 4 mm × 4 mm sterile adhesive tape. Yolk removal resulted in ~20% (18–26%) mass reduction in 1 group of manipulated eggs (N = 44), and ~10% (7–12%) mass reduction in the other group (N = 44). Sham-manipulated eggs (N = 44) were pierced with a needle but no yolk was removed. Finally, control eggs (N = 33) were incubated without any kind of manipulation.

Eggs were individually incubated in jars (250 mL) with a substrate of moist vermiculite (~80 mm depth) at a water potential of ~220 kPa (Ji and Du 2001). Jars were kept in 2 incubators (Binder Inc., Germany) at 30.0°C. We shuffled the jars within and between incubators every other day to minimize effects of thermal gradients. However, we noticed minor temperature gradients (0.3°C) within incubators. These gradients were measured using Thermochron iButtons (Maxim Integrated Products, USA) that were placed inside jars. We individually weighed jars every 4 days, and added water to the substrate when necessary to compensate for evaporative losses and water absorbed by eggs. Incubation length, defined as the time period between egg laying and pipping, was recorded for each hatched egg (Li et al. 2012; Lu et al. 2012).

Sixty-nine manipulated, 39 sham-manipulated, and 30 control eggs hatched. All hatchlings were collected, weighed, measured for snout–vent length (SVL), and tail length and sexed by manual eversion of hemipenes (Ji and Du 2001) <6 h post-hatching. Eighty-eight hatchlings, 8 (1 male and 1 female from each of the 4 treatments) from each clutch, were frozen at –20°C on the day of hatching. The remaining 50 hatchlings and their respective mothers were released back to the field in mid-September. Frozen hatchlings were later thawed, dissected, and separated into carcass, residual yolk, and fat bodies. The 3 hatching components were dried in an oven at 60°C for 48 h to obtain dry mass.

We used 2-way ANOVA (for egg mass at laying, incubation length, and hatching SVL and body mass) or ANCOVA (for other hatching traits covarying with egg mass) using egg mass at laying (for control and sham-manipulated eggs) or after yolk removal (for manipulated eggs) as a covariate to test if the effects of treatment (manipulated, sham-manipulated, or control), sex and their interaction were significant. Prior to ANCOVAs, we assessed if slopes of the relationship between an examined dependent variable and the covariate were parallel. All analyses were performed with Statistica 8.0 for PC (StatSoft Inc., Tulsa, OK, USA). All values were presented as means ± SE and range. We used a significance level of 0.05.

Figure 1. Hypothetical relationships between yolk-free body mass (green line or curve) at hatching and yolk quantity (blue curve) in yolk-reduced (miniaturized) eggs that produce 1) normal-sized hatchlings (unchanged yolk-free body mass) with no or dramatically reduced residual yolk (A), 2) smaller hatchlings with normal-sized residual yolk (B), or 3) smaller hatchlings of which yolk-free body mass and residual yolk both are reduced (C). Green line or curves show yolk-free body mass at hatching; blue curve shows yolk quantity; red line shows the relationship between yolk-free body mass at hatching and the quantity of yolk used to support somatic tissue growth during the embryonic stage.
Fat body dry mass 0.52
6
6
6
6
6

shorter in SVL, 17% lighter in wet mass, and 16% lighter in dry
reduction in egg mass resulted in hatchlings that were about 7%

Table 1. Descriptive statistics, expressed as mean ± SE and range, for size, mass and composition of hatchlings derived from manipulated (miniaturized), sham-manipulated and control eggs

