Hold on for One more Day: Oviductal egg retention as a mechanism for flexible reproductive phenology in Eastern Musk Turtles (*Sternotherus odoratus*)

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ABSTRACT

In oviparous reptiles, parental care is often limited to the energy allocated to embryos prior to oviposition. Energy used by reproducing females is allocated towards, nutrient acquisition, fertilization, vitellogenesis, determining the number and size of eggs, eggshell calcification, oviductal egg retention (retaining eggs within the oviduct after fertilization), and nesting activities. Oviductal egg retention in turtles ranges from two weeks to half a year and permits flexibility in the timing of oviposition and is a pivotal mechanism in determining reproductive phenology. The energetic cost of oviductal egg retention in Eastern Musk Turtles (*Sternotherus odoratus*) was investigated by measuring the metabolism of females prior to and following oviposition. Gravid female metabolic rates were adjusted by subtracting clutch metabolic rates and were elevated relative to males and non-gravid females, indicating an associated energetic cost for egg retention. The metabolism of gravid females was relatively constant across the period of oviductal egg retention, but 40% higher pre-oviposition than post-oviposition. Metabolic costs associated with egg retention were correlated with clutch mass and female body mass, but not with clutch size or number of days leading up to oviposition. These results suggest the strategy of oviductal egg retention, has considerable energetic costs for Eastern Musk Turtles, but likely provides critical flexibility in nesting phenology.

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Chapter I: Introduction

Reproductive output is constrained jointly by energy availability and limitations imposed by the environment (James and Whitford 1994). Life history theory suggests that species adopt optimized allocation strategies to partition available energy to the competing processes of maintenance, growth, and reproduction (Fisher 1930; Stearns 1976; Congdon et al. 1982). Since energetic resources available for reproduction are finite, tradeoffs often exist among the competing reproductive processes (Congdon and Tinkle 1982; Congdon 1989). In reproducing individuals these tradeoffs 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).

Theoretical models suggest that energetic costs of reproduction in a species can be categorized as put in two major components: 1) parental investment, the total resources available to be allocated to reproductive activities prior to birth or deposition of eggs and 2) parental care, the component of reproductive effort that is allocated towards individual offspring after birth of hatching (Congdon et al. 1982). Parental investment can be further partitioned into embryogenesis, embryo development, and care of offspring pre or post birth (Congdon and Gibbons 1985; Congdon 1989). Parental investment and care have most often been studied in species where offspring survival is primarily influenced by investment post-birth, as in many altricial mammals and birds (Broderick et al. 2003; Rafferty and Reina 2012). In oviparous reptiles, however, the investment of energy to parental investment is primarily limited to eggs prior to oviposition (Packard and Packard 1988). Available energy is budgeted primarily towards embryogenesis, oviductal egg retention (post-fertilization), and nest site selection (Congdon et al 1983; Congdon 1989; Wallace et al. 2006).

Oviductal egg retention is a pivotal reproductive strategy for regulating the timing of oviposition in many oviparous species (Renfree and Shaw 2000; Lopes et al. 2004; Rafferty and Reina 2012). Egg retention allows flexible reproductive phenology by permitting females the ability to prolong oviposition until environmental conditions are suitable or until they can locate 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 egg retention (Ewert 1991), and retention durations vary greatly across taxa (Andrews and Mathies 2000).The oviductal egg retention period and associated developmental arrest for most squamates is about 25%- 40% of the total embryonic period, but can be more prolonged in turtles (DeMarco 1993; Andrews and Mathies 2000; Rafferty and Reina 2012). Timing of oviductal egg retention differs within chelonians from a couple weeks to many months (Plotkin et al. 1997; Buhlmann et al. 1995).

