

The functional significance of residual yolk in hatchling lizards *Amphibolurus muricatus* (Agamidae)

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Summary

1. Although post-hatching parental care is uncommon in reptiles, reproducing females may none the less contribute to the nutritional state of their offspring by depositing more yolk into the egg than is needed for embryogenesis. This ‘extra’ yolk (i.e. residual yolk) is drawn into the offspring’s body prior to hatching and is widely assumed to serve as an energy resource for early life activities. However, empirical data on the functional significance of residual yolk are rare.

2. We surgically removed residual yolk from hatchling lizards *Amphibolurus muricatus* to evaluate its effects on offspring growth and survival over 4 weeks under two environmental conditions: low or high food abundance.

3. Unsurprisingly, higher food abundance enhanced growth rates of the young lizards. However, experimental removal of residual yolk did not affect any of the traits that we measured in either nutritionally harsh or benign post-hatching environments.

4. Overall, our results challenge the common assumption that residual yolk is an important source of energy during early life in lizards, and suggest instead that residual yolk is of trivial nutritional significance (especially, relative to prey availability). Residual yolk in *A. muricatus* may instead have a different (non-nutritive) function, or be a nonfunctional relict retained through phylogenetic conservatism from ancestral taxa.

Key-words: body size, endogenous nutrition, exogenous nutrition, growth, resource availability

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Introduction

The source of energy for newly born offspring is a critical component of life-history variation. Vertebrates provide energy to their neonates in a variety of ways, ranging from nutrients provided directly by the parents (as occurs in most birds and mammals) to the provision of energy stores within the egg at hatching (as seen in many fish, amphibians and reptiles: Mitchell 2001; Thompson & Speake 2004). Thus, parents can satisfy the energy requirements of their newly born offspring with either an exogenous source of energy (i.e. nutrients ingested directly by the offspring), an endogenous source (i.e. yolk reserves), or both. The source of energy provided to the offspring can affect the type or quality of nutrients available, with significant consequences for offspring growth, performance and, ultimately, fitness (Avery *et al.* 1993; Morafka, Spangenberg & Lance 2000).

The provision of endogenous sources of energy to offspring is widespread (perhaps ubiquitous) in oviparous

vertebrates, such as birds and most reptiles. In these taxa, mothers typically deposit more yolk into individual eggs than is needed to maintain embryogenesis. Prior to hatching, the portion of yolk that remains unutilized after completion of morphogenesis is drawn into the body of the offspring. This ‘residual yolk’ can serve as an energy source for the hatchlings during their early post-hatching life to support activity and growth (Kraemer & Bennett 1981; Troyer 1983; Murakami, Akiba & Horiguchi 1992; Turro *et al.* 1994; Tucker *et al.* 1998; Ar *et al.* 2004). In most birds, residual yolk is supplemented by exogenous nutrition that is provided by the parents throughout the time when offspring remain in the nest (and sometimes, for prolonged periods thereafter: Yoerg 1998). In contrast, parental care of post-hatching offspring is rare among reptiles, and is not known to involve any cases of direct supply of exogenous nutrients from parent to offspring (Shine 1988; Somma 2003). Hence, hatchling reptiles are completely reliant upon their residual yolk and/or their ability to capture prey in order to satisfy their energy demands immediately after hatching.

The amount of residual yolk available at hatching varies considerably among reptiles, not only among

lineages (Ar *et al.* 2004; Belinsky *et al.* 2004) but also among clutches within a single population (Troyer 1983; Rhen & Lang 1999). Even within a single clutch, minor variations in substrate moisture content (and hence, in water uptake by eggs) can strongly affect the quantity of yolk that remains at hatching. Relatively wet incubation conditions typically produce large-bodied hatchlings with small yolk reserves, whereas dry conditions produce small hatchlings with large yolk reserves (Packard & Packard 1988; Janzen *et al.* 1990; Packard 1991; Janzen, Ast & Paukstis 1995; Belinsky *et al.* 2004). In still drier conditions, desiccation of the yolk mass may preclude internalization by the embryo, so that the residual yolk remains attached to the eggshell and hence, is unavailable to the offspring (Aubret *et al.* 2003). Accordingly, the amount of residual yolk available to a hatchling reptile displays substantial variation both among and within species, so that the functional consequences of that variation warrant detailed examination.

