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Residual yolk energetics and postnatal shell growth in Smooth Softshell Turtles, *Apalone mutica*

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ABSTRACT

We examined functions of residual yolk (RY[1]) in hatchling Smooth Softshell Turtles (*Apalone mutica*). Removal of RY did not affect survival, shell growth, or resting metabolic rates of turtles for 40 d after hatching. Our estimates of metabolic rate suggest that RY can fuel maintenance and activity metabolism for approximately 25 days. *A. mutica* absorb more than 1 g of water in the first 2 weeks of life, which appears to be the basis of post-hatch shell expansion rather than yolk-provisioned growth. Post-hatch growth may be limited by the magnitude of RY remaining at hatching, but RY protein and lipid proportions do not differ from those of freshly-laid eggs. In addition, *A. mutica* did not use RY to fuel nest emergence. Our results suggest that RY does not fulfill several hypothetical functions in *A. mutica*, including postnatal growth, catabolic fuel for nest emergence, and long-term nutritional sustenance for maintenance, activity, or hibernation. Instead, *A. mutica* appear to absorb most yolk prior to hatching, and are left with a minimum of RY. Variation in RY mass with incubation regime in other species suggests that mothers may overprovision their eggs to ensure successful development across a diversity of possible incubation conditions.

1. Introduction

Offspring survival is a key component of both population dynamics and individual parental fitness (Cole, 1954). Offspring traits often correlated with survival, including size, growth rate, and locomotor performance, are constrained by the magnitude of parental provisioning to each offspring (Sinervo, 1990; Sinervo and Huey, 1990; Sinervo et al., 1992; Sinervo, 1993). The magnitude of parental provisioning per offspring encompasses a trade-off between offspring size and offspring number (Smith and Fretwell, 1974; Sinervo and Licht, 1991), so the consequences of parental allocation include not only the "quality" of individual offspring, but the number of offspring. As a result, mechanisms of parental allocation to offspring have direct implications for the fitness of organisms.

Post-embryogenic provisioning is presumed to be especially important in species that continue development, or are restricted from foraging, as neonates. In reptiles, post-embryonic provisioning is provided by residual yolk (Cagle, 1950; Ernst, 1971), a portion of the ovum yolk body which is not absorbed during embryonic development. Reptilian residual yolk is composed of lipid and amino acid fractions left over after embryogenesis (Congdon et al., 1983; Speake and Thompson, 2000; Nagle et al., 2003; Speake et al., 2003). Consumption of these metabolic substrates likely follows the pattern suggested for most energy budgets, in which maintenance metabolism, Specific Dynamic Action (SDA), activity, allocation to biomass, and biomass production compete for available resources (Congdon et al., 1982). While the structure and biochemical composition of residual yolk have been well-studied in some reptiles (Nagle et al., 2003; Speake et al., 2003), few studies have examined the degree to which residual yolk composition might constrain allocation to competing functions. Theoretically, residual yolks rich in fat content might better support post-hatch activity or maintenance metabolism, while those rich in protein content might better support post-hatch growth (Congdon et al., 1983; Speake and Thompson, 2000; Thompson et al., 2001; Speake et al., 2003; Thompson and Speake, 2003).

Yolkectomy has become a standard tool for manipulating the amount of yolk available to an embryo or neonate organism (Sinervo, 1990). Though methods vary, yolkectomy generally involves a minimally invasive removal of some subset of yolk, after which the embryo or neonate is allowed to develop or behave normally. Yolkectomy of freshly-laid eggs reduces hatchling body size in several lizard species (Sinervo, 1990; Sinervo et al., 1992; Sinervo, 1993; Radder et al., 2004), but does not reduce residual yolk mass in hatchling Oriental Garden Lizards, *Calotes versicolor*, suggesting that residual yolk may be critical to survival (Radder et al., 2004). Post-hatch experimental reductions of residual yolk resulted in reduced post-hatch growth in neonate Green Iguanas, *Iguana iguana* (Troyer, 1987) and Yellow-Bellied Sliders, *Trachemys scripta* (Yeomans, 1999),

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[1] RY: residual yolk.

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but not in Jacky Dragons, *Amphibolurus muricatus* (Radder et al., 2007) or Domestic Chickens, *Gallus gallus* (Murakami et al., 1992; Turro et al., 1994). Hatchling Red-Eared Sliders, *Trachemys scripta*, use residual yolk to continue growth after hatching while overwintering inside their nests (Filoramo and Janzen, 1999), and could use it as a metabolic fuel for maintenance metabolism as well (Cagle, 1950; Gibbons and Nelson, 1978). However, the residual yolks of hatchling Painted Turtles, *Chrysemys picta* and Common Snapping Turtles, *Chelydra serpentina* contained too little calcium to support the growth of ossified skeletal tissues (Packard and Packard, 1986, 1989).

Hatchling Loggerhead Sea Turtles, *Caretta caretta*, consume up to half of their residual yolk dry mass during emergence from the nest (Kraemer and Bennett, 1981), which is a troubling result because most laboratory studies of hatchling growth and post-embryonic nutrition incubate unburied eggs and thus ignore potential metabolic costs of nest emergence. Consumption and catabolism of residual yolk have also been hypothesized to contribute to post-partum elevated metabolic rates in neonate Timber Rattlesnakes, *Crotalus horridus* (Beaupre and Zaidan, 2001). Hatchling Smooth Softshell Turtles, *Apalone mutica*, have been hypothesized to use residual yolk reserves to fuel maintenance and activity metabolic costs in habitats of low prey abundance (Nagle et al., 2003), but even these reserves are completely consumed before winter (T.N. Lee et al., 2007). Morris et al. (1983) and Finkler et al. (2002) found that wetter incubation conditions produce larger offspring and smaller residual yolks, *C. serpentina*. In addition, residual yolks of wet-incubated *C. serpentina* and Ornate Box Turtles, *Terrapene ornata* contain less lipid and protein than those incubated in drier conditions (Packard et al., 1985, 1988; Janzen et al., 1990). Thus, residual yolk may be a by-product of mothers allocating a maximum amount of yolk to sustain offspring development across variable incubation conditions, and may not always be a factor necessary to post-hatch survival. The lack of residual yolk in neonate skinks of several Australian genera (Speake and Thompson, 2000; Thompson et al., 2001) supports this hypothesis.

While most investigations have studied the hypothetical uses of residual yolk piecemeal, few have examined multiple uses in a single species. Here, we report an investigation of residual yolk dynamics and postnatal growth in unfed hatchling *A. mutica*. We yolkectomized freshly-hatched turtles to examine two primary questions: first, whether or not *A. mutica* allocate residual yolk to growth, and second, whether or not residual yolk absorption and catabolism incur a metabolic cost analogous to SDA (Beaupre and Zaidan, 2001). We also estimated metabolic rates of developing eggs in order to determine whether egg and neonate metabolic rates followed an altricial or precocial pattern of increase (Vleck et al., 1980; Hoyt, 1987; Whitehead and Seymour, 1990).

After post-hatch yolkectomy, all hatchling turtles grew in shell size and live mass at similar rates, regardless of treatment and despite not being fed. Because post-hatch yolkectomy did not affect post-hatch growth, we hypothesized that yolk protein may be disproportionately consumed during development and would be less abundant in residual yolk than in the yolk of freshly laid eggs. Therefore, we compared residual yolk crude protein and crude fat contents to those of freshly laid eggs to determine if hatchling growth or activity could be functionally constrained by substrate availability in residual yolk. Residual yolk calcium deficiencies have been hypothesized to constrain growth in hatchling *C. serpentina* due to the ossification necessary for shell growth (Packard and Packard, 1989). We did not investigate this effect because *A. mutica* does not have a heavily ossified shell, so calcium deficiencies should be a less relevant constraint of shell growth.

