Hold on for One More Day: Energetic Costs of Oviductal Egg Retention in Eastern Musk Turtles (*Sternotherus odoratus*)

Lyranda R. Thiem*
C. M. Gienger
Department of Biology and Center for Excellence for Field Biology, Austin Peay State University, Clarksville, Tennessee 37044

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ABSTRACT

In oviparous reptiles, parental care is often limited to the energy allocated to embryos before oviposition. Reproducing females can allocate energy toward vitellogenesis, determining the number and size of eggs, fertilization, eggshell calcification, retention of eggs within the oviduct after fertilization (oviductal egg retention), and nesting activities. Oviductal egg retention in turtles ranges from 2 wk to half a year, permitting flexibility in the timing of oviposition. The energetic cost of oviductal egg retention in eastern musk turtles (*Sternotherus odoratus*) was investigated by measuring the metabolism of females before and after oviposition. Gravid female metabolic rates were elevated relative to male and nongravid female metabolic rates, indicating an associated energetic cost for egg retention. Metabolism of gravid females was 40% higher before oviposition than after oviposition, and it was relatively constant across the period of oviductal egg retention. Metabolic costs associated with egg retention were correlated with clutch mass and female body mass but not with clutch size or the number of days leading up to oviposition. These results suggest that the strategy of oviductal egg retention has considerable energetic costs for eastern musk turtles but that it likely provides critical flexibility in nesting phenology.

Keywords: oviductal egg retention, chelonians, reproduction, developmental arrest, energetics, metabolism.

Introduction

Reproductive output is constrained jointly by energy availability and limitations imposed by the stressors in the environment (James and Whitford 1994). Life history theory suggests that species adopt optimized allocation strategies for partitioning available energy to the competing processes of maintenance, growth, storage, and reproduction (Fisher 1930; Stearns 1976; Congdon et al. 1982). Since increased allocation of energy to one component of an energy budget requires reduced energy to another, the energetic resources available for reproduction are finite, and trade-offs often exist among the competing reproductive processes (Congdon and Tinkle 1982; Congdon 1989; Garland et al. 2021). In reproducing individuals, trade-offs can come at the cost of increased food requirements, increased mortality, slowed growth, and reduced mobility (Shine 1980; Tallamy and Denno 1982; Coleman and Whittall 1988).

Studies of life history theory suggest that energetic costs of reproduction can be categorized into two major components: (1) parental investment, the total resources required for reproductive activities before offspring birth or deposition of eggs and (2) parental care, the component of reproductive effort that is used in maintaining individual offspring after birth or hatching (Congdon et al. 1982). Parental investment can be further partitioned into embryogenesis and embryo maintenance and development (Congdon and Gibbons 1985; Congdon 1989). Parental reproductive costs have most often been studied in species where offspring survival is influenced primarily by their energetic requirements after birth, as in many altricial mammals and birds (Broderick et al. 2003; Rafferty and Reina 2012). However, in most oviparous reptiles, the primary investment of energy occurs before oviposition in the production of eggs (Packard and Packard 1988).

Oviductal egg retention is an important reproductive strategy for regulating the timing of oviposition (Renfree and Shaw 2000; Lopes et al. 2004; Rafferty and Reina 2012). Egg retention allows flexible reproductive phenology by permitting females the ability to delay oviposition until environmental conditions are suitable (e.g., soil hydric conditions) or until they can locate and travel to appropriate nesting sites. The strategy may also be used to time oviposition to increase the chances of synchronized hatching of clutches among conspecifics, thereby increasing nest predator dilution effects (Ewert 1979; Andrews 2004; Rafferty and Reina 2012). Squamates, crocodilians, and chelonians have the greatest diversity in prolonging oviductal egg retention (Ewert 1991), and retention durations vary greatly across taxa (Andrews and Mathies 2000). The oviductal egg retention period for most squamates is about 25%–40% of the total embryonic developmental period, but it can be more prolonged in turtles (DeMarco 1993; Andrews and Mathies 2000; Rafferty and Reina 2012), lasting up to several months (Buhlmann et al. 1995; Plotkin et al. 1997). After fertilization occurs eggs rapidly develop a calcified shell and are then retained in the oviduct where embryonic development is arrested, with the eggs remaining

*Corresponding author; email: lrthiem@gmail.com.

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at the undeveloped gastrula stage until oviposition (Ewert 1985; Rafferty and Reina 2012).