Previous studies of residual yolk in oviparous vertebrates have dealt primarily with nutritional or physiological aspects (Kraemer & Bennett 1981; Troyer 1983; Tucker *et al.* 1998), rather than experimentally evaluating potential fitness benefits. None the less, a few studies have convincingly demonstrated the importance of residual yolk for hatchling growth in chickens (Murakami *et al.* 1992; Turro *et al.* 1994) and as an energy source during overwintering and hatchling migration in turtles (Kraemer & Bennet 1981; Nagle, Burke & Congdon 1998; Willette, Tucker & Janzen 2005). In addition, residual yolk contributes to growth in *Iguana iguana* (Troyer 1987), and may serve as an energy reserve for *Calotes versicolor* hatchlings when competition for food is intense (Radder, Shanbhag & Saidapur 2002). Despite these two latter studies on lizards, however, the general lack of research on fitness consequences of residual yolk in this group of reptiles is surprising because these animals offer excellent models for numerous reasons. First, they begin independent feeding and activity immediately after hatching. Secondly, hatchling lizards exhibit major intra- and interspecific variation in the relative amounts of residual yolk (Troyer 1983; Radder *et al.* 2002). Third, the quantity of residual yolk is highly sensitive to minor variations in the incubation environment (Janzen *et al.* 1990; Belinsky *et al.* 2004).

Intuition suggests that residual yolk is most likely to be important in the earliest stages of an animal's life, before it has the opportunity to gather significant energy resources from its own feeding behaviour. Similarly, it seems likely that the significance of endogenous energy reserves will depend upon the nutritional environment that the hatchling encounters early in life. Spatial and temporal variation in prey availability is substantial in many habitats, such that some cohorts encounter plentiful food whereas others do not (Ballinger 1977; Madsen & Shine 2000). Presumably, endogenous reserves may play a trivial role if prey is abundant, but be critical to offspring survival and growth if the young animals

have limited access to other energy reserves. The objective of our study was to test this idea.

To explore the functional significance of residual yolk for growth, performance and survival of hatchling lizards, we developed a surgical technique to experimentally manipulate the quantity of residual yolk in newly hatched lizards. We then raised these manipulated lizards and their control-treatment siblings under conditions that mimicked habitats with low or high food abundance. Thus, our experimental design allowed us to evaluate the degree to which growth and survival rates of hatchling lizards were affected by endogenous (residual yolk) reserves vs. exogenous (prey) sources of nutrition, as well as the interactive effects of these two sources of offspring nutrition.

Materials and methods

STUDY SPECIES

The jacky dragon *Amphibolurus muricatus*, is a medium-sized [to *c.* 100 mm snout-vent length (SVL)] agamid lizard that is abundant throughout coastal habitats of south-east Australia (Cogger 2000). This species provides an excellent model for addressing the above issues because: (1) jacky dragons are easily maintained in captivity and nest readily under seminatural conditions in outdoor enclosures, thus eggs and hatchlings can be easily obtained for experimental studies; (2) protocols for successful incubation and hatchling husbandry are well established (Warner & Shine 2005); and (3) the life history of this species has been well-studied (e.g. Harlow & Taylor 2000).

SOURCE OF HATCHLING LIZARDS

During October–December 2005, we obtained jacky dragon eggs from a captive colony of lizards housed in outdoor enclosures (for husbandry details see Warner & Shine 2005). In total, we obtained 107 eggs from 27 clutches (from 24 different females; thus, double clutches from the same female were used in this experiment in only three cases). Each egg was weighed within 48 h of oviposition and placed in an individual glass jar (125 mL) containing moist vermiculite (−200 kPa), which was covered with plastic foodwrap and sealed with a rubber band. Jars were placed in one of three incubators set at a constant 28 °C. Previous studies demonstrate that 28 °C yields high hatching success and also yields a 50 : 50 offspring sex ratio (jacky dragons have temperature-dependent sex determination: Harlow & Taylor 2000; Warner & Shine 2005). Jars were rotated within and among the incubators three times per week to minimize thermal differentials. Incubators were checked twice daily for hatchlings.