Because all hatchling turtles in the yolkectomy experiment grew in shell dimensions and wet mass, despite not being fed and regardless of treatment, we also compared wet masses of fresh hatchling turtles to those at 2 weeks of age to determine if water uptake contributed to apparent growth in live mass and shell dimensions immediately after hatching. Finally, we determined whether residual yolk served as a significant energy source for metabolic costs of nest emergence by comparing yolk dry mass between fresh hatchlings and those emerging from artificial nests.

2. Materials and methods

2.1. Egg collection and incubation

Freshly-laid *A. mutica* eggs were collected from 10 to 20 June in 2006 (8 clutches), 2008 (9 clutches), and 2009 (12 clutches), from nests constructed on sandbars of the White River near Georgetown, White County, Arkansas, USA. Mean clutch size ± SE was 11.6 ± 1.67 in 2006, 13.1 ± 1.51 in 2008, and 14.4 ± 0.91 in 2009. Mean egg mass ± SE was 9.26 ± 0.08 in 2006, 8.98 ± 0.12 in 2008, and 8.52 ± 0.07 in 2009.

All eggs were individually marked with clutch and egg numbers and were packed in moist sand for transport to the laboratory at the University of Arkansas. All eggs were weighed to the nearest 0.01 g (Sartorius, model BP3100S, Goettingen, Germany) and half-buried in 600 g of a 1:1 vermiculite/water mixture in a covered 70 × 200 × 270 mm (H × W × L) plastic tray. Up to 20 eggs were placed in each tray. Water potential of this mixture was approximately −200 kPa (Plummer and Snell, 1988), and was maintained by periodically weighing trays and replacing evaporated water. Eggs in each tray were incubated at 29 °C in Hovobator incubators (GFQ Corporation, Savannah, GA, USA). Two trays could be incubated in each incubator, and clutches were always maintained together in single incubators to control for the combined effects of maternal allocation and maternal nest selection. Because this procedure confounds clutch and incubator effects, all further references to "clutch effects" should be interpreted to include incubator effects as well.

When pipping began, wire cages were placed over eggs to contain hatchlings for identification. Post-hatching disposition of each individual was dependent on the specific question under consideration and random treatment assignment. Eggs that did not hatch were discarded. Unless otherwise noted, all hatchling turtles were maintained in 740 mL plastic containers filled with 400 mL of water. Water was changed twice per week and turtles were not fed for the duration of each experiment. Temperature was maintained at 27.5 °C, and there was a 12:12 light/dark cycle. Mean hatchling mass ± SE was 6.80 ± 0.06 in 2006, 5.77 ± 0.09 in 2008, and 5.04 ± 0.05 in 2009.

2.2. Egg metabolic rate and yolkectomy effects on hatchling metabolic rate and growth

In 2006, egg metabolic rates were estimated every 7–10 days using open-flow respirometry of CO_2 production. In late July, 62 of 93 eggs hatched from 8 clutches. All hatchling turtles were assigned to one of three treatments, yolkectomy ($n = 18$), sham ($n = 21$), and control ($n = 23$). In six clutches, at least 9 turtles hatched, so at least three could be assigned to each treatment from each clutch, and all clutches were represented in all treatments. Control turtles were not manipulated in any way. Because *A. mutica* hatchlings pip and often emerge in the laboratory before residual yolk is fully retracted into the body cavity, yolkectomy was performed by cutting a small incision into the protruding yolk extra-embryonic membrane. Next, all yolk was squeezed out of the yolk sac by manually applying gentle pressure to the external yolk membrane. Finally, the empty yolk sac membrane and surrounding epidermis were gently pushed and folded into the yolk scar. The vitelline vein was avoided as much as possible during incisions, but five turtles visibly bled after yolkectomy and did not survive 24 h after the procedure. All 13 surviving turtles developed no apparent side effects of the procedure during the study. Mean wet mass of removed residual yolk ± SE was 0.65 ± 0.15 g. Extra-embryonic yolk membranes of sham turtles were incised identically to those of yolkectomized turtles, but no yolk was removed.

Immediately after manipulations, and at 10-day intervals for 40 days, all turtles were weighed to the nearest 0.01 g. Carapace and

plastron lengths and widths were measured with digital calipers to the nearest 0.01 mm on the same schedule. After measurements were recorded, turtle CO_2 production rates were estimated using open-flow respirometry. The study was conducted for 40 days because *A. mutica* hatchlings have been predicted to completely exhaust their residual yolk over that time (T.N. Lee et al., 2007).

Carbon dioxide production was measured following the methods of Beaupre and Zaidan (2001) and Zaidan and Beaupre (2003), with minor modification, using a Sable System TR-3 open-flow system. Eggs were placed in 125 mL respirometry chambers with approximately 50 g of sterile sand to maintain stability. Hatchling turtles were placed in 125 mL respirometry chambers with approximately 25 mL of water to avoid desiccation. Incurrent air from an 80-psi line was scrubbed of CO_2 and water with a Whatman purge gas generator (model FT-IR 75-45 purge-gas generator, Whatman, Haverhill, MA). Clean air was then split into eight equal flows with a Sable System MF-8 airflow manifold (Sable Systems, Las Vegas, NV, USA). Flow rates in lines were matched to 200 ± 10 mL min^{-1} using a Sable System mass flow-meter and needle valves for each line on the MF-8 manifold. All but the first of the eight flow-matched lines were connected to one port of the seven respirometry chambers. No eggs or turtles were measured in the first line, so that it could serve as a reference for baselining. A separate line carried excurrent gas from each chamber to a syringe barrel for subsampling.

Subsampling was controlled by a Sable System eight-channel multiplexer that cycled through all seven chambers sequentially, once per hour. The unoccupied baseline line was measured for 3.3 min at the beginning and end of each sampling sequence to compensate for baseline drift. Each chamber was sampled for 6.7 min in each sampling sequence. In total, a sampling sequence of seven chambers and baseline lasted 1 h. This allowed the measurement of CO_2 production of 7 turtles or eggs per hour. Eggs were only measured during a single sampling sequence (1 h) because CO_2 production was invariant within a 6.7 min sampling period, and no circadian differences were observed. Turtles were measured over 20 sampling sequences (20 h).

Subsampled gas was drawn at a flow rate of 90 mL min^{-1} by negative pressure through two 30-mL vials of Drierite to remove all water. Samples were then drawn into a Li-Cor CO_2 infrared gas analyzer (IRGA, model LI 6251, Li-Cor, Lincoln, NE, USA). All gas-flow connections were fabricated with low-permeability Pharmed NSF-51 tubing. Data from the IRGA were downloaded through the Sable System Universal Interface and DATACAN V software (Sable Systems 1991). During measurements, time (h:min:s) and CO_2 concentration (ppm) were stored to disk every hour. Flow rate used in $\dot{V}CO_2$ calculation was the excurrent flow rate through respirometry chambers. Temperature was maintained by placing respirometry chambers in a Percival environmental chamber. Temperature was set to 27.5 °C and light was provided at 12 L:12D beginning at 0700 h CST. We used a thermocouple to continuously monitor the temperature inside the Percival environmental chamber, and the chamber was turned on at least 1 h prior to measurement to ensure temperature stability. The Li-Cor CO_2 infrared gas analyzer was calibrated daily at two points with the CO_2-free incurrent air and a 500 ppm CO_2 span gas standard.