On average, eastern mud turtles (*Kinosternon subrubrum*) retain eggs for 26–50 d, pond sliders (*Trachemys scripta*) retain eggs for 23–39 d (Kuchling 1999), and olive ridley sea turtles (*Lepidochelys olivacea*) and Kemp's ridley sea turtles (*Lepidochelys kempii*) delay oviposition for 14–75 d (Kuchling 1999). Prolonging oviductal egg retention potentially increases the survivorship of offspring by allowing scheduling of nesting for when resource availability would be optimal for survival of eggs within the nest and upon hatching (Buhlmann et al. 2009; Rafferty and Reina 2012). For the chicken turtle (*Deirochelys reticularia*), gravid females regularly overwinter with calcified eggs in the oviduct and lay the clutch the following spring after 4–6.5 mo of retention (Congdon et al. 1983; Buhlmann et al. 1995). Chicken turtles typically inhabit wetlands that dry in the summer before females are ready to nest (Buhlmann et al. 1995, 2009).

Despite pronounced differences in duration of egg retention, no studies have yet focused on the energetic costs of this important life history strategy for turtles; rather, most studies have primarily focused on the material costs of physically producing a clutch of eggs (Schwarzkopf and Shine 1992; Stewart 2013). Thus, the aim of this study is to assess the energetic cost of oviductal egg retention in eastern musk turtles, a species that employs the strategy across multiple clutches within a single reproductive season (Risley 1933; McPherson and Marion 1981). We hypothesize that oviductal egg retention is a beneficial reproductive strategy because it permits flexibility in timing of oviposition at a relatively low energetic cost to the mother. Because the embryos undergo developmental arrest during the oviductal egg retention period and because they remain at a relatively early and metabolically quiescent gastrula stage, we predict that the energetic cost to females maintaining the embryos in the oviduct should be relatively low compared with other processes involved in reproduction, such as vitellogenesis or mating activities. These energetic costs should also be influenced by the relative trade-offs in the allocation of reproductive materials within a clutch, and therefore costs of retention should increase as a function of increased clutch mass and clutch size.

Methods

Field Collection and Care

Eastern musk turtles were captured from backwater sloughs along the Cumberland River, Cheatham County, Tennessee, using Jones traps (Chandler et al. 2017). Traps were baited with canned tuna or sardines and placed in water deep enough to be approximately three-fourths submerged, which allowed traps to be left overnight and checked the following day. Collection was conducted during the reproductive season from mid-May to October (Mitchell 1985; Ernst 1986; Ford and Moll 2004). Weights of individuals were recorded (± 1 g), and individuals were given a unique identification code by notching marginal scutes (Cagle 1939). Sex was determined by examining tail length and thickness, amount of exposed skin along the medial plastron seam, and by presence or absence of small patches of raised scales behind the knee (Ernst and Barbour 1972; Smith and Iverson 2002). All females greater than 70 g and 66 mm in carapace length were considered adults and potentially gravid. To determine reproductive state, adult females were X-ray radiographed (AMRAD model E7239GX, Summit Industries, Chicago, IL) to determine the presence of shelled eggs. Females that were not gravid during the initial radiograph were then scanned a second time 7–10 d later to verify the absence of shelled eggs. It is unlikely that we misclassified an individual as nongravid because calcification of eggshells begins immediately after the albumen layer is secreted onto the ova, 12–36 h after fertilization (Kuchling 1999). Shelling of eggs may differ with clutch size, and turtles that produce large clutches (such as *Chelonia mydas*; clutches with >20 eggs) show the first calcified eggs within 72 h following fertilization but may take as long as 7 d for the entire clutch to become completely shelled (Kuchling 1999). Shelling of eggs typically occurs simultaneously for the entire clutch (*Pseudemydura umbrina*; Kuchling 1999).

Turtles were housed individually in 32-L (60 × 43 × 17-cm) translucent plastic tubs with ~10 cm of water depth and sphagnum moss provided as floating surface cover. A plastic box containing a mixture of sand, soil, leaves, and twigs was placed on top of a brick in each enclosure to provide a dry refuge and a nesting location. To permit voluntary body temperature regulation, individuals were provided a basking lamp with a 60-W light bulb set on a 12L : 12D photoperiod. Turtles were fed an ad lib. diet of earthworms, mealworms, and sinking mud and musk turtle pellets (Zoo Med ZM-68) every 2 d. All collection, care, and experimental procedures were performed in accordance with Austin Peay State University institutional animal care and use committee guidelines (protocol 19-013).