We obtained a total of 101 hatchlings from these eggs (thus, hatching success = 94.5%). On the day of hatching, offspring were weighed and measured (SVL and tail length), and sexed by manual eversion of

hemipenes (Harlow 1996). Each hatchling was marked with a unique combination of toe-clips. Hatchlings from each clutch were then assigned to one of six experimental treatments in a split-clutch design.

EXPERIMENTAL DESIGN

We used a 2×3 factorial design to evaluate the relative influence of endogenous (i.e. residual yolk) vs. exogenous nutrition (i.e. prey) on offspring growth, performance, and survival over a 1-month period post-hatching. Our experimental design involved three levels of yolk manipulation (yolk removal, sham control and procedural control), and two levels of food manipulation (low vs. high food availability). Descriptions of each experimental treatment are given below.

Treatment 1: Residual yolk absent & Low food availability

This treatment represents the lowest nutrient availability for hatchlings. To nondestructively remove residual yolk from the abdomen of hatchling lizards, we developed the following procedure. In newly hatched lizards, the connection between the yolk sac and intestine through the abdominal skin does not heal until about 3–4 days post-hatching. This abdominal fistula allows access to the residual yolk when creating a small incision next to the fistula. Prior to yolk removal, hatchlings were cooled in an ice-box until they became immobile. Once immobilized, all residual yolk was removed by inserting the tips of a pair of forceps through the abdominal fistula (after making a small incision). The abdominal opening from where the residual yolk was removed was then cleaned with 100% ethanol, dressed with anti-infection agents and tissue sealing liquid (Naxaband® tissue adhesive). Hatchlings were weighed before and after the residual yolk-removal procedure to quantify the amount of residual yolk that was removed. After this procedure, hatchlings in this treatment ($n = 22$) were offered crickets once per week. Because of this infrequent feeding regime, all crickets were eaten within the first 48 h of feeding (i.e. low food availability; more details on feeding are below).

Treatment 2: Residual yolk absent & High food abundance

Residual yolk was removed using the above procedures ($n = 16$), and these individuals were then offered crickets three times per week (on alternate days). Because of this high-frequency feeding regime, live crickets were always available within the enclosures.

Treatment 3: Residual yolk present (sham surgical control) & Low food abundance

Hatchlings assigned to this treatment ($n = 17$) experienced the same surgical procedure as described above,

but no residual yolk was removed. Similar to treatment 1, these hatchlings were fed crickets once per week (low food availability). Thus, hatchlings in this treatment served as a sham control for treatment 1 to determine if the yolk-removal procedure had any effect on the offspring.

Treatment 4: Residual yolk present (sham surgical control) & High food abundance

These hatchlings also experienced sham yolk removal (as for treatment 3; $n = 17$) and were then offered crickets three times per week. Thus, this group served as a sham control for treatment 2.

Treatment 5: Residual yolk present (no surgical procedures) & Low food abundance

No surgical procedures were performed on these hatchlings ($n = 16$) and all were fed crickets once per week.

Treatment 6: Residual yolk present (no surgical procedures) & High food abundance

Hatchlings in this treatment ($n = 13$) served as another experimental control; no surgical procedures were performed, and hatchlings were fed crickets three times per week.

HATCHLING HUSBANDRY

After surgery, all hatchlings were transferred to outdoor enclosures (30 cm high \times 36 cm wide \times 50 cm long). To avoid overcrowding, enclosures housed no more than five individuals at a given time (and all were from the same treatment). Each enclosure contained branches for perching, shelters for hiding, and water bowls. All enclosures were exposed to natural sunlight, photoperiod and temperature. The treatment groups were randomly assigned to pre-prepared enclosures and enclosures were rotated to avoid any confounding position or environmental effects. Hatchlings were offered small crickets dusted with calcium and vitamins; feeding frequency depended upon treatment (see above). All hatchlings were maintained under these conditions for 1 month. We measured morphological traits (SVL, tail length, body mass), locomotor performance and assessed survival status (live vs. dead) twice, at 2 and 4 weeks of age.