Carbon dioxide ppm was recorded every 5 s during each sampling period. Sable Systems DATACAN V software adjusted the data for baseline. The data were processed with a QuickBasic (Microsoft, 1989) program designed to associate values in the output data file with appropriate individual variables and calculate hourly averages of CO_2 concentration (ppm) for each turtle. Hourly CO_2 concentrations were then used to calculate hourly CO_2 production rates (mL h^{-1}) using the following equation:

$$\dot{V}CO_2 = (f_e - f_i) \times FR \times 60$$

Where $\dot{V}CO_2$ is in milliliters per hour, f_e is the fractional concentration of CO_2 in the chamber excurrent line, f_i is the fractional concentration of CO_2 in the chamber incurrent line, FR is the flow rate in milliliters per minute, and the factor 60 converts data to hourly rates. Values for $\dot{V}CO_2$ are reported at standard temperature and pressure.

Because turtles can limit apparent gas exchange rates by not breathing for long periods of time, we did not calculate resting CO_2 production rates by averaging only the lowest hourly $\dot{V}CO_2$ values from the entire sample sequence. Instead, we discounted the first 3 h as a period for acclimation to respirometry conditions, and averaged all subsequent nightly (19:00 h to 7:00 h) hourly $\dot{V}CO_2$ values. The final grand average $\dot{V}CO_2$ is reported as the individual turtle's resting CO_2 production rate for that specific sampling period.

Growth in mass and gas exchange data were analyzed using repeated-measures analysis of covariance (rANCOVA) in SAS PROC MIXED (SAS Institute, Cary, NC, USA). Gas exchange data were $\log_{10}$-transformed and analyzed with $\log_{10}$-transformed hatchling mass as the covariate, and clutch was included in the model as a random effect. Mass was $\log_{10}$-transformed and analyzed using rANCOVA using $\log_{10}$- initial mass (pre-yolkectomy) as the covariate. Log transformation of gas exchange and mass data was necessary to meet the assumption of linearity necessary for ANCOVA. We examined interaction effects to test for slope homogeneity prior to analysis of treatment and covariate effects. In PROC MIXED, repeated-measures covariance structure was specified as Compound Symmetry in the metabolic rate comparison, and Autoregressive, Type 1 in the mass comparison. Random effects covariance structure was specified as Variance Components in all comparisons. All covariance models were found to best fit the data using Akaike's Information Criterion (AIC). Examinations of residuals showed that the assumptions of parametric statistics (independent and normally distributed errors) were met in both analyses.

Carapace length and width, and plastron length and width were all $\log_{10}$-transformed and analyzed using a repeated-measures multivariate analysis of covariance (rMANCOVA) in SAS PROC GLM using $\log_{10}$-initial mass (pre-yolkectomy) as the covariate. Log transformation of shell and mass data was necessary to meet the assumption of linearity necessary for ANCOVA. Clutch was included in the model as a random effect. Although examinations of residuals showed that the assumptions of univariate parametric statistics were met, the assumption of multivariate normality could not be directly tested. Pillai's Trace was used as the test statistic because it is the most robust to violations of multivariate normality (Scheiner, 2001). Interaction effect tests were used to test for slope homogeneity before treatment and covariate effects were analyzed. Univariate pairwise ANOVA comparisons then tested for age differences on each dimension of shell growth, using Bonferroni-corrected $\alpha = 0.05/4 = 0.0125$.

We also estimated the duration of time during which residual yolk could provide energy sustenance to hatchling *A. mutica*, assuming an energy density of 21.66 kJ g^{-1} (T.N. Lee et al., 2007). We followed Gessaman and Nagy (1988), and assumed that the respiratory exchange ratio = 0.72 for a ureotelic carnivore metabolizing a mixed substrate of 20% protein, 75% fat, and 5% carbohydrate, which yielded a conversion factor of 26.88 J mL^{-1} CO_2. These assumptions were based on the lipid-rich content of hatchling *A. mutica* residual yolk and carcass (Nagle et al., 2003), the low RQs commonly observed in hatchling aquatic turtles (Steyermark and Spotila, 2000; Litzgus and Hopkins, 2003), and the ureotelic excretory system found in most trionychid turtles (Baze and Horne, 1970; Schmidt-Nielsen, 1988; S. M.L. Lee et al., 2007).

2.3. Residual yolk protein and fat content

In 2008, 33 eggs of 8 clutches (2–5 eggs per clutch) were frozen immediately after collection at −20 °C. Twenty-six hatchling turtles from the same clutches (2–6 turtles per clutch) were sacrificed by isoflurane inhalation immediately after hatching. All eggs and turtles were dried in a freeze dryer (Labconco, Kansas City, MO, USA)

at −50 °C and at pressures below 200×10^{-3} mbar for 7 days. After drying, turtle residual yolks were dissected from carcasses and were pooled by clutch in order to produce samples large enough for analysis at the University of Arkansas Central Analytical Laboratory (UACAL). UACAL recommends that samples for crude protein and crude lipid content analyses weigh 7 g, and requires that samples weigh 1 g. Individual egg yolks averaged only 1.88 g, while residual yolks averaged only 0.197 g. To maximize the sensitivity of chemical analyses, and because yolks were only available for 2 eggs or 2 turtles in some clutches, we elected to pool both egg and residual yolks within each clutch.

Both residual and egg yolk samples were then analyzed for crude fat and crude protein contents at the UACAL. Crude protein was measured by combustion analysis, using mass spectrometry of nitrogen content to estimate total protein content. Crude fat content was measured by ether extraction. Egg and residual yolk crude protein and crude fat content were compared using multivariate analysis of covariance (MANCOVA) of protein and fat contents, using yolk mass as a covariate, in SAS PROC GLM. Clutch effects could not be analyzed since clutches were pooled to gain sufficient material for analysis at UACAL. While pooling samples is a form of pseudoreplication and reduces the statistical power of our experiment (Hurlbert, 1984), the resulting relationships between fat/protein masses and pooled yolk masses were tightly linear ($r^2=0.88$). This suggests that potential differences among individual turtles, eggs, and clutches were minimal, and likely varied on the basis of total yolk mass, rather than yolk composition. Mass and yolk content data were not log-transformed because relationships were linear in all cases. We examined interaction effects to test for slope homogeneity prior to analysis of treatment and covariate effects. Although residual analysis showed that the assumptions of univariate parametric statistics were met, the assumption of multivariate normality could not be directly tested. As a result, Pillai's Trace was used as the test statistic because it is the most robust to violations of multivariate normality assumptions (Scheiner, 2001).

2.4. Post-hatch water uptake

In 2008, 40 freshly-hatched turtles of 8 clutches (2–4 turtles per clutch) were randomly assigned to control ($n=26$) and watered treatments ($n=14$). All turtles were weighed to the nearest 0.01 g. Control turtles were sacrificed immediately by isoflurane inhalation, without access to water prior to sacrifice. Watered turtles were maintained in 740 mL plastic containers filled with 400 mL of water. Water was changed twice per week and turtles were not fed for the duration of each experiment. Temperature was maintained at 27.5 °C, and there was a 12:12 light/dark cycle. After 2 weeks, watered turtles were re-weighed to the nearest 0.01 g and sacrificed by isoflurane inhalation. All turtle carcasses were freeze-dried for 7 days. After drying, all carcasses were weighed to the nearest 0.01 g.