Most turtles exhibit some degree of oviductal egg retention (Ewert 1985), and once eggs have been fertilized, they rapidly develop a calcified shell and are retained in the oviduct. Embryonic development is then arrested, and eggs remain at the Gastrula stage until oviposition (Ewert 1985; Rafferty and Reina 2012). Despite pronounced differences in duration of egg retention, no studies have yet focused on the energetic costs of this important life history strategy, 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 (*Sternotherus odoratus*), a species that actively uses this strategy over the reproductive season (Risley 1933). I 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 relative to other processes within reproduction (i.e. Vitellogenesis). 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 the energetic cost to females maintaining the embryos in the oviduct should be relatively low compared to other processes within reproduction. These energetic costs should also be influenced by the relative tradeoffs 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.

Chapter II: Methods

Field Collection and Care

Musk Turtles *(Sternotherus odoratus)* 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 ¾ submerged, which allowed traps to be left overnight and be checked the following day. Collecting was conducted during the reproductive season from mid-May to October (Mitchell 1985, Ernst 1986, Ford and Moll 2004). Weights of individuals were recorded (+/- 1g). 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 66mm in carapace length were considered adults and potentially gravid. To determine reproductive state, potentially gravid females were taken to a lab and radiographed to determine the presence of shelled eggs. Females that were not gravid during the initial radiograph were then scanned a second time 7-10 days later to verify the absence of shelled eggs. It is unlikely that we misclassified an individual as non-gravid, because calcification of eggshells begins immediately after the albumen layer is secreted onto the ova, 12-36 h after fertilization (Kutchling 1999). Shelling of eggs may differ with clutch size and turtles that produce large clutches (such as *Chelonia mydas*; clutches with > 20 eggs) show calcified eggs within 72 h following fertilization but calcification can take as long as 7 days (Kutchling 1999). Shelling of eggs typically occurs simultaneously for the entire clutch indicated by ultra-sound examination rarely if at all recording embryos at different stages of development (*Pseudemydura umbrina*; Kutchling 1999).

Turtles were housed individually in 32-liter (60 x 43 x 17 cm) habitat enclosures (translucent plastic tubs) with water depth ~10 cm 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 12:12 hour photoperiod (light: dark cycle). Turtles were fed an *ad libitum* diet of earthworms, mealworms, and musk turtle pellets every two days. All collection, care, and experimental procedures were performed in accordance with Austin Peay State University IACUC guidelines (Protocol # 19-013).

Measurement of Adult Turtle Energy Expenditure

Carbon dioxide production (VCO_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 non-gravid females (NGMR), while both gravid metabolic rates (GMR) and post-oviposition metabolic rates (POMR) were determined for each gravid female. Before initiating a metabolic trial, turtles were fasted for 72 hours to allow food to clear the gut and ensure a post-absorptive digestive state. Individual turtles were placed into clear plastic respirometry chambers (OXO Rectangle Pop Container) and were then placed within a precision incubator (Percival Scientific Series 942 Microprocessor). Temperature was controlled at a constant 25°C and a 12:12 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 and then through a manifold that distributed air into mass flow controllers (Sierra Mass Trak). Mass flow controllers maintained flow rates into each chamber at rates between 25 to 50 mL/min, depending on size of turtle. A Sable Systems MUX flow multiplexer was used to measure four turtles and a control (chamber

without a turtle) sequentially. Each turtle was measured for 30 minutes with a 10-minute baseline between sample. Thus, each turtle was sampled 18 times within the 48 h run. Excurrent air from each respirometry chamber was passed through a Drierite column to scrub water vapor, and then through a CO_2 analyzer (Sable Systems CA-10a).

Carbon dioxide production (VCO_2) was calculated at 3 sec intervals for the 48 h testing cycle, and the most level 15 min period from each 30 min sample was used to calculate the rate of gas exchange using the equations of Withers (1977), implemented in the program Lab Analyst (Warthog Systems). 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 gravid metabolic rate, metabolism was measured approximately every 14 days throughout the pre-oviposition period.