|                     | Control                       | Sham-manipulated               | Miniaturized by 10% | Miniaturized by 20% |
|---------------------|-------------------------------|--------------------------------|---------------------|---------------------|
|                     | Females Males                 | Females Males                  | Females Males       | Females Males       |
| Initial egg mass    | 17.4 ± 1.1 17.8 ± 1.0         | 17.5 ± 1.0 17.6 ± 1.0          | 18.0 ± 0.9 16.6 ± 0.7 | 18.0 ± 0.9 17.2 ± 1.0 |
| 11.9 – 22.8         | 12.2 – 23.3                   | 12.1 – 23.2 12.6 – 23.5        | 13.3 – 22.2 12.4 – 21.0 | 12.2 – 23.1 12.1 – 22.5 |
| Miniaturized egg mass| –                             | –                              | 16.2 ± 0.7 14.8 ± 0.6 | 14.4 ± 0.7 13.9 ± 0.8 |
|                    | 12.0 – 19.5 11.1 – 19.0       | 10.0 – 18.4 9.6 – 18.5         |                     |                     |
| Snout-vent length   | 26.1 ± 0.6 26.3 ± 0.4         | 26.4 ± 0.5 26.4 ± 0.5          | 26.0 ± 0.3 24.8 ± 0.6 | 24.9 ± 0.3 24.1 ± 0.4 |
| 21.5 – 29.3         | 24.8 – 28.8                   | 23.8 – 29.8 23.8 – 29.4        | 24.4 – 27.4 20.8 – 28.2 | 23.6 – 26.7 21.4 – 25.8 |
| Tail length         | 4.6 ± 0.1 4.8 ± 0.1           | 4.5 ± 0.1 4.9 ± 0.1           | 4.3 ± 0.1 4.6 ± 0.1 | 4.2 ± 0.1 4.4 ± 0.1 |
| 3.9 – 5.3           | 4.6 – 5.2                     | 4.5 – 5.6 |                     |                     |
| Wet body mass       | 13.1 ± 0.8 13.7 ± 0.7         | 13.7 ± 0.7 14.0 ± 0.8          | 13.3 ± 0.7 11.4 ± 0.8 | 11.6 ± 0.6 11.3 ± 0.6 |
|                    | 9.7 – 17.3 10.5 – 17.2        | 10.6 – 17.6 10.7 – 18.5        | 9.2 – 16.0 6.2 – 14.8 | 9.0 – 14.7 8.0 – 14.9 |
| Dry body mass       | 3.42 ± 0.21 3.38 ± 0.21       | 3.43 ± 0.19 3.48 ± 0.17        | 3.28 ± 0.21 2.78 ± 0.20 | 2.92 ± 0.17 2.86 ± 0.18 |
|                    | 2.44 – 4.31                   | 2.40 – 4.63 2.68 – 4.38        | 2.52 – 4.15 |                     |                     |
| Carcass dry mass    | 1.87 ± 0.14 1.94 ± 0.11       | 1.89 ± 0.10 2.03 ± 0.10        | 1.79 ± 0.08 1.65 ± 0.11 | 1.63 ± 0.08 1.59 ± 0.08 |
|                    | 1.09 – 2.45                   | 1.48 – 2.44 1.37 – 2.53        | 1.41 – 2.50 |                     |                     |
| Residual yolk dry mass| 1.04 ± 0.09 0.94 ± 0.08       | 1.00 ± 0.08 0.91 ± 0.05        | 1.00 ± 0.12 0.68 ± 0.08 | 0.84 ± 0.09 0.83 ± 0.09 |
|                    | 0.66 – 1.55                   | 0.54 – 1.51 0.65 – 1.51        | 0.70 – 1.18 |                     |                     |
| Fat body dry mass   | 0.52 ± 0.05 0.50 ± 0.04       | 0.54 ± 0.04 0.54 ± 0.03        | 0.50 ± 0.03 0.46 ± 0.04 | 0.45 ± 0.03 0.44 ± 0.03 |
|                    | 0.31 – 0.76                   | 0.34 – 0.70 0.32 – 0.76        | 0.38 – 0.72 |                     |                     |
|                    |                                |                                | 0.30 – 0.66 0.21 – 0.64    | 0.35 – 0.63 0.33 – 0.66 |

All mass units are in g, and length units in cm.