To determine how much water *A. mutica* absorbs in the first 2 weeks of life, we compared wet masses of control turtles to those of watered turtles using ANCOVA in SAS PROC GLM. Carcass dry masses served as the covariate, and clutch was included as a random effect. Prior to ANCOVA, interaction effect tests were used to test for slope homogeneity before treatment and covariate effects were analyzed. Mass data were not $\log_{10}$-transformed because relationships between dry and wet masses were linear in all cases. Residual analysis showed that assumptions of parametric statistics were met.

2.5. Effects of metabolic costs of nest emergence on residual yolk dry mass

In 2009, 131 freshly-hatched turtles of 13 clutches (4–6 turtles per clutch) were randomly assigned to control ($n=63$) and nest emergence treatments ($n=68$). Control turtles were weighed to the nearest 0.01 g and were sacrificed by isoflurane inhalation. Emergence-treatment turtles were buried individually in artificial nests as soon after emergence from their shells as possible. Artificial nests were constructed by dividing 10 gallon glass aquaria into two 257 mm × 267 mm (L × W) chambers. A polyvinyl chloride (PVC) pipe 200 mm in length and 50 mm in diameter was placed in the center of each chamber, and the chamber was filled with enough sand to be level at 120 mm once the pipe was removed. One hundred and twenty millimeters is the mean depth, to the topmost egg, of nests *A. mutica* constructs at the Georgetown site on the White River, Arkansas (Plummer et al., 1994). Emergence-treatment turtles were gently slid down the PVC pipe to the bottom of the chamber, and the pipe was pulled out of the sand, covering the turtle at the bottom. The surface was smoothed to be exactly 120 mm deep over the entire chamber. After burial, emergence-treatment turtles were allowed to emerge under their own power and were checked every 15 min until they successfully emerged. Emergence was counted as complete once the turtle's head was observed above the surface. Times to emergence were recorded as the end of the quarter-hour during which the turtle was visible on the surface. Emergence-treatment turtles were re-weighed to the nearest 0.01 g and were sacrificed by inhalation of isoflurane.

After sacrifice, all turtles of both treatments were dissected to remove their residual yolks. Residual yolk bodies and yolk-free carcasses were weighed to the nearest 0.0001 g (Scaltec, model SBA32, Goettingen, Germany), and were individually packaged and labeled in vinyl scintillation vials and aluminum foil, respectively. Residual yolks and carcasses were then dried in a drying oven at 60 °C for at least 48 h, the amount of time necessary for masses to equilibrate to a low asymptote. After drying, carcasses and residual yolks were re-weighed to the nearest 0.0001 g.

To test whether the effort of emergence influenced residual yolk consumption, residual yolk dry mass was compared between control and emergence-treatment turtles using ANCOVA in SAS PROC GLM, using yolk-free carcass dry mass as a covariate. Clutch was included as a random effect. Prior to ANCOVA, interaction effect tests were used to test for slope homogeneity before treatment and covariate effects were analyzed. Carcass and yolk mass data were not $\log_{10}$-transformed because the relationship was found to be linear. Examinations of residuals showed that the assumptions of parametric statistics were met. Finally, we used a Pearson correlation to determine if residual yolk dry mass was linearly correlated with emergence time in turtles forced to emerge from artificial nests. Log-transformation of both yolk mass and emergence times was necessary to meet the assumptions of normality in order to test the hypothesis of significant correlation between yolk mass and emergence time.

Unless reported otherwise, statistical significance was judged at a 0.05 Type I error rate in all analyses, and all means are reported as ± SE. While clutch effects were included in all statistical models to account for maternal effects, proportions of variance attributable to clutch were determined independently using PROC VARCOMP in SAS. All animal care and use procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (Protocol # 07005).

3. Results

3.1. Egg metabolic rates

We measured egg CO_2 consumption rates over embryonic development to determine whether the associated increase in metabolic rate followed an altricial or precocial pattern. Egg metabolic rates differed significantly among ages (rANCOVA $F_{6,313}=167.55$, $P<0.0001$; Fig. 1). Egg metabolic rates did not vary significantly with egg mass (rANCOVA $F_{1,313}=3.76$, $P=0.0534$). Egg CO_2 production rates increased from a minimum of 0.12 ± 0.02 mL h^{-1} 47 days prior to hatching to a maximum of 0.75 ± 0.05 mL h^{-1} at 10 days prior to

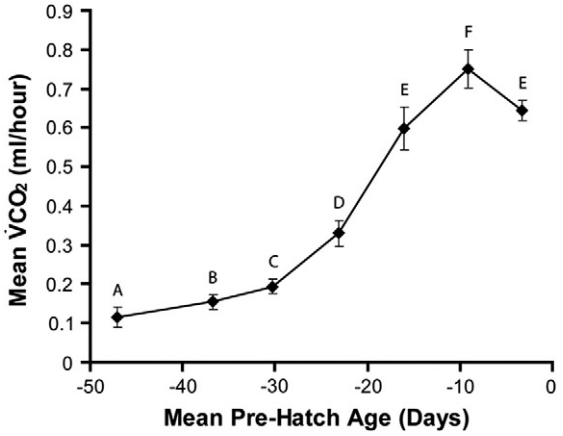

Fig. 1. Means and 95% confidence intervals of *Apalone mutica* egg $\dot{V}CO_2$ from 47 days to 3 days prior to hatching. *A. mutica* egg CO_2 production increased from 47 days prior to hatching to approximately 10 days prior to hatching, and then declined immediately prior to hatching. Letters indicate significant differences among pre-hatch ages found by post-hoc orthogonal contrasts.

hatching, and then decreased to 0.65 ± 0.03 mL h^{-1} 3 days prior to hatching. Clutch effects were responsible for only 1.3% of the total variance in egg metabolic rate.

3.2. Yolkectomy effects on resting metabolic rate

We measured CO_2 consumption rates of control, sham, and yolkectomized hatchling *A. mutica* to determine whether or not there was a metabolic cost of residual yolk absorption and catabolism. Yolkectomy did not significantly affect resting metabolic rate in *A. mutica* (rANCOVA $F_{2,54} = 0.31$, $P = 0.7380$). There was also no significant relationship between $\log_{10}$-mass and $\log_{10}$-$\dot{V}CO_2$ (rANCOVA $F_{1,208} = 1.37$, $P = 0.2434$), though this could result from there being such a small range of body masses, from 5.46 g to 8.11 g (6.80 ± 0.06). Metabolic rates of all turtles significantly decreased with age after hatching (rANCOVA $F_{4,208} = 10.35$, $P < 0.0001$), with mean $\dot{V}CO_2$ at 40 days of age being 43% that of $\dot{V}CO_2$ at hatching (Fig. 2). Less than 0.001% of the variance in metabolic rate was associated with clutch. Because mass and treatment were not significant, all $\dot{V}CO_2$ values were regressed against age. The relationship between $\dot{V}CO_2$ and age ($\dot{V}CO_2 = -0.151(\text{age}) + 12.762$) was then integrated to find the total mean $\dot{V}CO_2$ from age 0 to age 40 days. The total mean $\dot{V}CO_2$ for the 40-day period was calculated to be 389.76 mL CO_2 for a 6.80 ± 0.06 g hatchling *A. mutica*, which, following Gessaman and Nagy (1988), estimates a total energy expenditure of 10.48 kJ.