Gravid females were allowed to lay eggs naturally and following oviposition, individual egg metabolism was measured using closed circuit O_2 respirometry (Lighton 2008). Prior to obtaining a post-oviposition metabolic measurement, post-partum females were fed for 2-3 days, fasted for 72 hours to again ensure a post-absorptive digestive state, and measured again in the same respirometry chamber. Metabolic rates of males and non-gravid females were measured using the same protocol, but individuals were only measured once.

Egg Metabolic Measurements

After oviposition, eggs were given a unique identification number and metabolism was recorded 24 hours post-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 3-way stopcock. The stopcock allowed sub-samples of chamber air to be injected into an air

stream passing through the oxygen analyzer (Figures 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 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 measurement for each egg was obtained by injecting a 40 mL gas sample with a 6 sec count from the syringe through the 3-way stopcock into the middle of a 2 m section of Tygon tubing connecting to a Ametek R-1 flow controller and Ametek S-3A/I Oxygen Analyzer (Dynamic Injection Technique; Lighton 2008). Air from the syringe was scrubbed of water vapor and CO_2 and passed through the oxygen analyzer at ~70 mL min^{-1}. Oxygen concentration was recorded at 1 sec 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}) for eggs were calculated using the closed systems calculations for the rate of O_2 consumption (Vleck 1987; Lighton 2008; LabHelper, Warthog Systems). Energy expenditure of eggs within a single clutch were 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 gravid female metabolic rate 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}) (McDonald 1976, Gessaman and Nagy 1988, Reid et. al.

2009) and a factor of 24.32 J mL^{-1} of CO_2 was used for each turtle (Gessaman and Nagy 1988). For each individual the metabolic rate measurement was reported as both the whole-animal metabolic rate (mL CO2 h^{-1}) and mass-adjusted metabolic rate (mL CO_2 g^{-1} h^{-1}). Mass-adjusted metabolic rate was calculated by fitting a common allometric slope between body mass and whole-animal metabolic rate and then adjusting each individual to the mean mass of all turtles (gravid female, non-gravid female, male, Fig. 1). This procedure accounts for the allometric scaling of metabolism with body mass rather than assuming isometric scaling (often termed mass-specific metabolism). The energetic cost of retaining eggs in the oviduct was then calculated by taking the difference between post-oviposition metabolic rate (POMR) and the adjusted metabolic rate (AGMR) (Angilletta and Sears 2000).

Statistical Analyses

I used a Gaussian log-link Generalized Linear Mixed Model (GLMM) fit with all combinations of predictors variables (female body mass, clutch mass, days leading to oviposition, and clutch metabolic rate) and an individual-level random effect of Turtle ID to determine influences on mass-adjusted metabolic rates. The supported models (delta AICc less than 2) were compared by using Akaike' Information Criterion adjusted for a small sample size (AICc; Burnham and Anderson 2002). The best model was then used to determine whether days leading up to oviposition influenced the metabolism of gravid females. An analysis of covariance (ANCOVA) using a Restricted Maximum Likelihood with female body mass as a covariate was used to determine differences between GMR and POMR; indicating the energetic costs of oviductal egg retention. We used an analysis of covariance (ANCOVA) with the emmeans package to compare differences in metabolism among males, non-gravid females, and gravid females (adjusted clutch mass and clutch metabolism) using body mass of each individual was used as a

covariate. 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).

Chapter III: Results

Metabolic rates were measured for 15 gravid females, 18 non-gravid females, and 17 males. For gravid females, post-oviposition metabolic rates (POMR) and whole-clutch metabolic rates were obtained along with gravid metabolic rates. Mean body mass of gravid females was 137.9 ± 24.4 g with a mean clutch mass of 13.9 ± 3.1 g. Non-gravid females and males had mean body masses of 118.2± 29.9 g and 114.1± 36.2 g respectively. After adjusting gravid female metabolic rates by subtracting clutch mass and energy expenditure, mean metabolic rates of gravid females were 103.4 ± 5.6 J h^{-1}, 74.1 ± 4.7 J h^{-1} for non-gravid females, and 83.0 ± 4.8 J h^{-1} for males (mass-adjusted rates for each; Table 1, Fig.2; $F_{3,45}$ 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 non-gravid females (Table 2; $t_{3,45}$=4.02, P=0.001). Males had about 11% higher metabolism than non-gravid females, but the significance of the relationship is unclear (Table 2; $t_{3,45}$= 1.35, P=0.38).