Results

Table 1 shows descriptive statistics for size, mass, and composition of hatchlings from manipulated (miniaturized), sham-manipulated and control eggs. Eggs assigned to the 4 treatments did not differ from each other in mean mass at laying ($F_{3,80} = 0.05, P = 0.983$) or mean incubation length ($F_{3,80} = 1.39, P = 0.251$). We found that eggs producing male and female hatchlings did not differ in mean mass ($F_{1,80} = 0.40, P = 0.529$) or mean incubation length ($F_{1,80} = 0.03, P = 0.859$). We determined that the sex × treatment interaction was not a significant source of variation in egg mass at laying ($F_{3,80} = 0.41, P = 0.745$) or incubation length ($F_{3,80} = 0.80, P = 0.497$). Data pooled for the 4 treatments and sexes showed that incubation lengths ranged from 43.4 to 49.9 days, with a mean of 47.0 days.

None of the examined hatching traits differed among the 4 treatments after accounting for egg mass at laying (for control and sham-manipulated eggs) or after yolk removal (for manipulated eggs) ($P > 0.240$). The tail length was the only trait that differed between male and female hatchlings, with males having a longer tail than females ($F_{1,79} = 31.46, P < 0.0001$). The sex × treatment interaction was not a significant source of variation in all examined hatching traits ($P > 0.372$). The 10% reduction in egg mass resulted in production of hatchlings that were about 3% shorter in SVL, 8% lighter in wet mass and 10% lighter in dry mass. The 20% reduction in egg mass resulted in hatchlings that were about 7% shorter in SVL, 17% lighter in wet mass, and 16% lighter in dry mass.

Discussion

We found that yolk-reduced eggs produce smaller hatchlings that were shorter and lighter than controls. None of the examined hatching traits differed among the 4 treatments (manipulated [2], sham-manipulated [1] and control [1]) after accounting for yolk quantity at laying (for controls) or after yolk removal (for the manipulated treatments). Therefore, our results are consistent with the hypothesis that yolk-reduced eggs produce smaller hatchlings of which both yolk-free body mass and residual yolk are proportionally reduced (Figure 1C).

The proportions of yolk allocated to produce either larger hatchlings with smaller yolk reserves or smaller hatchlings with larger amounts of residual yolk vary considerably among studied vertebrate species and even between the sexes of the same clutch (Allsteadt and Lang 1995; Nagle et al. 2003; Gao et al. 2010; Spencer and Janzen 2014; Koláčková et al. 2015). There is also evidence that yolk partitioning in embryonic snakes is phylogenetically related or species-specific (Wu et al. 2017). For instance, the relative size of residual yolk is far smaller in the short-tailed pit viper *Gloydius breviceadus* (~3% of the body dry mass; Gao et al. 2010) than in the 5-paced pit viper *Deinagkistrodon acutus* (~33% of the body dry mass; Lin et al. 2005). While larger residual yolks provide sustenance for longer periods and perhaps support early growth better, larger hatchlings with smaller yolk reserves but more developed bodies are favored when resources are abundant or selection for high performance is strong (Booth 2000; Ji and Sun 2000; Radder et al. 2004, 2007; Van Dyke et al. 2011; Pezaro et al. 2013).

Natural selection for a given relative size of residual yolk is influenced by the feeding ability of newborn offspring or, probably, the period when they have a negative energy balance (Lance and Morafka 2001; Pezaro et al. 2013; Spencer and Janzen 2014). Here, we found that body size (both SVL and tail length) and mass were the only traits affected by experimental miniaturization of egg size. All other examined hatching traits including yolk-free dry body mass (carcass dry mass + fat body dry mass) and residual yolk dry mass were not affected by the manipulations of egg size after accounting for yolk quantity. Below, we summarize the main consequences of our findings on yolk partitioning strategies in *N. atra*.

*Naja atra* is not one of the species where the advantages of maximizing body size prior to hatching is favored over the advantages of maximizing residual yolk (see *I. iguana*; Troyer 1983). In these cases, yolk-reduced eggs are more likely to produce hatchlings with unchanged yolk-free body mass (Troyer 1983). *Naja atra* is also not
of yolk reserves to maximize body size at hatching.