3.3. Yolkectomy effects on growth

We measured shell dimensions and live mass of control, sham, and yolkectomized hatchling *A. mutica* to determine whether or not residual yolk contributed to early post-hatch growth. Yolkectomy significantly reduced *A. mutica* hatchling mass between ages 0 and 40 days (rANCOVA $F_{2,228} = 15.13$, $P < 0.0001$). Yolkectomized mass averaged 0.3 g less than that of sham and control turtles due to yolk removal (Fig. 3). Hatchling $\log_{10}$-mass scaled significantly with $\log_{10}$-initial mass (rANCOVA $F_{1,5} = 38.90$, $P = 0.0016$), and increased with age (rANCOVA $F_{4,28} = 4.11$, $P = 0.0096$), regardless of treatment. Clutch effects accounted for 39.40% of the variance in hatchling *A. mutica* mass increase. Over 40 days, masses of control and sham turtles increased by 0.91 ± 0.06 g (14%; Fig. 3), while masses of yolkectomized turtles increased by 0.77 ± 0.10 g (13%; Fig. 3).

Hatchling *A. mutica* shell dimensions were not affected by yolkectomy (Pillai's Trace = 0.313, rMANCOVA $F_{8,74} = 1.71$, $P = 0.1092$), did not scale with initial mass (Pillai's Trace = 0.156, rMANCOVA $F_{4,36} = 1.66$, $P = 0.1801$), but significantly increased with age (Pillai's Trace = 0.966, rMANCOVA $F_{16,27} = 47.41$, $P < 0.0001$). Clutch effects were responsible for 19.64% of the variance in shell size. Mean carapace length increased by 1.62 ± 0.27 mm (6%) between age 0 and 10 days (Fig. 4A). Carapace length did not increase after age 10 days (Fig. 4A). Mean carapace width increased by 2.64 ± 0.13 mm between age 0 and 10 days (Fig. 4B), 1.05 ± 0.12 mm between ages 10 and 20 days (Fig. 4B), and 0.55 ± 0.20 mm between ages 20 and 30 days (Fig. 4B). Carapace width did not increase after day 30, indicating an asymptotic increase in carapace width over time. Total increase over 30 days in mean carapace width was 4.18 ± 0.20 mm (13%).

Mean plastron length increased by 1.72 ± 0.18 mm between age 0 and 10 days (Fig. 4C), 0.67 ± 0.14 mm between ages 10 and 20 days (Fig. 4C), and 0.43 ± 0.17 mm between ages 20 and 30 days (Fig. 4C). Plastron length did not increase between ages 30 and 40 days (Fig. 4C), indicating an asymptotic increase in plastron length over time. Total increase in plastron length over 30 days was 2.93 ± 0.20 mm (11%). Mean plastron width increased 2.10 ± 0.16 mm from age 0 to 10 days (Fig. 4D), 1.43 ± 0.20 mm from age 10 to 20 days (Fig. 4D), and 1.42 ± 0.24 mm from age 20 to 30 days (Fig. 4D). Plastron width did not increase from age 30 to 40 days (Fig. 4D),

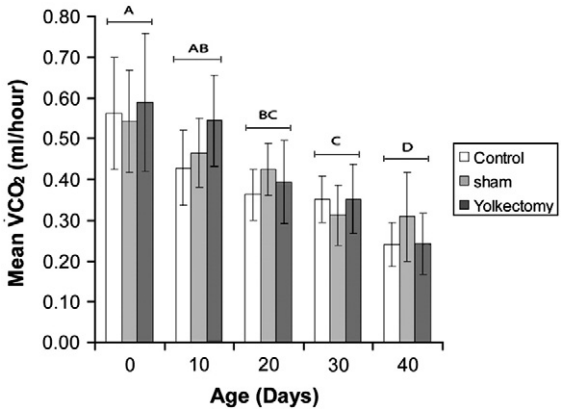

Fig. 2. Least-squares means and 95% confidence intervals of unfed hatchling *Apalone mutica* VCO_2 in response to control, sham, and yolkectomy treatments, from age 0 to 40 days. All least-squares mean $\dot{V}CO_2$ are mass-adjusted using the significant relationships between VCO_2 and mass. Yolkectomy had no significant effect on mean VCO_2 of *A. mutica* from age 0 to 40 days. Mean VCO_2 decreased from 0 to 40 days old. Letters indicate significant differences among ages found by post-hoc orthogonal contrasts.

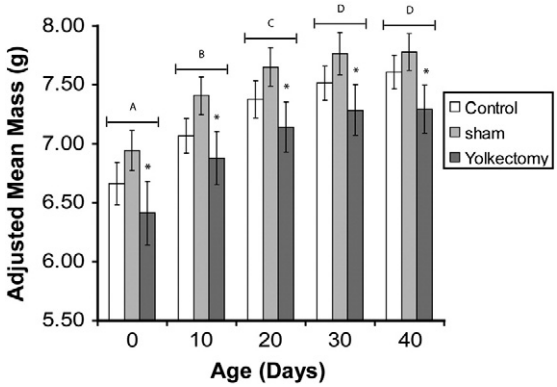

Fig. 3. ANCOVA-adjusted least-squares means and 95% confidence intervals of unfed hatchling *Apalone mutica* body mass growth in response to control, sham, and yolkectomy treatments, from age 0 to 40 days. Yolkectomy significantly reduced mean mass of unfed *A. mutica* at all ages. Mean masses of all treatments increased from 0 to 30 days, but did not increase after day 30. Error bars represent 95% confidence intervals. Asterisks represent significant differences between yolkectomized and both control and sham turtles found by post-hoc orthogonal contrasts. Letters indicate significant differences among ages found by post-hoc orthogonal contrasts.