Measurement of Adult Turtle Energy Expenditure

Carbon dioxide production ($\dot{V}_{CO_2}$) was measured for each individual using flow-through respirometry (Lighton 2008). Metabolic measurements (mL CO_2 h^{-1}) were taken once for males and nongravid females, while both gravid metabolic rates (GMRs) and postoviposition metabolic rates (POMRs) were determined for each gravid female. Before initiating a metabolic trial, turtles were fasted for 72 h to allow food to clear the gut and ensure a postabsorptive digestive state. Individual turtles were placed in clear plastic respirometry chambers (OXO 2.3-L rectangle POP container) and were then placed within a precision incubator (Percival Scientific, series 942 microprocessor, Dallas, IA). Temperature was controlled at a constant 25°C, and a 12L : 12D photoperiod was used for the duration of a 48-h testing cycle. Room air was pumped through a Drierite drying column using an Ametek R-1 flow controller (Ametek, Berwyn, PA) and then through a manifold that distributed air into mass-flow controllers (Sierra Mass-Trak, Sierra Instruments, Monterrey, CA). Flow rates into each chamber were maintained at rates between 25 and 50 mL min^{-1}, depending on the size of the turtle. A flow multiplexer (Sable Systems, Las Vegas, NV) was used to cycle through measurements of four turtles and a control (chamber without a turtle) sequentially. Each turtle was measured for 30 min with a 10-min baseline between samples; each turtle was sampled nine times within the 48-h run. Excurrent air from each respirometry chamber was passed through a Drierite

Table 1: Mean whole-animal metabolic rates, standard error (SE), and number of samples (*N*) of *Sternotherus odoratus* gravid females, postoviposition females, adjusted gravid females, males, nongravid females, and whole-clutch metabolism

Group	Metabolic rate (J h^{-1})	SE	*N*
Gravid females	103.4	5.6	15
Egg clutch[a]	.30	.03	15
Gravid females, adjusted (AGMR)	103.1	4.9	15
Postoviposition females	73.3	3.7	15
Nongravid females (NGMR)	74.1	4.7	18
AGMR − NGMR	32.7	4.4	15
Males	83.0	4.8	17

[a]Mean clutch size was four eggs. Eggs from each clutch were weighed and measured for metabolic rate 24 h after oviposition.

column to scrub water vapor and then through a CO_2 analyzer (Sable Systems CA-10a).

$\dot{V}_{CO_2}$ was calculated at 3-s intervals for the 48-h testing cycle. We used the most level 15-min period from each 30-min sample (lowest sum of absolute deviations from the interval mean) to calculate the rate of gas exchange using the equations of Withers (1977), implemented in the program LabAnalyst (Warthog Systems, Riverside, CA). Individual metabolic rate was considered to be the mean of the three lowest and most level 15-min measurements during the 48-h test period. To obtain a time sequence for GMR throughout the oviductal egg retention period, metabolism of gravid females was measured approximately every 14 d until oviposition. Gravid females were allowed to lay eggs naturally. Before obtaining a POMR, postpartum females were fed for 2–3 d, fasted for 72 h to again ensure a postabsorptive digestive state, and measured again in the same respirometry chamber.

Egg Metabolic Measurements

After oviposition, eggs were given a unique identification number, and egg metabolism was measured 24 h after oviposition using a closed-circuit O_2 respirometer. The respirometer consisted of a 50-mL syringe containing two ventilation holes drilled near the opening of the barrel and a three-way stopcock. The stopcock allowed subsamples of chamber air to be injected into an airstream passing through the oxygen analyzer (figs. 4.3 and 4.8 of Lighton 2008). Each egg was placed in a separate syringe and flushed with dry and CO_2-free air (scrubbed via Drierite and Ascarite columns) at a rate of 45 mL min^{-1} for 10 min. After being flushed, the syringe plunger was adjusted to 50 mL (enough to cover ventilation holes), the stopcock was sealed, and the syringe was placed into a precision incubator at 25°C for 24 h (sufficient time for measurable O_2 consumption to occur).

Oxygen consumption for each egg was obtained by injecting a 40-mL gas sample (scrubbed of water vapor and CO_2) through the three-way stopcock into the middle of a 2-m section of Tygon tubing connecting an Ametek R-1 flow controller and Ametek S-3A/I oxygen analyzer (dynamic injection technique; Lighton 2008). Oxygen concentration was recorded at 1-s intervals for the duration of the measurement (LabHelper, Warthog Systems). Eggs were weighed to the nearest 0.1 g, and the effective volume of the syringe was calculated by subtracting the volume of the egg assuming 1 g = 1 mL volume (Henen 1997). Metabolic rates (mL O_2 min^{-1}) of eggs were calculated using closed-system calculations for the rate of O_2 consumption (Vleck 1987; Lighton 2008; LabHelper). Energy expenditure of eggs within a single clutch was summed to give whole-clutch metabolic rates.