Reproductive females of *N. atra* can adjust egg size in response to fluctuations in resource availability (Ji and Wang 2005) or clutch size (Ji et al. 2009). Based on our findings, the following prediction can be extrapolated to wild populations of *N. atra*. Changes in egg size may influence hatching linear size and mass, but not proportional amounts of major hatching components (including yolk reserves for post-hatching utilization). In fact, snakes cannot control the use or sequestration of yolk, although the residual yolk has a functional role in buffering the embryos from unpredictable environments and its quantity is affected by the environmental conditions, extremely hot and/or dry environments in particular, experienced during embryonic development (Lee et al. 2007; Van Dyke et al. 2011; Pezaro et al. 2013; Wu et al. 2017). For example, high incubation or gestation (for viviparous species) temperatures often result in the production of smaller offspring that characteristically have less developed bodies and contain more unutilized yolk in a diverse array of reptilian species (Packard et al. 1988; Booth and Astill 2001; Gao et al. 2010; Warner et al. 2012). This is also the case in *N. atra* (Ji and Du 2001; Lin et al. 2008). For a given snake species, the size of residual yolk is variable but such a variability does not have anything to do with yolk partitioning strategies.

Our study also shows that yolk removal does not affect the rate of embryonic development, substantiating an earlier conclusion that egg size is not a determinant of incubation length in *N. atra* (Ji and Du 2001; Lin et al. 2008; Ji et al. 2009). Overall, this has been shown in *S. undulates* (Warner and Andrews 2002; Storm and Angilletta 2007), the green anole lizard *Anolis carolinensis* (Goodman 2010), and the Mexican axolotl *Ambystoma mexicanum* (Brain and Landberg 2017). However, our findings are inconsistent with studies of *S. occidentalis* (Simerov 1990), *C. versicolor* (Radder et al. 2004; but see also Ji et al. 2002b) and different salamander species (e.g., *Ambystoma barbouri* and *A. maculatum*; Landberg 2014, 2015) where yolk removal actually induce fast embryonic development and thus early hatching.

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Authors’ Contributions

Y.-F.Q. and X.J. conceived and designed the experiments. X.J. supervised the study. Y.-F.Q., S.-Z.Z., X.-F.J., and L.-H.L. performed the experiments. Y.-F.Q. and X.J. analyzed the data. Y.-F.Q. and X.J. wrote the manuscript. All authors reviewed and contributed to editing of the manuscript and approved its final publication.

References

Alava JJ, Keller JM, Kucklick JR, Wynneken J, Crowder L et al., 2006. Loggerhead sea turtle *Caretta caretta* egg yolk concentrations of persistent organic pollutants and lipid increase during the last stage of embryonic development. *Sci Total Environ* 367:170–181.

Allsteadt J, Lang JW, 1995. Incubation temperature affects body size and energy reserves of hatching American alligators *Alligator mississippiensis*. *Physiol Zool* 68:76–97.

Booth DT, 2000. Incubation of eggs of the Australian broad-shelled turtle *Chelodina expansa* (Testudinata: chelidae) at different temperatures: effects on pattern of oxygen consumption and hatching morphology. *Austr J Zool* 48:369–378.

Booth DT, Astill K, 2001. Incubation temperature, energy expenditure and hatching size in the green turtle *Chelonia mydas*, a species with temperature-sensitive sex determination. *Austr J Zool* 49:389–396.

Brain C, Landberg T, 2017. Embryonic yolk removal affects neither morphological nor escape performance of larval axolotls. *Zoology* 122:7–15.

Carpentier AS, Booth DT, Arthur KE, Limpus CJ, 2013. Stable isotope relationships between mothers, eggs and hatchlings in loggerhead sea turtles *Caretta caretta*. *Mar Biol* 162:783–797.

Condon JD, Gibbons JW, 1989. Posthatching yolk reserves in hatching American alligators. *Herpetologica* 45:305–309.

Filoarmoni NI, Janzen EJ, 1999. Effects of hydric conditions during incubation on overwintering hatchlings of the red-eared slider turtle *Trachemys scripta elegans*. *J Herpetol* 33:29–35.