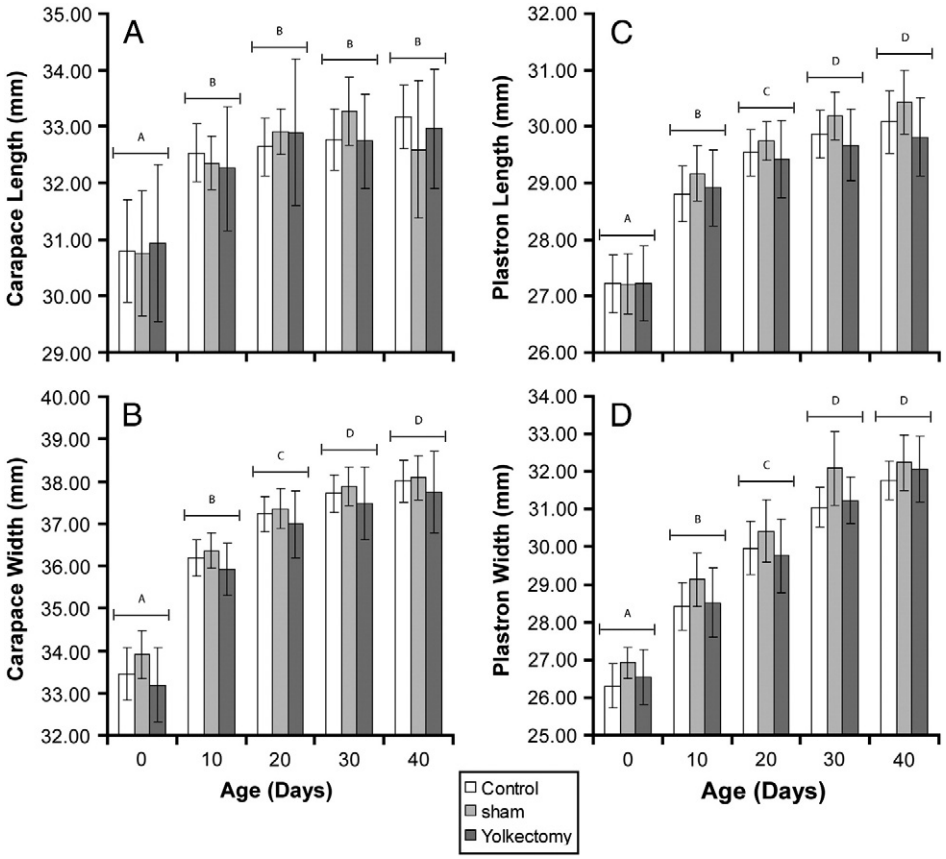

Fig. 4. Least-squares means and 95% confidence intervals of unfed hatchling *Apalone mutica* shell growth in response to control, sham, and yolkectomy treatments, from age 0 to 40 days. Letters indicate significant differences among ages found by post-hoc univariate pairwise ANOVAs. (A) Yolkectomy had no significant effect on mean carapace length of unfed *A. mutica* from age 0 to 40 days. In all treatments, mean carapace length increased from 0 to 10 days old, but did not increase after day 10. (B) Yolkectomy did not significantly affect mean carapace width of unfed *A. mutica* from age 0 to 40 days. Mean carapace width increased from 0 to 30 days old, but did not increase after day 30. (C) Yolkectomy had no significant effect on mean plastron length of unfed *A. mutica* from age 0 to 40 days. Mean plastron length increased from 0 to 30 days old, but did not increase after day 30. (D) Yolkectomy had no significant effect on mean plastron width of unfed *A. mutica* from age 0 to 40 days. Mean plastron width increased from 0 to 30 days old, but did not increase after day 30.

indicating an asymptotic increase in plastron width over time. Total increase in plastron width over 30 days was 5.30 ± 0.23 mm (20%).

3.4. Comparison of egg and residual yolk crude protein and crude fat

Because yolkectomy did not affect post-hatch growth, we compared the protein and fat contents of *A. mutica* residual yolk to those of freshly-laid egg yolk to determine if protein was disproportionately consumed during development and thus would not be available for post-hatch allocation to growth. In freshly-laid eggs, mean yolk dry mass (±SE) was 1.88 ± 0.26 g, while mean residual yolk dry mass was 0.20 ± 0.02 g, representing approximately 10.4% of the amount originally allocated to eggs. Pooled egg and residual yolk protein and fat contents significantly covaried with their respective whole yolk dry masses (Pillai's Trace = 0.998, MANCOVA $F_{2,12} = 2664.48$, $P < 0.0001$). The relationships between pooled protein and fat contents and residual yolk dry mass were linear and accounted for a very large percentage of the overall variance (overall $r^2 = 0.88$). After accounting for mass effects via ANCOVA, pooled residual yolk protein and fat contents did not significantly differ from those of freshly-laid eggs (Pillai's Trace = 0.035, MANCOVA $F_{2,12} = 0.22$, $P = 0.8706$).

3.5. Effects of Water Uptake on Wet Mass

All turtles grew in live mass and shell size during the yolkectomy experiment, despite not being fed. We compared wet masses of 14-day old turtles to those of freshly-hatched turtles to determine if that growth could be the result of water uptake. *A. mutica* wet mass did not scale with dry mass (ANCOVA $F_{1,39} = 0.33$, $P = 0.5698$), but significantly differed between turtles at age 0 and age 14 days (ANCOVA $F_{1,39} = 12.61$, $P = 0.00013$). Mean wet mass of 14-day old turtles was 0.78 g greater than that of turtles at the day of hatching (Fig. 5). Dry mass also differed significantly between turtles at age 0 and age 14 days (ANOVA $F_{1,\ 39} = 33.82$, $P < 0.0001$). Dry mass at 14 days averaged 0.20 g less than dry mass at hatching (11% reduction; Fig. 5). Correspondingly, dry mass at hatching accounted for 30.3% of live mass, while that of 14-day old turtles accounted for only 26.0% of live mass. Clutch accounted for 26.1% of the variance in wet mass and 22.8% of the variance in dry mass.

3.6. Effects of emergence effort on yolk mass

We compared residual yolk dry masses of freshly-hatched *A. mutica* to those of turtles forced to emerge from artificial nests to determine if residual yolk fueled the energetic demands of nest emergence activity. *A. mutica* nest emergence times varied widely among turtles, from a minimum of 2 min, to a maximum of 20 h (mean ± SE = 138.68 ± 30.57 min). Only 13% of the variance in nest emergence time was attributable to clutch. Residual yolk dry mass was not significantly correlated with emergence time in emerging turtles ($r_{66} = 0.1750$, $P = 0.1535$; Fig. 6). Residual yolk dry-mass significantly covaried with carcass dry-mass (Fig. 7; ANCOVA $F_{1,107} = 8.86$, $P = 0.0036$), but did not significantly differ between freshly hatched and emerged turtles (ANCOVA $F_{1,107} = 3.06$, $P = 0.0833$). Clutch effects accounted for 50.1% of the variance in residual yolk dry mass. Residual yolk dry mass averaged 48.5% of residual yolk wet mass.

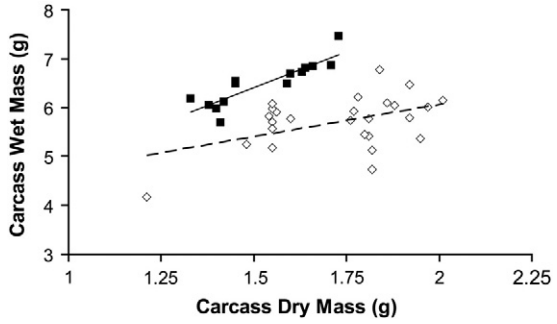

Fig. 5. Body wet masses of freshly-hatched and 14-day old *Apalone mutica* regressed on carcass dry mass. Freshly-hatched *A. mutica* are represented by open diamonds and a dashed line, while 14-day old *A. mutica* are represented by solid squares and a solid line. Wet masses of 14-day old *A. mutica* were significantly greater than that of freshly-hatched turtles. Dry masses of 14-day old *A. mutica* were also significantly less than that of freshly-hatched turtles. Lines represent best-fit regressions of wet mass to dry mass by treatment, but these regressions were not significant.

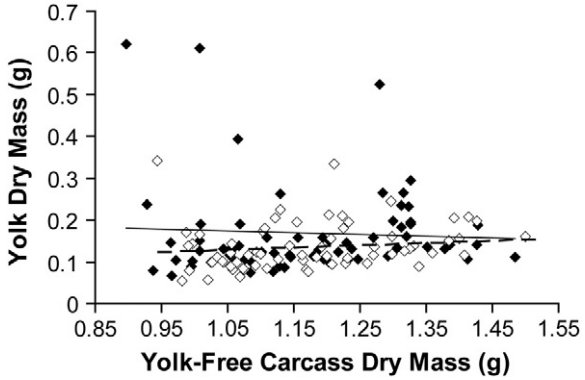

Fig. 7. Residual yolk dry masses of freshly-hatched *Apalone mutica* and those that emerged from artificial nests regressed on carcass dry mass. Residual yolks of freshly-hatched *A. mutica* are represented by solid diamonds and a solid line, while those of *A. mutica* that emerged from artificial nests are represented by open diamonds and a dashed line. Residual yolk dry-masses of freshly hatched *A. mutica* were not significantly different from residual yolk dry-mass of *A. mutica* that emerged from artificial nests. Lines represent best-fit regressions of residual yolk dry mass to carcass dry mass.