To account for the energy expenditure of eggs in each gravid female, the whole-clutch metabolic rate was subtracted from the total GMR to yield an adjusted estimate of female gravid metabolic rate (AGMR). Since CO_2 production was measured for adult turtles and O_2 consumption was measured for eggs, a conversion factor was used to convert both rates of gas exchange to common rates of energy use (J h^{-1}). A conversion factor of 20.1 J mL^{-1} of O_2 was used for each egg (mL O_2 h^{-1}; assuming respiratory quotient = 0.8; McDonald 1976; Gessaman and Nagy 1988; Reid et. al. 2009), and a factor of 24.32 J mL^{-1} of CO_2 (assuming respiratory quotient = 0.8) was used for each turtle (Gessaman and Nagy 1988). The energetic cost of retaining eggs in the oviduct was then calculated by taking the difference between the POMR and the AGMR (Angilletta and Sears 2000).

Statistical Analyses

We used ANCOVA for comparisons of metabolic rates among males, nongravid females, and gravid females (adjusted for clutch mass and clutch metabolism) and for comparing females before and after oviposition. Body mass was used as a model covariate in comparisons, and individual ID was modeled as a random effect

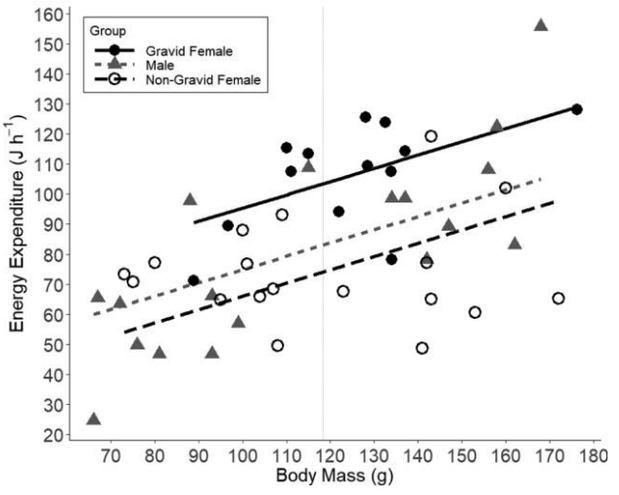


Figure 1. Metabolic rates (J h^{-1}) and fitted relationships for 15 gravid *Sternotherus odoratus* females (filled circles), 17 males (triangles), and 18 nongravid females (open circles). The metabolic rate for one of the gravid females is plotted behind another in the figure. The vertical line indicates the grand mean for the three groups (118 g). Gravid female body mass was adjusted by subtracting the total clutch mass.

when there were repeated measurements of individuals (e.g., pre- and postovipositional females). Post hoc comparisons among groups were conducted using the emmeans package (Lenth 2020). Linear regression was used to determine whether whole-clutch mass, clutch size (number of eggs per clutch), whole-clutch metabolic rate, female body mass, or mean egg width significantly influenced the total cost of oviductal egg retention (AGMR − POMR). All analyses were conducted using R version 3.6 (R Core Team 2020).

Results

Metabolic rates were measured for 15 gravid females, 18 nongravid females, and 17 males. Mean body mass of gravid females was 137.9 ± 24.4 g, with a mean clutch mass of 13.9 ± 3.1 g. Nongravid females and males had mean body masses of 118.2 ± 29.9 g and 114.1 ± 36.2 g, respectively. After adjusting GMRs by subtracting clutch mass and clutch energy expenditure, mean metabolic rates were 103.4 ± 5.6 J h^{-1} for gravid females, 74.1 ± 4.7 J h^{-1} for nongravid females, and 83.0 ± 4.8 J h^{-1} for males (table 1; fig. 1; $F_{3, 45} = 8.08$, $P = 0.001$). Gravid females had approximately 20% higher energy expenditure than males (table 2; $t_{3, 45} = 2.72$, $P = 0.02$) and 33% higher metabolism than nongravid females (table 2; $t_{3, 45} = 4.02$, $P = 0.001$). Males had about 11% higher metabolism than nongravid females, but the difference was not statistically significant (table 2; $t_{3, 45} = 1.35$, $P = 0.38$).

Mean GMR (before oviposition) was 103.1 ± 4.9 J h^{-1}, and mean POMR (after oviposition) was 73.3 ± 3.7 J h^{-1} (fig. 2; $t_{2, 27} = 5.62$, $P = 0.0001$). Mean whole-clutch energy expenditure was low at 0.30 J h^{-1} compared with mean GMR of 103.1 J h^{-1} (table 1). Throughout the egg retention period (days leading up to oviposition), there was no marked change in metabolic rate (fig. 2; $t_{1, 41} = -1.67$, $P = 0.10$). Timing of oviposition varied among females, with observed egg retention periods of 14–78 d between capture and oviposition, and most females had decreased energy investment after oviposition (fig. 3). The energetic costs of oviductal egg retention yielded a 40% increase in energy expenditure for gravid females ((AGMR − POMR)/POMR) relative to their POMR (table 1; fig. 4).