There was a clear effect of oviductal egg retention on whole-female metabolic rate; mean mass-adjusted GMR (pre-oviposition) was 103.1 ± 4.9 J h^{-1} and mean POMR (post-oviposition) was 73.19 ± 3.7 J h^{-1} (Fig. 1 and 3, $t_{2,27}$=5.62, P=0.0001). Mean whole clutch energy expenditure was low at 0.30 J/h compared to mean GMR at 103.1 J h^{-1} (Table 1). The energetic costs of oviductal egg retention yielded a 40% increase in energy expenditure for gravid females (AGMR-POMR)/POMR) to NGMR (Table 1, Fig.5). Timing of oviposition varied among females with the shortest egg retention period being 14 days and longest lasting for 78 days before oviposition. Throughout the egg retention period (days leading up to oviposition) there was no marked change in GMR for gravid females (Table 3 Gaussian models, Fig. 3 and Fig.4, $t_{1,41}$= -1.67, P=0.10).

Comparing the peak GMR, the highest measurements of each individual while gravid, to mean GMR and to mean POMR revealed that peak GMR was not statistically higher than mean GMR (paired $t_{1,14}$= -1.78, P= 0.09), and peak GMR was significantly elevated relative to each individuals POMR (paired $t_{1,14}$= -6.32, p= 0.0001).

The energetic cost of oviductal egg retention (AGMR-POMR) was related to whole-clutch mass but there was no clear significance of female body mass (Fig. 6a, $F_{1,14}$ = 6.86, P=0.03 and Fig. 6c, $F_{1,14}$ = 0.98, P=0.34) such that heavier clutches and heavier females increased gravid female metabolic rate. There was no clear significance of clutch size on GMR (Fig.6b, $F_{1,14}$= 2.15, P=0.18) but clutch mass did influence whole-clutch metabolic rates (Fig. 6d, $F_{1,14}$= 5.31, P=0.03).

Chapter IV: Discussion

Female 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 would have a high energetic cost to the mother. The lack of parental care in turtles may necessitate a high energetic investment to the clutch before oviposition because this enhances offspring survival and fitness (Rafferty and Reina 2012; Foucart et al 2018). Gravid females would have to build up sufficient resources in order to compensate for the increased energetic costs of oviductal egg retention and each processes that provide embryos with vital nutrients and eggshells that provide protection from the microclimate of the nests after oviposition (Ewert 1979). Therefore, when determining reproductive costs, oviductal egg retention should be considered because prolonged clutch retention causes a high energetic cost to the gravid females.

Gravid metabolic rates were either relatively constant or decreased slightly across 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 of cell growth which ensures that egg development is synchronized and that they will be laid at the same stage of development (Ewert 1985; Rafferty and Reina 2012). Recommencement of embryo development typically occurs within hours of being deposited into the nest if environmental conditions are suitable (Miller 1997). The first sign of continuing development typically occurs 1-4 days after oviposition with a distinct opaque white spot occurring on the shell, indicating the adhesion of vitelline membrane and inner shell membrane (Ewert 1991). While being retained within the oviduct, small amounts of calcium can continue to be added to the eggshells (Ewert et al. 1984). We might then expect metabolic rates of gravid females would rise, thus increasing the energetic

costs of maintaining the clutch during oviductal egg retention. However, since gravid metabolic rates did not change across this time period (or slightly decreased), this energetic cost of continued calcium deposition may be negligible.