LOCOMOTOR PERFORMANCE

We measured locomotor performance of all hatchlings by chasing them along an electronically timed racetrack. The racetrack (1-m long) contained five infrared photocells, spaced at 25-cm intervals, connected to an electronic stopwatch. Prior to racing trials, hatchlings were given at least 30 min to acclimate to room temperature (32 °C). Hatchlings were placed at one end of the racetrack and encouraged to run by gently

prodding their tails with a paintbrush. Walls along the sides of the track prevented hatchlings from jumping out during racing trials. We recorded each 25-cm split time and the number of pauses hatchlings made when running over the entire 1-m distance. Each hatchling was raced three times with at least 10 min of rest between successive trials. For our analyses of locomotor performance, we only used the fastest 25-cm split to represent maximal sprinting performance.

DATA ANALYSES

Relationships among the quantity of residual yolk, egg size, and hatchling traits were analysed with correlation analyses. For correlation analyses we used data collected on the day of hatching (i.e. before assigning them to different feeding regimes) for hatchling's traits. In most analyses of hatchling body mass, we used the mass of individuals after the yolk was surgically removed. Preliminary analyses demonstrated that offspring sex had no effect on the quantity of residual yolk [unpaired *t*-test; $t = 1.4$, $P = 0.457$, d.f. = 37 (mean \pm SD) 0.075 ± 0.026 and 0.061 ± 0.021 g for males and females, respectively] or on any other hatchling trait that we measured (all $P > 0.100$). Thus, data for male and female offspring were combined for all further analyses.

To explore the relative importance of endogenous (yolk) vs. exogenous (food) sources of nutrition, we used a mixed model two factor analyses of covariance (ANCOVA) to evaluate the effects of our residual yolk manipulation, feeding regime, and their interaction (fixed effects) on offspring size, growth and locomotor performance (dependent variables). Egg mass was used as a covariate in our analyses of hatchling SVL and mass. SVL was used as a covariate for analyses of tail length and locomotor performance, as SVL has previously been shown to be a significant predictor of these variables (Warner & Shine 2006). Treatment effects on hatchling body condition were evaluated using body mass as the dependent variable and SVL as the covariate. Individual growth rates were calculated as the change in SVL or mass from hatching to 2 weeks and during the period from 2 weeks to 4 weeks of age divided by the number of days between measurements. To avoid pseudo-replication, clutch means for each trait were used in cases when more than one clutch-mate was allocated to the same treatment. Maternal identity was defined as a random effect in the above analyses. Random effects on offspring traits were evaluated with likelihood ratio tests (Littell *et al.* 1996). Treatment effects on offspring survival were evaluated with logistic regression. Statistical significance was accepted at $P = 0.05$. All analyses were performed using SAS software (SAS Institute 1997).

Results

All offspring contained measurable quantities of residual yolk at hatching. The amount of residual yolk

removed was averaged 6.21% of hatchling mass and 6.48% of egg mass at oviposition, with no significant correlation between egg mass and quantity of internalized yolk ($r = 0.06$, $P = 0.703$, $n = 38$). In addition, the amount of residual yolk was not significantly correlated with any aspect of hatchling morphology that we measured (vs. SVL: $r = 0.03$, $P = 0.853$, $n = 38$; vs. body mass: $r = 0.25$, $P = 0.135$, $n = 38$; vs. tail length: $r = 0.04$, $P = 0.833$, $n = 38$). The quantity of residual yolk (relative to hatchling mass) was not related to oviposition or hatching date ($r = -0.23$, $P = 0.172$, $n = 38$).