The energetic cost of oviductal egg retention (AGMR − POMR) was positively related to whole-clutch mass (fig. 5*A*; $F_{1, 14} = 6.86$, $P = 0.03$), but there was no effect of female body mass (fig. 5*B*; $F_{1, 14} = 0.98$, $P = 0.34$) such that heavier clutches and heavier females did not increase GMR. There was no apparent influence of clutch size on GMR (fig. 5*C*; $F_{1, 14} = 2.15$, $P = 0.18$), but clutch mass did influence whole-clutch metabolic rates (fig. 5*D*; $F_{1, 14} = 5.31$, $P = 0.03$).

Table 2: Pairwise comparisons of mean metabolic rates for groups of *Sternotherus odoratus* turtles

Contrast	Estimate (J h^{-1})	SE	*t*	*P*
Gravid female/male	20.80	6.06	3.43	.003
Gravid female/nongravid female	29.73	6.01	4.95	.001
Male/nongravid female	8.93	7.06	1.26	.420

Note. Estimates are allometrically adjusted to the mean body mass of all individuals (118 g).

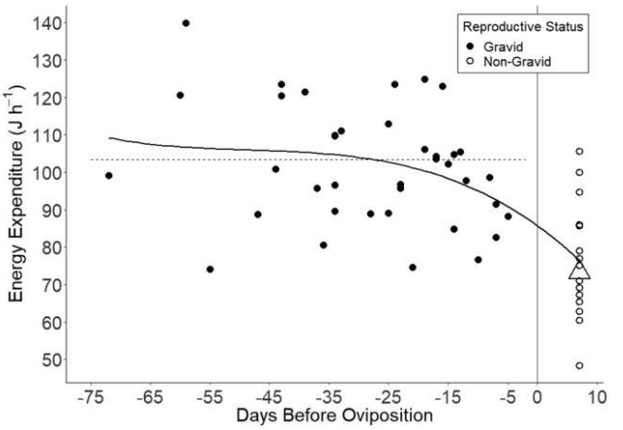


Figure 2. Metabolic rates (J h^{-1}) for 15 gravid *Sternotherus odoratus* females repeatedly measured throughout the oviductal egg retention period and after oviposition. The solid vertical line indicates a break between metabolic rates measured before oviposition and after oviposition (day 0). The dashed horizontal line indicates mean gravid metabolic rate before oviposition, and the triangle indicates mean nongravid metabolic rate after oviposition.

Discussion

Female eastern musk turtles had a ~40% increase in energy expenditure while gravid, a substantial energetic cost of reproduction associated with oviductal egg retention. Thus, counter to our hypothesis, retaining eggs for a prolonged period of time appears to have a high energetic cost to the mother. Lack of parental care in most turtles may necessitate a high energetic investment to the clutch before oviposition to ensure offspring survival and fitness (Rafferty and Reina 2012; Foucart et al. 2018). When determining reproductive costs, oviductal egg retention should be considered because it may potentially come with a high energetic cost to gravid females. For example, the longest egg retention period in our trial was 82 d, which resulted in 65% energetic increase relative to the nongravid state, whereas the shortest egg retention period was 19 d, which resulted in 58% energetic increase.

Metabolic rates of gravid females were either relatively constant or decreased slightly across the days leading up to oviposition, potentially due to the state of developmental arrest of the embryos. During developmental arrest the embryos remain in the gastrula stage, and if environmental conditions are suitable, recommencement of embryo development typically occurs within 12–16 h of being deposited into the nest as eggs experience an increase in oxygen availability (Miller 1997; Williamson et al. 2017). The first sign of continuing development is the appearance of a distinct opaque white spot occurring on the shell, indicating the adhesion of vitelline membrane and inner shell membrane (Ewert 1991). Since