The energetic cost of oviductal egg retention was influenced by clutch mass, such that females with retaining clutches with heavier masses 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). Smaller hatchlings typically have lower fitness (or perform poorer in performance-related activities) than larger conspecifics (Ewert 1979; Valenzuela 2001). Investment into larger, heavier eggs within the clutch would increase energetic investments made by the female during the pre-oviposition stages, leading to increased costs of oviductal egg retention. During this study, for every 1 g increase in clutch mass there is an increase in 69 J/h. The individual with the largest clutch mass (19.8 g) had a 29% increase in energetic investment relative to the female with the smallest clutch mass (10g).

Clutch size did not appear to play a role in energetic costs of oviductal egg retention in gravid female *S. odoratus*. Eastern musk turtles typically produce a mean of four eggs per clutch, with a range of two to six (Ernst 1986, and this study). Adding an additional egg may not appreciably increase the costs of oviductal egg retention because development is arrested. 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 out of 15) or 4 eggs (8 out of 15) and clutches of 2,5,or 6 eggs were each produced by a single individual.

After accounting for mass of individuals, metabolic rates of gravid females were significantly elevated from males and non-gravid females due to energetic requirements for a

clutch. During a given year females may choose to invest energy toward either reproduction or towards growth (Kunz and Orrell 2004), and many chelonians have bi-annual (every other year) reproductive cycles (Pearse and Avise 2001). By allocating available energy to continued growth, females can overcome reduce morphological constraints on egg size because larger females have larger pelvic apertures and caudal gaps (space between the carapace and plastron through which the eggs pass) (Iverson and Smith 1993; Clark et al. 2001). Larger females would have an advantage over smaller females because they would be capable of producing larger and more eggs, thereby potentially increasing fitness and offspring survival (Rowe 1994). Moreover, smaller females are at a disadvantage because larger females tend to be preferred by breeding males because they would have the ability to produce larger and greater number of eggs (Pearse et al. 2002).

Eastern Musk Turtles *(Sternotherus odoratus)* energetic costs of retaining oviductal eggs were more than double that observed for Eastern Box Turtles *(Terrapene carolina)*; 16% increase in energy compared to non-gravid energy investments (Clinger 2016). This difference could be attributed to prolonging of shell growth of rigid-shelled eggs in *S. odoratus* versus the early termination of shell growth in flexible-shelled eggs in *T. Carolina* . (Iverson 1978; Packard et al. 1984; Deeming and Whitefield 2010). The development of rigid eggshells requires continued shell growth contributing to a more columnar egg shape, a more advanced form of shell growth than with rounded flexible eggshells (Packard et al. 1984). As calcium can be continuously deposited during oviductal egg retention, females would be required to invest a greater amount of energy than would be needed for flexible-shelled eggs (Packard et al. 1984; Kutchling 1999). Thus rigid-shelled eggs would be heavier in mass compared to flexible shelled eggs leading to an increased energy requirement. Rigid-shell eggs contain a lower density of pores and a greater calcified shell,

which contributes to reduced water loss in drier environments (Packard and Demarco 1991; Thompson and Speake 2004; Zhao et al. 2013). This reserve of water increases consumption of yolk by the embryos in the nest, allowing them to grow larger before hatching (Packard 1999). However, the thick layer of calcium surrounding embryos in rigid-shelled eggs that helps prevent water loss also precludes water intake (Booth and Yu 2009). At oviposition these eggs need to already contain the water required for development (Belinsky et al. 2004); eggs are typically composed of 64% water, 40.8% shell, and 25.84% yolk (Congdon and Gibbons 1985). Flexible eggshells develop a thinner mineralized layer of calcium, making the eggs more hydrophilic for increased water uptake (Packard 1991; Brown and Shine 2005). Unlike rigid shells, flexible eggshells contain less water and more yolk because they take up water from the surrounding environment (Pike et al. 2012). Since rigid-shelled eggs contain all the necessary water needed for development, gravid females would not need to seek out nests that will sustain high moisture levels throughout the incubation period.