4. Discussion

Taken together, the results of our experiments refute several hypothesized uses of residual yolk in *A. mutica*. Yolkectomy did not affect post-hatch metabolic rates, suggesting that residual yolk catabolism and absorption do not incur a metabolic response similar to SDA. In addition, total energy consumption of unfed, captive hatchling *A. mutica* over a 40-day period, calculated from CO_2 production, exceeded the total energy content of residual yolk. This suggests that residual yolk cannot fulfill energetic demands for long after hatching, especially those of hibernation. Yolkectomy did not affect post-hatch growth in shell size, suggesting that residual yolk does not contribute to post-hatch growth. Yolkectomy reduced live mass initially, but live mass increased at similar rates among all treatments over the 40 days after hatching. Residual yolk protein and fat proportions were not significantly different from those of freshly-laid egg yolks, suggesting that neither fat nor protein were used in rates disproportionate to their abundance. However, the smaller mass of residual yolk (10% of egg yolk dry mass) suggests that the raw amounts of protein and fat available to hatchlings may be limited by raw amount rather than a change in proportion. Finally, nest emergence did not reduce residual yolk dry mass, suggesting that residual yolk is not a source of energetic fuel for nest emergence activity in this species.

A. mutica egg metabolic rates peaked 10 days before hatching, then declined until 40 days after hatching. The pattern of egg and fasted neonate metabolic expenditure thus appears to follow a precocial pattern, in which metabolic rates peak prior to hatching, and then slowly return to a resting asymptote days or weeks afterward (Vleck et al., 1980; Hoyt, 1987; Thompson, 1989; Whitehead and Seymour, 1990; Thompson, 1993; Peterson and Kruegl, 2005; Jones et al., 2007). Resting metabolic rates of hatchlings were not affected by the removal of residual yolk, suggesting that yolk catabolism and absorption do not incur measurable metabolic costs similar to those observed during digestion as SDA. Given the vascularization of the yolk sac (Yeomans, 1999), and the slow transport of yolk-derived nutrients into the bloodstream, it is possible that the metabolic costs of residual yolk absorption, transport, and utilization may not be detectable using current means of respirometry. Unfortunately, little is known about the mechanisms of yolk catabolism or their metabolic costs, especially in reptiles (Thompson and Speake, 2003). An upregulated metabolic rate immediately after hatching may be necessary to maximize exertion effort during nest emergence and/or early foraging (Kraemer and Bennett, 1981), and has also been hypothesized to serve some function in the synchronization of hatching in variable thermal environments in other reptiles (Thompson, 1989). Because turtles were not fed during the study, the post-hatch 56% reduction in metabolic rate in all treatments may have been caused by catabolism of metabolic tissues for maintenance and activity requirements. However, this hypothesis would still predict an earlier reduction in metabolic rate in the yolkectomized turtles than the control or sham turtles, which we did not observe. Alternatively, the post-hatch metabolic reduction we observed may be a continuation of the reduction that began in the 10 days prior to hatching (Fig. 1), or may be related to the energetic demands of hatching.

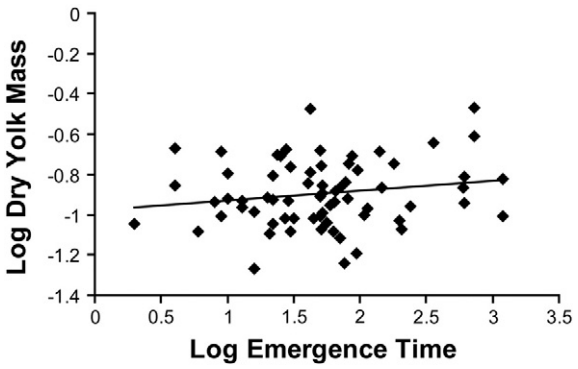

Fig. 6. Log-residual yolk dry masses of *Apalone mutica* emerged from artificial nests correlated against log-time to emergence. Residual yolk dry mass was not significantly correlated with emergence time in hatchling *A. mutica*. The line represents a best-fit regression of log-yolk dry mass to log-emergence time, but this regression was not significant ($r^2 = 0.03$).

We estimated total energy consumption of a 6.80 ± 0.06 g *A. mutica* to be 10.48 kJ over the 40-day study period. Because total yolk energy density is approximately 21.66 kJ g^{-1} (T.N. Lee et al., 2007), our estimates of yolk dry mass (0.32 g in 2006, 0.15 g in 2009) predict total yolk energy content to range from 3.25 to 6.93 kJ. Assuming the 26.88 J mL^{-1} conversion factor cited in the methods (Gessaman and Nagy, 1988), catabolism of 0.15–0.32 g of dry yolk would produce 120.91–257.81 mL of CO_2. Based on our calculated rate of CO_2 production, we predict this range of residual yolk mass to be exhausted in 10.07–23.46 days. As a result, it does not appear that hatchling *A. mutica* are able to fuel hibernation metabolism with the energy available in residual yolk, unlike in *Trachemys scripta* (Filoramo and Janzen, 1999).

Furthermore, our estimate of residual yolk energy density is approximately 21.7 kJ g^{-1} (T.N. Lee et al., 2007). *A. mutica* residual yolk energy density is therefore similar to that of Leatherback Sea Turtles, *Dermochelys coriacea* (30.71 kJ g^{-1}; Jones et al., 2007), Murray Short-Necked Turtles, *Emydura macquarii* (27.9 kJ g^{-1};

Thompson et al., 1999), and Olive Ridleys, *Lepidochelys olivacea* (28.2 kJ; Silas et al., 1984). Total energy content (7.22 kJ) is also more than twice that of the residual yolk of *C. serpentina* (2.22 kJ; Wilhoft, 1986), but is smaller than that of *E. macquarii*, (10.9 kJ; Thompson et al., 1999) likely due to differences in residual yolk mass in addition to energy density.

It seems remarkable that yolkectomized *A. mutica* survived 40 days without this extra energy source, but that may be due to the high concentration of non-yolk triacylglycerol already stored in the body (Nagle et al., 2003). Nearly 25% of hatchling dry mass is non-polar lipid, including nearly 75% of the non-polar lipid originally allocated to the egg. Furthermore, residual yolk accounts for only 28% of the non-polar lipid found in freshly laid eggs, while the yolk-free carcass includes 46.6% (Nagle et al., 2003). Eighty percent of leftover non-polar lipid is triacylglycerol (Nagle et al., 2003), an energy-rich fat that forms the primary metabolic fuel in most embryonic and neonate reptiles (Rowe et al., 1995; Thompson et al., 1999; Speake and Thompson, 2000; Speake et al., 2003; Thompson and Speake, 2003). Like some Australian skinks, developing *A. mutica* appear to absorb the majority of yolk contents prior to hatching, and only hatch with a minimal amount of residual yolk. This is likely a primary reason why the removal of residual yolk did not affect hatchling *A. mutica* survivorship in our study. The relative contributions of residual yolk and stored reserves to hatchling energy budgets, and its variation among taxa, are clearly questions in need of further research.