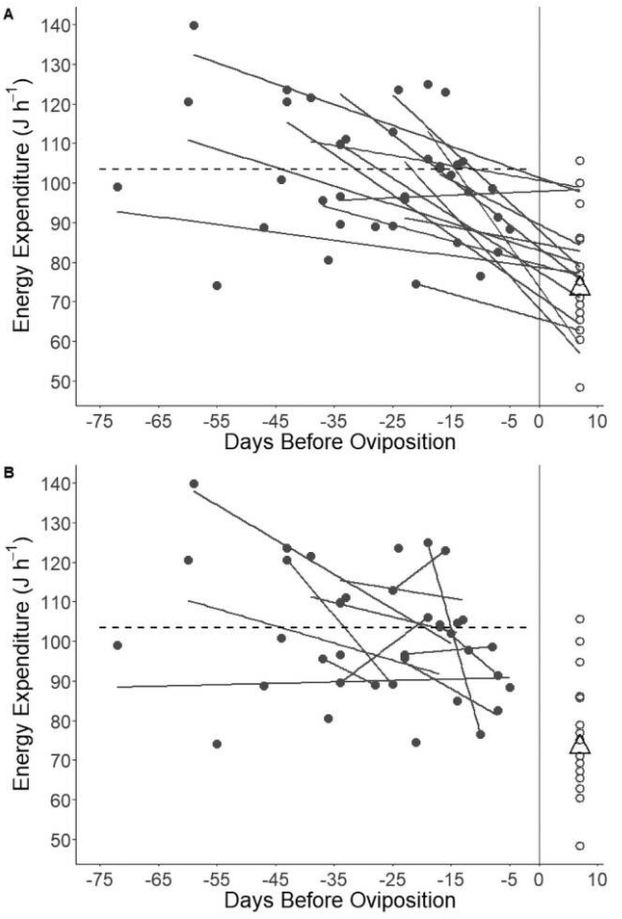


Figure 3. *A*, Metabolic rates for 15 gravid females throughout the oviductal egg retention period and after oviposition in eastern musk turtles. The solid vertical line at zero indicates a break between metabolic rates taken before oviposition and after oviposition. The dashed line represents mean gravid metabolic rate before oviposition, and the triangle indicates mean gravid metabolic rate after oviposition. The sloped lines represent each of the gravid females measured during this study. *B*, Whole-animal metabolic rates for 15 gravid females throughout the oviductal egg retention period in eastern musk turtles. The regression lines do not include the postoviposition metabolic rate. The solid vertical line at zero indicates a break between metabolic rates taken before oviposition and after oviposition. The dashed line indicates mean gravid metabolic rate before oviposition, and the triangle indicates mean gravid metabolic rate after oviposition. The sloped lines represent each of the 15 gravid females.

the energetic demands for the developing embryo are arrested and because embryo development does not continue until after eggs are within the nest, the energetic investment by gravid females remains relatively constant throughout the oviductal egg retention period.

Metabolic rates of gravid females were significantly elevated relative to nongravid females and males because of the energetic requirements of retaining a clutch. Continued calcium mobilization during egg retention may be a contributing factor to the elevated metabolic rates for gravid females (Ewert et al. 1984; Rafferty and Reina 2012). While retaining eggs within the oviduct, small amounts of calcium can be continually added to the eggshells by the Ca^{+} ATPase pump in the oviductal tissue (after the initial shell layer has been added), potentially representing a continuing energetic demand for gravid females throughout the egg retention period (Ewert et al. 1984; Thompson et al. 2007). Further activities associated with arresting embryo development (i.e., production of oviductal secretions) could result in an increased energetic demand for gravid females (Rafferty and Reina 2012; Rafferty et al. 2013).

The energetic cost of oviductal egg retention was influenced by clutch mass, such that females retaining more massive clutches had increased energetic expense. Producing heavier clutches may yield offspring that are larger, which putatively increases survivorship by increasing competitive abilities and predator avoidance (Brockelman 1975; Janzen and Warner 2009). Smaller hatchlings typically have lower fitness or perform poorer in performance-related activities than larger conspecifics (Ewert 1979; Valenzuela 2001). Producing larger, heavier eggs within the clutch would increase energetic investments made by the female during the preoviposition stages, leading to increased costs of oviductal egg retention. We determined that for every increase of 1 g in clutch mass, there is an increase of 2.85 J h^{-1} in energy expenditure for gravid females.

Clutch size (number of eggs) did not appear to play a role in energetic costs of oviductal egg retention. Eastern musk turtles typically produce a mean of four eggs per clutch, with a range of two to six (McPherson and Marion 1981; Ernst 1986; this study). The production of an additional egg would require gravid females to invest more available resources to egg development; thus, we would expect to see a positive trend, with females producing larger clutch sizes having a higher energetic demand. Contrary to this expectation, no obvious trend between metabolic rate and clutch size was observed. However, the sample size may have been insufficient to determine the effects of adding an additional egg, particularly since most of our females produced either 3 (4 of 15 individuals) or 4 eggs (8 of 15 individuals), and clutches of 2, 5, or 6 eggs were each produced by a single individual.