As expected, egg mass at oviposition was positively correlated with all morphological traits of hatchlings (as measured on the day of hatching, vs. SVL: $r = 0.51$, $P < 0.001$; vs. body mass: $r = 0.78$, $P < 0.001$; vs. tail length: $r = 0.54$, $P < 0.001$). Hatchling morphology did not differ significantly among treatments ($P > 0.156$ for all hatchling traits). However, after surgery, hatchlings with yolk removed were significantly lighter than those without yolk removed ($F_{1,100} = 7.8$, $P = 0.006$).

Hatchlings that were offered more food grew more rapidly and thus, averaged larger body sizes by two weeks of age (Fig. 1). In addition, hatchlings from the high food treatment exhibited better body condition (mass relative to SVL) at 2 weeks of age, compared with siblings from the low food treatment. However, neither the removal of residual yolk, nor our surgical

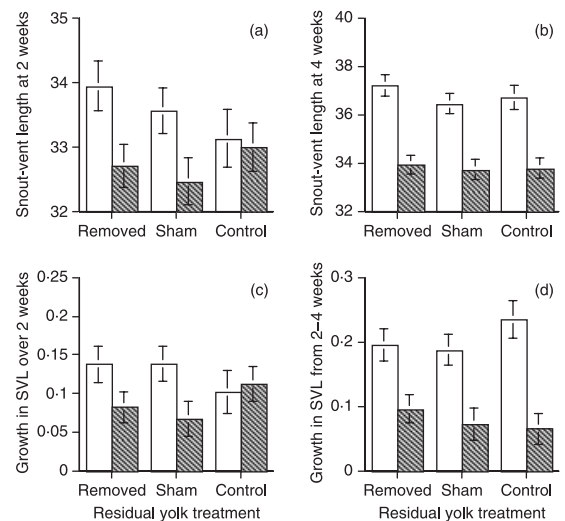


Fig. 1. Effect of residual yolk and feeding regime on snout-vent length (SVL) and growth rate of hatchling jacky dragons at 2 and 4 weeks of age. Open bars represent the high feeding treatment. Hatched bars represent the low feeding treatment. (a) SVL of hatchlings at 2 weeks of age (bars represent least-squares means adjusted for egg mass). (b) SVL of hatchling at 4 weeks of age (bars represent least-squares means adjusted for egg mass). (c) Growth rate in SVL over the first 2 weeks after hatching (bars represent mean values). (d) Growth rate in SVL over the 2–4-week period (bars represent mean values). Error bars represent 1 SE. Statistical results are in Table 1. Similar patterns were observed with respect to hatchling mass.

procedures *per se*, had any significant effect on the body size of hatchlings at 2 weeks of age despite a significant effect on growth rate in mass (Table 1, Fig. 1); in contrast to our a priori prediction, however, hatchlings from the yolk-removed treatment grew faster than did the hatchlings in the control treatments. Feeding treatment did not modify the (non)effect of residual yolk on offspring morphology at this time, as indicated by nonsignificant interaction terms (Table 1). Locomotor performance was not significantly influenced either by yolk removal or by prey availability, and was not dependent upon offspring body size ($P = 0.987$). Hatchling running speed was highly repeatable (fast runners remained fast, slow runners remained slow) as indicated by a positive correlation between speed at 2 and 4 weeks of age ($r^2 = 0.15$, $P < 0.001$). Clutch (maternal) effects contributed significantly to variation in most phenotypic traits of hatchlings (Table 1).

In general, the patterns evident at 2 weeks of age were also seen when the young lizards were retested at 4 weeks of age (Table 1, Fig. 1). As before, our treatments significantly affected rates of growth in mass over 2–4 weeks of age; however, growth rate differed only between hatchlings from the sham control vs. the procedural control. Thus, overall, residual yolk had no impact on any morphological or performance trait, but feeding regime strongly influenced the growth rate

(and thus body size) of the lizards. Interestingly, the impact of feeding regime on hatchling condition had disappeared by 4 weeks of age. Clutch effects on offspring phenotypes at 4 weeks of age were similar to those observed earlier (Table 1).