In lizards, yolkectomy of freshly-laid eggs significantly reduced hatchling body size (Sinervo, 1990; Sinervo et al., 1992; Sinervo, 1993; Radder et al., 2004). In contrast, post-hatch growth of hatchling *A. mutica* was not apparently affected by post-hatch yolkectomy. Yolkectomy significantly reduced wet mass, but only by an amount similar to the total mass of residual yolk removed, and the trajectory of wet mass increase appeared similar across all treatments. Growth in carapace length and width, and plastron length and width were also not affected by yolkectomy. All measurements of size, including mass, exhibited significant increases after hatching, regardless of treatment. After hatching, no turtles were fed, but all turtles were housed in water-filled dishes. Therefore, we hypothesized that water absorption during the first 2–3 weeks of life was responsible for the increase in body mass. Our follow-up experiment showed that 14-day old hatchling wet mass exceeded that of freshly-hatched turtles by more than 1 g. Over the same period, dry mass decreased by 0.2 g, so hatchling *A. mutica* appear to uptake at least 1.2 g of water in the first 2 weeks of life.

Though we did not measure changes in dry mass of turtles in our yolkectomy experiment, removal of yolk could only have reduced dry mass of yolkectomized turtles. Because residual yolk dry mass averages 48.5% of residual yolk wet mass at hatching (this study, 2009 data), the mean yolkectomized reduction of 0.65 g (wet mass) would result in a dry mass reduction of approximately 0.32 g. Because no hatchling turtles in our yolkectomy experiment were fed at any point, all observed increases in total wet mass must have been the result of water uptake rather than dry mass allocation, and the 0.32 g of dry yolk material could not have been replaced in yolkectomized turtles. That apparent growth in shell size occurred at similar rates in both yolkectomized and control turtles strongly suggests that anabolic materials for shell growth are not provided by residual yolk. In fasted turtles, early post-hatch shell growth may not be related to anabolic tissue production at all. Instead, concomitant increases in shell size and water content, by more than 1 g, suggest that early post-hatch increases in shell dimensions could actually be expansion as a result of increased hydration.

The lack of residual yolk contribution to growth could be caused by a lack of anabolic substrate left over after embryogenesis, specifically crude protein (Congdon et al., 1983; Thompson et al., 2001). Residual yolk dry mass makes up approximately 24% of the amount of yolk originally allocated to freshly laid eggs (Nagle et al., 2003), but after accounting for total yolk mass using ANCOVA, neither crude protein content nor crude fat content differed between residual yolk and freshly-laid egg yolk. The small amount of residual yolk (2009 mass = 0.15 ± 0.01 g; 11% of total body dry mass), of which only 50% is crude protein, suggests that only a maximum of 5.5% of a hatchling's dry mass (~0.07 g in 2009) is available for anabolic production of new tissues. The amount of anabolic substrate potentially available to post-hatch growth may be further constrained because embryonic and neonate turtles often use yolk protein to supplement lipid as metabolic fuel (Thompson and Speake, 2003). The small amount of crude protein remaining in residual yolk is likely not enough to produce significant anabolic growth. Similarly small magnitudes of residual yolk have also been suggested to prevent long-term reliance on residual yolk for significant post-hatch energetic or growth demands, *C. serpentina* (Wilhoft, 1986; Finkler et al., 2002). It is also possible that non-organic yolk constituents that are necessary for growth, especially calcium, may have been exhausted during embryogenesis, thus limiting post-hatching growth (Packard and Packard, 1986).

Emergence effort did not significantly reduce dry mass in hatchling *A. mutica*, nor was residual yolk mass significantly correlated with time to emergence. Unlike Loggerhead Sea Turtles, *Caretta caretta*, which consume up to half of their residual yolk dry mass during emergence (Kraemer and Bennett, 1981), *A. mutica* hatchlings do not apparently rely on residual yolk to fuel metabolic costs of nest emergence. Furthermore, re-settling the sand between each emergence trial should remove any effect of social facilitation, in which the combined emergence effort of siblings reduces the energetic expenditure of each individual (Carr and Hirth, 1961). As a result, we forced individual turtles to exert maximum emergence effort in every trial, yet did not observe a reduction in residual yolk. The lack of emergence effect also shows that residual yolk stores are not solely an artifact of artificial incubation conditions that, by design, do not incur emergence costs. That some turtles were able to emerge only minutes after burial suggests that nest emergence is likely fueled via glycolysis rather than catabolism of residual yolk, which should take much longer. However, we cannot easily explain the exceptionally large variance in nest emergence times among all turtles. It is possible that individual turtles differed in their ability to recuperate from hatching stress prior to emerging, or perhaps turtles varied in the some aspect of yolk internalization which we were unable to observe. Regardless, the lack of significant correlation between emergence time and residual yolk mass suggests that the variance in emergence time did not significantly impact residual yolk consumption.

In some reptile species, residual yolk has been shown to function as a source of material for post-hatching growth (Troyer, 1987; Yeomans, 1999) and as an energy reserve for metabolic costs of nest emergence (Kraemer and Bennett, 1981) and hibernation (Filoramo and Janzen, 1999), and has been hypothesized to function as an energy reserve for post-hatch survival (Nagle et al., 2003; Radder et al., 2004). Turtles that hibernate within the nest have been reported to continue growth after hatching using residual yolk resources (Filoramo and Janzen, 1999), and may also use it as a source of energy (Cagle, 1950; Gibbons and Nelson, 1978). In addition, post-hatch absorption and catabolism has also been hypothesized to incur a metabolic cost, as a portion of costs of growth (Beaupre and Zaidan, 2001). Each of these observations and hypotheses encompass key components of a neonate animal's energy budget: maintenance metabolism, activity, biomass energy content, and biomass production. The results of this study show that *A. mutica* residual yolk does not contribute to growth or metabolic costs of nest emergence, and that consumption of residual yolk does not incur a measurable metabolic cost. Residual yolk also probably does not serve as an energy reserve for hibernation in *A. mutica* because it is exhausted long before hibernation (T.N. Lee et al., 2007).

By process of elimination, residual yolk in *A. mutica* likely serves primarily as a source of energy for maintenance metabolism and

activity prior to successful foraging. However, the magnitude of residual yolk reserves, relative to those already absorbed into the carcass, is relatively small, so the energetic contribution of residual yolk may be limited. Variation in residual yolk mass with different incubation conditions in Common Snapping Turtles, *Chelydra serpentina,* and Ornate Box Turtles, *Terrapene ornata* (Morris et al., 1983; Packard et al., 1985; Finkler et al., 2002), suggests that mothers may allocate a maximum amount of yolk to each offspring to ensure that embryogenesis can be completed in even the most taxing of incubation environments. In contrast, nest hydration has not been shown to affect the wet mass of hatchlings of a closely related species, the Spiny Softshell Turtle, *Apalone spinifera* (Packard et al., 1979, 1981). However, these studies only investigated wet mass, and did not examine the size or content of residual yolk, shell dimensions, or dry mass of hatchlings, and also did not investigate the effects of incubation temperature in addition to hydration.

A bet-hedging strategy of yolk allocation could maximize incubation success in stochastic incubation environments, and suggests that, in some species, residual yolk may simply be a consequence of incubation conditions being less demanding than the maximum anticipated by the mother. Thus, the magnitude of residual yolk we observed could have been an experimental artifact of our carefully controlled incubation conditions that mimicked the optimum observed in nature (Plummer et al., 1994). However, if incubation environments are not stochastic, then our results suggest that residual yolk may be better invested by increasing clutch size rather than maximizing individual offspring survival. Thus, the presence of unnecessary post-embryonic provisioning could further complicate the trade-off between offspring size and number (Bernardo, 1996), especially in a lecithotrophic species where unused residual yolk cannot be reclaimed by a parent. Variation in maternal allocation, in conjunction with incubation environment stochasticity and local selection on hatchling survival, likely drives the evolution of residual yolk functions along different trajectories among different species.

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