The energetic costs of retaining oviductal eggs in eastern musk turtles were more than double that observed for eastern box turtles (*Terrapene carolina*), which have a ~16% increase in energy use for gravid females compared with nongravid individuals (Clinger 2016). This difference could be attributed to the duration of calcification for rigid-shelled eggs in eastern musk turtles versus flexible-shelled eggs in eastern box turtles (Iverson 1978; Packard et al. 1984; Deeming and Whitfield 2010). The development of rigid eggshells requires continued shell deposition into an advanced stage of calcification after fertilization, contributing to a more columnar egg shape (Packard et al. 1984). Increasing the duration of calcification causes rigid-shelled eggs to contain a lower density of pores and a thicker calcified shell, which contributes to reduced water loss in dry environments, unlike flexible-shelled eggs that are hydrophilic for increased water uptake (Packard and Demarco 1991; Thompson and Speake 2004; Brown and Shine 2005; Zhao et al. 2013). The thick layer of calcium in rigid-shelled eggs that helps prevent water loss also precludes water intake (Packard 1999; Booth and Yu 2009). At oviposition rigid-shelled eggs need to already contain the water required for development (Belinsky

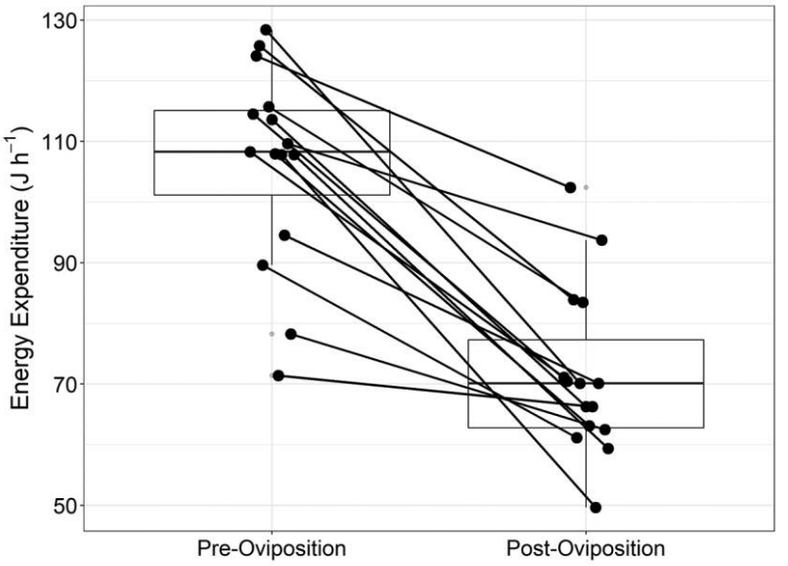


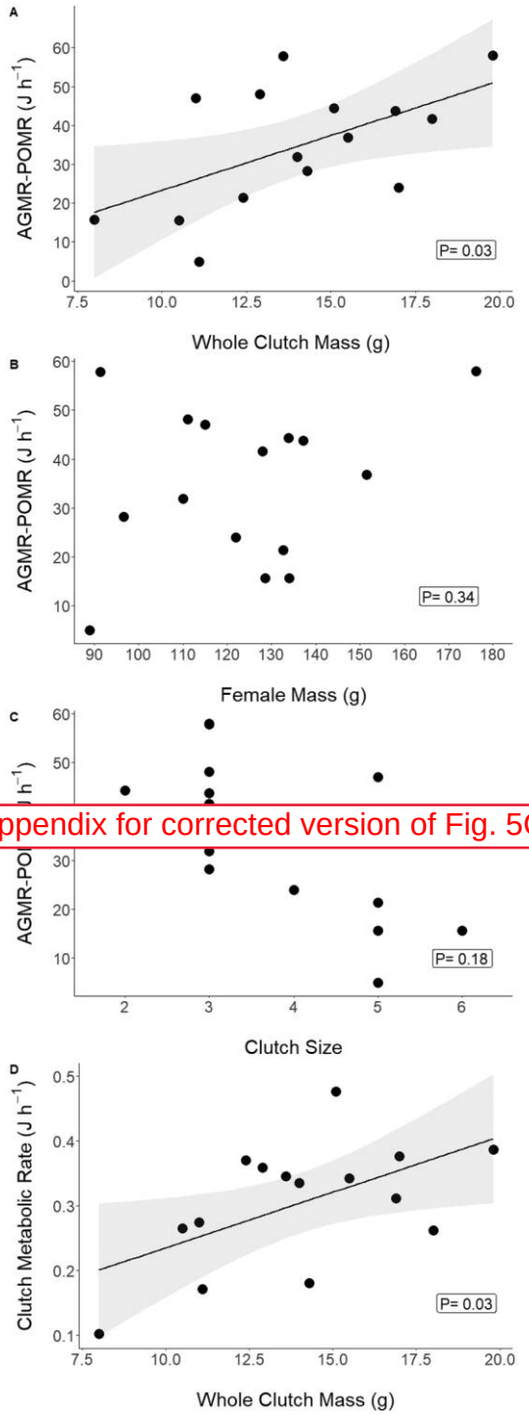
Figure 4. Whole-animal metabolic rates before and after oviposition for 15 gravid females. Box and whiskers plot (1.5 times interquartile range) depicting the range of metabolic rates of the preovipositional or postovipositional state.

et al. 2004); eggs are composed of 40.8% shell and 25.84% yolk (Congdon and Gibbons 1985). Rigid-shelled eggs that retain available water at oviposition typically have larger embryos (as a proportion of total egg mass) compared with flexible-shelled eggs, consume their yolk more quickly, grow faster, and typically have higher metabolism than flexible-shelled eggs that are laid in drier environments (Miller and Packard 1992).