Offspring survival was high overall (88.1%), and was not influenced by yolk removal ($\chi^2 = 0.9$, $P = 0.634$), feeding regime ($\chi^2 = 0.1$, $P = 0.772$), or their interaction ($\chi^2 = 0.9$, $P = 0.641$). This pattern was consistent at 2 and 4 weeks of age.

Discussion

Published discussions of residual yolk in hatchling reptiles typically have accepted the (highly plausible) assumption that residual yolk is an important source of nutrition during the first few weeks post-hatching (Congdon, Tinkle & Rosen 1983; Congdon & Gibbons 1989; Morafka *et al.* 2000; Ar *et al.* 2004). Contrary to this common assumption, however, our experiment demonstrated that the role of residual yolk is trivial compared with that of exogenous sources of nutrition in hatchling jacky dragons. Surprisingly, the quantity of residual yolk had only a minimal impact on growth in mass, and our impression is that this statistically significant effect may well be spurious. For example: (1) unlike growth in mass, growth in SVL was not affected by yolk removal; (2) contrary to prediction,

Table 1. Effects of feeding regime, yolk removal, and their interaction on morphology, growth and locomotor performance of hatchling jacky dragons at 2 and 4 weeks of age. Analyses were carried out with mixed model two-factor ANOVAs using maternal identity as a random effect. Random effects (i.e. maternal identity effects) were evaluated with likelihood ratio tests (Littell *et al.* 1996). *P*-values in bold face type are statistically significant

Phenotype	Covariate	Feeding effect	Yolk removal effect	Interaction	Maternal effect
SVL (mm)					
At 2 weeks	Egg mass	$F_{1,62} = 8.7$, $P = 0.005$	$F_{2,62} = 0.5$, $P = 0.600$	$F_{2,62} = 1.4$, $P = 0.260$	$\chi^2 = 4.4$, $P = 0.036$
At 4 weeks	Egg mass	$F_{1,59} = 83.8$, $P < 0.001$	$F_{2,59} = 0.9$, $P = 0.434$	$F_{2,59} = 0.3$, $P = 0.780$	$\chi^2 = 5.3$, $P = 0.021$
Body mass (g)					
At 2 weeks	Egg mass	$F_{1,62} = 17.6$, $P < 0.001$	$F_{2,62} = 2.2$, $P = 0.121$	$F_{2,62} = 2.4$, $P = 0.100$	$\chi^2 = 12.0$, $P < 0.001$
At 4 weeks	Egg mass	$F_{1,59} = 77.1$, $P < 0.001$	$F_{2,59} = 0.7$, $P = 0.482$	$F_{2,59} = 0.5$, $P = 0.625$	$\chi^2 = 0.7$, $P = 0.403$
Tail length (mm)					
At 2 weeks	SVL	$F_{1,62} = 0.5$, $P = 0.507$	$F_{2,62} = 2.0$, $P = 0.148$	$F_{2,62} = 1.0$, $P = 0.392$	$\chi^2 = 42.8$, $P < 0.001$
At 4 weeks	SVL	$F_{1,59} = 1.4$, $P = 0.239$	$F_{2,59} = 2.2$, $P = 0.126$	$F_{2,59} = 1.7$, $P = 0.199$	$\chi^2 = 25.4$, $P < 0.001$
Condition (mass, g, relative to SVL, mm)					
At 2 weeks	SVL	$F_{1,62} = 5.9$, $P = 0.018$	$F_{2,62} = 2.1$, $P = 0.135$	$F_{2,62} = 1.1$, $P = 0.328$	$\chi^2 = 26.5$, $P < 0.001$
At 4 weeks	SVL	$F_{1,59} = 2.6$, $P = 0.111$	$F_{2,59} = 1.0$, $P = 0.384$	$F_{2,59} = 0.6$, $P = 0.569$	$\chi^2 = 12.8$, $P < 0.001$
Growth in SVL (Δ SVL day ⁻¹)					
Over 2 weeks	–	$F_{1,62} = 4.7$, $P = 0.0344$	$F_{2,62} = 0.1$, $P = 0.929$	$F_{2,62} = 1.8$, $P = 0.177$	$\chi^2 = 2.1$, $P = 0.147$
From 2 to 4 weeks	–	$F_{1,57} = 45.1$, $P < 0.001$	$F_{2,57} = 0.4$, $P = 0.676$	$F_{2,57} = 1.1$, $P = 0.327$	$\chi^2 = 3.1$, $P = 0.078$
Growth in mass (Δ mass day ⁻¹)					
Over 2 weeks	–	$F_{1,62} = 14.7$, $P < 0.001$	$F_{2,62} = 3.8$, $P = 0.028$	$F_{2,62} = 0.3$, $P = 0.763$	$\chi^2 = 5.0$, $P = 0.025$
From 2 to 4 weeks	–	$F_{1,57} = 72.8$, $P < 0.001$	$F_{2,57} = 3.9$, $P = 0.027$	$F_{2,57} = 1.3$, $P = 0.292$	$\chi^2 = 6.3$, $P = 0.012$
Running speed (m s ⁻¹)					
At 2 weeks	SVL	$F_{1,61} = 0.8$, $P = 0.374$	$F_{2,61} = 2.4$, $P = 0.104$	$F_{2,61} = 0.2$, $P = 0.830$	$\chi^2 = 13.3$, $P < 0.001$
At 4 weeks	SVL	$F_{1,55} = 0.5$, $P = 0.495$	$F_{2,55} = 0.6$, $P = 0.554$	$F_{2,55} = 0.0$, $P = 0.987$	$\chi^2 = 7.0$, $P = 0.008$