Gao JF, Qiu YF, Luo LG, Ji X, 2010. Evolution of reptilian viviparity: a test of the maternal manipulation hypothesis in a temperate snake, *Gloydias brevicaudus* (Viperidae). *Zool Sci* 27:248–255.

Goodman RM, 2010. Evidence of divergent growth rates among populations of the lizard *Anolis carolinensis*, based on experimental manipulations of egg size. *Popul Ecol* 52:113–122.

Gouiden CE, Henry L, Berrigan D, 1987. Egg size, postembryonic yolk, and survival ability. *Oecologia* 72:28–31.

Ji X, Du WG, 2001. Effects of thermal and hydric conditions on incubating eggs and hatching traits in the cobra *Naja naja atra*. *J Herpetol* 35:186–194.

Ji X, Du WG, Qu YF, Lin LH, 2009. Nonlinear continuum of egg size-number trade-offs in a snake: is egg-size variation fitness related? *Oecologia* 159:689–696.

Ji X, Huang HY, Hu XZ, Du WG, 2002a. Geographic variation in reproductive characteristics and egg incubation of *Eumeces chinensis*. *Chin J Appl Ecol* 13:680–684.

Ji X, Qiu QB, Dong CH, 2002b. Influence of incubation temperature on hatching success, embryonic use of energy, and size and morphology of hatchlings in the oriental garden lizard *Calotes versicolor* (Agamidae). *J Exp Zool* 292:649–659.

Ji X, Sun PY, 2000. Embryonic use of energy and post-hatching yolk in the gray rat snake *Ptyas korros* (Colubridae). *Herpetol J* 10:13–17.

Ji X, Sun PY, Fu SY, Zhang HS, 1997. Utilization of energy and nutrients in incubating eggs and post-hatching yolk in a colubrid snake *Elaphe carinata*. *Herpetol J* 7:7–12.

Ji X, Sun PY, Fu SY, Zhang HS, 1999. Utilization of egg energy and material during incubation and post-hatching yolk in a colubrid snake *Elaphe taeniuera*. *Asiat Herpetol Res* 8:53–59.

Ji X, Wang ZW, 2005. Geographic variation in reproductive traits and trade-offs between size and number of eggs of the Chinese cobra, *Naja atra*. *Biol J Linn Soc* 85:27–40.

Kamler E, Keckes H, Bauer-Nemeschkal E, 1998. Temperature-induced changes of survival, development and yolk partitioning in *Chondrostoma nasus*. *J Fish Biol* 53:658–682.

Kaplan RH, 1980. Ontogenetic energetics in *Ambystoma*. *Physiol Zool* 53:43–56.

Koláčková M, Prokůpková L, Albrecht T, Horák D, 2015. Incubation temperature influences trade-off between structural size and energy reserves in mallard hatchlings. *Physiol Biochem Zool* 88:1–10.

Lance VA, Morafka DJ, 2001. Postnatal lecithotroph: a new age class in the ontogeny of reptiles. *Herpetol Monogr* 15:124–134.
Landberg T, 2014. Embryonic yolk removal affects a suite of larval salamander life history traits. *J Exp Zool B* 322:45–53.

Landberg T, 2015. Evolution of maternal egg size effects in sister salamander species. *Int J Dev Biol* 58:908–916.

Lee TN, Plummer MV, Mills NE, 2007. Use of posthatching yolk and external forage to maximize early growth in *Apalone matica* hatchlings. *J Herpetol* 41:492–500.

Li H, Wang Z, Chen C, Ji X, 2012. Does the variance of incubation temperatures always constitute a selective force for the origin of reptilian viviparity? *Carr Zool* 58:812–819.

Lin LH, Li H, An H, Ji X, 2008. Do temperature fluctuations during incubation always play an important role in shaping the phenotype of hatching reptiles? *J Therm Biol* 33:193–199.