Comparing the energetics of oviductal egg retention to other reproductive processes, such as vitellogenesis, is difficult because other studies largely focus on total reproductive costs for gravid females or variability among species. Reproductive costs (egg production, courtship, mating, nesting, and parental care) for gravid painted turtles (*Chrysemys picta*) was estimated to be 48% of the total available energy (Congdon et al. 1982; Congdon and Gatten 1989; Iverson and Smith 1993), while the total annual reproductive efforts as a portion of body energy content was only about 15% in European tortoises (*Testudo graeca*; Hailey and Loumbourdis 1988). These studies do not distinguish between the various components of reproduction, and energy is an allocation constraint such that energy used for one component of reproduction should decrease the amount available for another (Garland et al. 2021). Further research is needed to scale the required energetics for specific reproductive processes among similar groups. Even among other oviparous reptiles, energetic costs of reproductive activities are highly variable. In squamates the reproductive effort ranges from an increase of 21% to 164% in metabolism while gravid (Demarco and Guillette 1992; Schultz et al. 2008), and costs associated solely with vitellogenesis range from an increase of 26.3% to 42.8% in metabolism (Van Dyke and Beaupre 2011).

Although egg retention has numerous benefits in terms of reproductive phenology, there may also be factors that limit this strategy. Gravid females risk the chance of becoming egg-bound if they retain eggs too long (Clark et al. 2001; Alkindi et al. 2006). After the initial shelling of the egg, calcium can continue to be deposited throughout the retention period (Kuchling 1999). If females wait too long to nest, the eggs could become larger than the pelvic aperture, preventing successful oviposition and jeopardizing survival of both the embryos and the mother. Furthermore, prolonging egg retention potentially increases mortality rates of gravid females because of increased predation rates. Gravid females are encumbered with increased body mass while carrying clutches, leading to the potential for lower mobility when escaping from predators (Miles et al. 2000; Ibáñez et al. 2015). Collectively, these studies suggest that the longer gravid females retain eggs, the greater the risk of losing the entire clutch to predation or other environmental factors.

Our study suggests that allocating reproductive resources toward extended oviductal egg retention is energetically substantial for eastern musk turtles. Though potentially costly, the benefits likely outweigh the costs. Prolonging egg retention after fertilization allows flexibility in timing of reproduction and in nest site selection. Nest site selection is critical in turtles, as placement of nest sites determines developmental rates of embryos, impacts hatchling phenotypes, and influences offspring fitness through effects on moisture content, temperature, and vegetation cover (Packard et al. 1987; Wilson 1998; Spencer 2002; Reid et al. 2009). Prolonging egg retention decreases hatchling mortality in olive ridley sea turtles (*Lepidochelys olivacea*), as environmental cues lead hundreds to thousands of gravid females to come ashore for synchronized nesting (arribada; Plotkin et al. 1997). Synchronized nesting increases the chance that offspring are able to escape predators because the amount of prey is more than what predators will be able to consume in a short time span (Eckrich and Owens 1995). The benefits of retaining eggs for longer than would be strictly necessary may outweigh the negative effects by increasing survival for offspring.



See appendix for corrected version of Fig. 5C.

Figure 5. Energetic costs (J h^{-1}) of oviductal egg retention (adjusted gravid metabolic rate [AGMR] − postovipositional metabolic rate [POMR]/POMR) for 15 gravid *Sternotherus odoratus* females in relation to whole-clutch mass (g; *A*), whole-clutch size (*B*), gravid female body mass (g; *C*), and whole-clutch metabolic rate (J h^{-1}) in relation to whole-clutch mass (g; *D*). The shaded area surrounding the regression line in *A* and *D* indicates a 95% confidence interval.

Many studies of reproductive costs in chelonians have largely focused on the total reproductive costs (which are considerably variable) rather than smaller, nuanced constituent processes that cumulatively dictate reproductive energetics. Further investigations should focus on obtaining more data on the costs of oviductal egg retention across Testudines and determine the mechanisms driving the energetic investment in oviductal egg retention.

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The correct version of Figure 5 is below. Note difference in Fig. 5C compared to published version.