SVL, snout-vent length.

hatchlings from the yolk-removed treatment grew faster not slower than their nonmanipulated siblings; (3) the difference in growth rate was not great enough to influence body mass at 2 or 4 weeks of age; (4) treatment differences in growth were not consistent over the first two weeks vs. the final 2 weeks; and (5) significant effects disappeared when analyses were corrected for multiple comparisons (Rice 1989). Thus, overall, residual yolk had little to no impact on hatchling growth even when very little exogenous energy was available to the hatchlings. This pattern was persistent up to 1 month post-hatching and we suspect that the effect would persist at least up to the onset of winter. Despite the strong effect of exogenous energy sources on growth and body size, hatchling locomotor performance and survival were not influenced by our manipulations. Clutch identity was another significant contributor to variation in the phenotypes that we measured. Thus, variation in offspring traits is due both to environmental conditions experienced by hatchlings (i.e. food availability) and maternal factors (perhaps genetic), but not by the quantity of residual yolk.

These patterns have important implications for maternal and offspring fitness. Our results suggest that mothers can do relatively little to enhance growth of their offspring by depositing more yolk into eggs than is needed for embryogenesis. Thus, maternal allocation of residual yolk appears to be of little importance for the traits that we measured. In contrast, offspring foraging ability and prey abundance will have direct consequences on hatchling growth, and hence body size at the onset of winter. Such an effect on body size could impact the likelihood of reproduction in the following spring (Olsson & Madsen 1998; Warner & Shine 2005). As jacky dragons can attain sexual maturity within 1 year of hatching (depending upon how early they hatch and how rapidly they grow: Warner & Shine 2005), any factor that enhances growth, such as food availability or maternal effects (other than yolk allocation), may have significant consequences for life-time fitness.

Overall, the nonsignificant effect of residual yolk on hatchling growth seen in our study runs counter to traditional perspectives on this issue, and to the conclusions of previous studies. Perhaps these contrasting results reflect the fact that most such studies have been conducted on species with very different life-histories than jacky dragons. For example, studies of turtles convincingly demonstrate the importance of residual yolk for providing nutrition during the extended periods of time that hatchlings spend within nests (sometimes over the entire winter season) prior to migrating to aquatic habitats (Congdon *et al.* 1983; Nagle *et al.* 1998; Willette *et al.* 2005). In addition, studies of green iguanas demonstrate that residual yolk influences hatchling growth (Troyer 1987). However, green iguanas differ from jacky dragons in that iguana hatchlings cannot break down their usual (plant-based) food until they have ingested faeces from other iguanas to obtain the gut microbes necessary for their herbivorous

diet (Troyer 1984). Thus, residual yolk during early life in these animals may be beneficial by providing them with energy prior to the time when they are themselves capable of digesting exogenous food. Also, the amount of residual yolk relative to body mass is much greater in hatchling iguanas (up to 17% of body mass) than in jacky dragons (6%). Jacky dragons often begin feeding on insects within 24 h of hatching, and thus such a small quantity of residual yolk may make very little difference to their energy budgets. That is, the advantage of having such yolk reserves may be outweighed by consuming even one or two insects.