Lin LH, Mao F, Chen C, Ji X, 2012. Reproductive traits of the gray ratsnake *Ptyas korros* from three geographically distinct populations. *Carr Zool* 58:820–827.

Lin ZH, Ji X, Luo LG, Ma XM, 2005. Incubation temperature affects hatching success, embryonic expenditure of energy and hatching phenotypes of a prolonged egg-retaining snake *Deinagkistrodon acutus* (Viperidae). *J Therm Biol* 30:289–297.

Lu HL, Gao JF, Ma XH, Lin ZH, Ji X, 2012. Tail loss affects fecundity but not offspring traits in the Chinese skink, *Eumeces chinesis*. *Carr Zool* 58:228–235.

Nagle RD, Plummer MV, Congdon JD, Fischer FU, 2003. Parental investment, embryo growth, and hatching lipid reserves in softshell turtles *Apalone matica* from Arkansas. *Herpetologica* 59:145154.

Niewiarowski PH, Angilletta AM Jr, 2008. Countergradient variation in embryonic growth and development: do embryonic and juvenile performances trade off? *Funct Ecol* 22:895–901.

Orr LP, Maple WT, 1978. Competition avoidance mechanisms in salamander larvae of the genus *Desmognathus*. *Copeia* 1978:679–685.

Packard GC, Packard MJ, Miller K, Boardman TJ, 1988. Effects of temperature and moisture during incubation on carcass composition of hatching snapping turtles *Chelydra serpentina*. *J Comp Physiol B* 158:117–125.

Pandav BN, Shanbhag BA, Saidapur SK, 2006. Functional significance of posthatching residual yolk in the lizard *Calotes versicolor*. *J Herpetol* 40:385387.

Pezaro N, Doody JS, Green B, Thompson MB, 2013. Hatching and residual yolk internalization in lizards: evolution, function and fate of the amnion. *Evol Dev* 15:87–95.

Radder RS, Shanbhag BA, Saidapur SK, 2004. Yolk partitioning in embryos of the lizard *Calotes versicolor*: maximize body size or save energy for later use? *J Exp Zool A* 301:783–785.

Radder RS, Warner DA, Cuervo JJ, Shine R, 2007. The functional significance of residual yolk in hatching lizards *Amphibolurus muricatus* (Agamidae). *Funct Ecol* 21:302–309.

Sinervo B, 1990. The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* 44:279–294.

Sinervo B, McEdward LR, 1988. Developmental consequences of an evolutionary change in egg size: an experimental test. *Evolution* 42:885–899.

Spencer RJ, Janzen FJ, 2014. A novel hypothesis for the adaptive maintenance of environmental sex determination in a turtle. *Proc R Soc B* 281:20140831.

Storment MA, Angilletta AM Jr, 2007. Rapid assimilation of yolk enhances growth and development of lizard embryos from a cold environment. *J Exp Biol* 210:3415–3421.

Troyer K, 1983. Posthatching yolk energy in a lizard: utilization pattern and interclutch variation. *Oecologia* 58:340–344.

Troyer K, 1987. Posthatching yolk in a lizard: internalization and contribution to growth. *J Herpetol* 21:102–106.

Van Dyke JU, Plummer MV, Beaupre SJ, 2011. Residual yolk energetics and postnatal shell growth in smooth softshell turtles, *Apalone matica*. *Comp Biochem Physiol A* 158:302–309.

Warner DA, Andrews RM, 2002. Laboratory and field experiments identify sources of variation in phenotypes and survival of hatching lizards. *Biol J Linn Soc* 76:105–124.

Warner DA, Moody MA, Telemedco RS, Kolbe JJ, 2012. Egg environments have large effects on embryonic development, but have minimal consequences for hatching phenotypes in an invasive lizard. *Biol J Linn Soc* 105:25–41.

Warner DA, Shine R, 2007. Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia* 154:65–73.

Wu YQ, Qu YF, Wang XJ, Gao JF, Ji X, 2017. Does the oviparity-viviparity transition alter the partitioning of yolk in embryonic reptiles? *BMC Evol Biol* 17:235.