The conclusion that offspring growth rates are influenced less by residual yolk than by post-hatching prey availability may depend upon the amounts of food provided in our study. For example, if the amount of food offered during our experiment vastly exceeded that usually available to young lizards in the field, then our results might inflate the significance of food supply relative to residual yolk effects in the field. That is, residual yolk may be significant in the field because feeding rates are generally low at that time; and we failed to detect this effect by providing much greater-than-normal amounts of food. However, our field data on growth rates from mark-recapture studies on free-ranging lizards (D. Warner and R. Shine, unpubl. data) argue against this hypothesis: growth rates of hatchlings measured under natural field conditions were similar to those from our high-feeding treatment, and much higher than those of captive hatchlings from the low-feeding treatments. This pattern suggests that prey availability in the low-feeding treatments was lower than is typically encountered under natural field conditions. Thus, if residual yolk is important for growth, especially when hatchlings are exposed to low exogenous energy, our experiment should have detected an effect of residual yolk on growth rates.

If yolk reserves are unimportant, why don't jacky dragon embryos utilize all their yolk prior to hatching? One possibility is that mothers cannot predict or control the exact amount of energy needed for embryogenesis, and thus provide slightly more than is likely to be needed to carry the embryo through to full-term. If this is the case, then residual yolk may have no specific function (at least for hatchling growth). Contrary to this hypothesis, however, experimental removal of yolk from *Calotes versicolor* eggs immediately after oviposition did not reduce the amount of residual yolk at hatching (Radder, Shanbhag & Saidapur 2004). If residual yolk was simply a consequence of poor maternal control over yolk allocation to the eggs, then we would not expect to see internalised yolk in hatchlings from eggs subject to post-oviposition yolk removal. A second possibility is that residual yolk in jacky dragons may be a nonfunctional consequence of phylogenetic constraints. Indeed, internalized residual yolk characterizes offspring of all or nearly all reptile lineages (birds included) (Ar *et al.* 2004). Third, residual yolk may be important for some trait that we did not measure in

our experiment. For example, residual yolk may provide hatchlings with the energy necessary for digging out of nests, as suggested for turtles (Kraemer & Bennett 1981); or the residual yolk may act as a 'plug' to prevent infection penetrating through the umbilical opening into the hatchling's peritoneal cavity. Fourth, residual yolk may enhance survival rather than growth *per se* in the critical first few days until the offspring find natural food sources. Contrary to this hypothesis, however, we did not find any significant difference in the survival rates of *A. muricatus* offspring between yolk-removed and control groups either at high or low food treatments.

Overall, we predict that the functional significance of residual yolk may vary considerably among reptiles. In keeping with this hypothesis, living reptiles display a remarkable range of variation in the quantity of neonatal residual yolk relative to body size. Some small species of lizards contain virtually no residual yolk at hatching, whereas residual yolk in crocodylians and some large-bodied lizards may comprise up to 17% of hatchling body mass (Ar *et al.* 2004; Belinsky *et al.* 2004; Thompson & Speake 2004). Because yolk is rich in energy, researchers have often assumed that it serves as significant reserve energy during early life. Although this may well be true in some reptile species, our data suggest that residual yolk plays a trivial role, at best, in the energy balance of hatchling jacky dragons. Clearly, this type of functional significance of residual yolk can no longer be assumed, and we need experimental studies on a broad variety of taxa to detect general patterns. The ease and simplicity of our yolk-removal method suggests that such studies should be entirely feasible.

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