Many studies of reproductive costs in Chelonians have largely focused on the total reproductive costs (which are considerably variable) rather than smaller nuanced processes that cumulatively dictate reproductive energetics. The total annual reproductive efforts as a portion of body energy content was only about 15% in European Tortoises (Hailey and Loumbourdis 1988), while the total reproductive costs (egg production, courtship, mating, nesting, and parental investment) for gravid painted turtles (*Chysemys picta*) was estimated to be 48% of the total available energy with less than 1% of available energy invested into nesting (Congdon et al. 1982; Congdon and Gatten 1989). Since about half of their yearly energy budget is spent towards reproduction, only about 50% of energy to be allotted towards maintenance and growth.

Even among other oviparous reptiles energetic costs of reproductive activities are highly variable. In squamates the reproductive effort ranges from 21% to 164% increase in metabolic rate while gravid, and costs associated with vitellogenesis ranging from 26.3% to 42.8% increase in metabolism (Demarco and Guillette 1992; Schultz et al. 2008; Van Dyke and Beaupre 2011). Further, within and among species reproductive energetics would change depending on age and available resources. Younger female turtles invest less energy into reproduction than older females because younger individuals benefit from allocating energy towards developmental growth rather than reproduction (Congdon et al. 2003). Since there is a high range of costs associated with reproduction and the duration of oviductal egg retention is variable, comparing oviductal egg retention in Eastern Musk Turtles to other taxa would be difficult.

Prolonged oviductal egg retention is variable among turtles, with some species choosing to retain eggs for weeks to months until suitable nesting conditions become available. On average most *Kinosternon subrubrum* (Eastern mud turtle) retain eggs for 26-50 days and *Trachemys scripta* (pond slider) retain eggs for 23-39 days (Kutchling 1999). Two species of sea turtle *Lepidochelys olivacea* (Olive Ridley Sea Turtle) and *Lepidochelys kempii* (Kemps Ridley Sea Turtle) have shown delayed oviposition for between 14-75 days (Kutchling 1999). Prolonging oviductal egg retention for these species increases the survivorship of offspring. For the chicken turtle (*Deirochelys reticularia*) gravid females regularly overwinter with calcified eggs in the oviduct and lay the clutch the following spring; 4-6.5 months (Buhlmann et al. 1995). Chicken turtles typically inhabit wetlands that dry up in the summer before females are ready to nest (Buhlmann et al. 2009). Having the ability to retain eggs for extended period of time would allow females to wait for optimal nesting conditions, potentially leading to increased nest success and increased reproductive fitness.

Although egg retention has numerous benefits in terms of reproductive phenology, there may also be factors that limit the benefits of this strategy. Gravid females risk the chance of becoming egg-bound if they retain eggs too long (Alkindni et al. 2006). When in the oviduct, calcium can continue to be deposited in small amounts to the eggshells of the embryos after the initial shelling of the eggs (Kutchling 1999). If females wait too long to nest the eggs will become larger than the pelvic aperture, potentially preventing successful oviposition and jeopardizing survival of both embryos and the mother. Further, prolonging egg retention increases mortality rates on gravid females because of the potential for increased predation rates. Gravid females are encumbered with increased body mass while carrying clutches, leading to potential for lower mobility when escaping from potential predators (Miles et al. 2000; Ibanez et al. 2015). The longer gravid females retain clutches the greater they are at risk of losing the entire clutch to predation or other environmental factors effecting turtles (disease).

Our study suggests that allocating reproductive resources towards extended oviductal egg retention is energetically substantial for *S. odoratus*. 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 moisture content, temperature, and vegetation cover (Wilson 1998; 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) (Poltkin et al. 1997). Synchronized nesting increases the chance offspring are able to flee predators because the amount of prey available to predators is significantly more than what predators will be able to consume in a short time span.

The benefits of retaining eggs for longer that would be strictly necessary may outweigh the negative effects by increasing survival and fitness for offspring.

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APPENDIX A: FIGURES

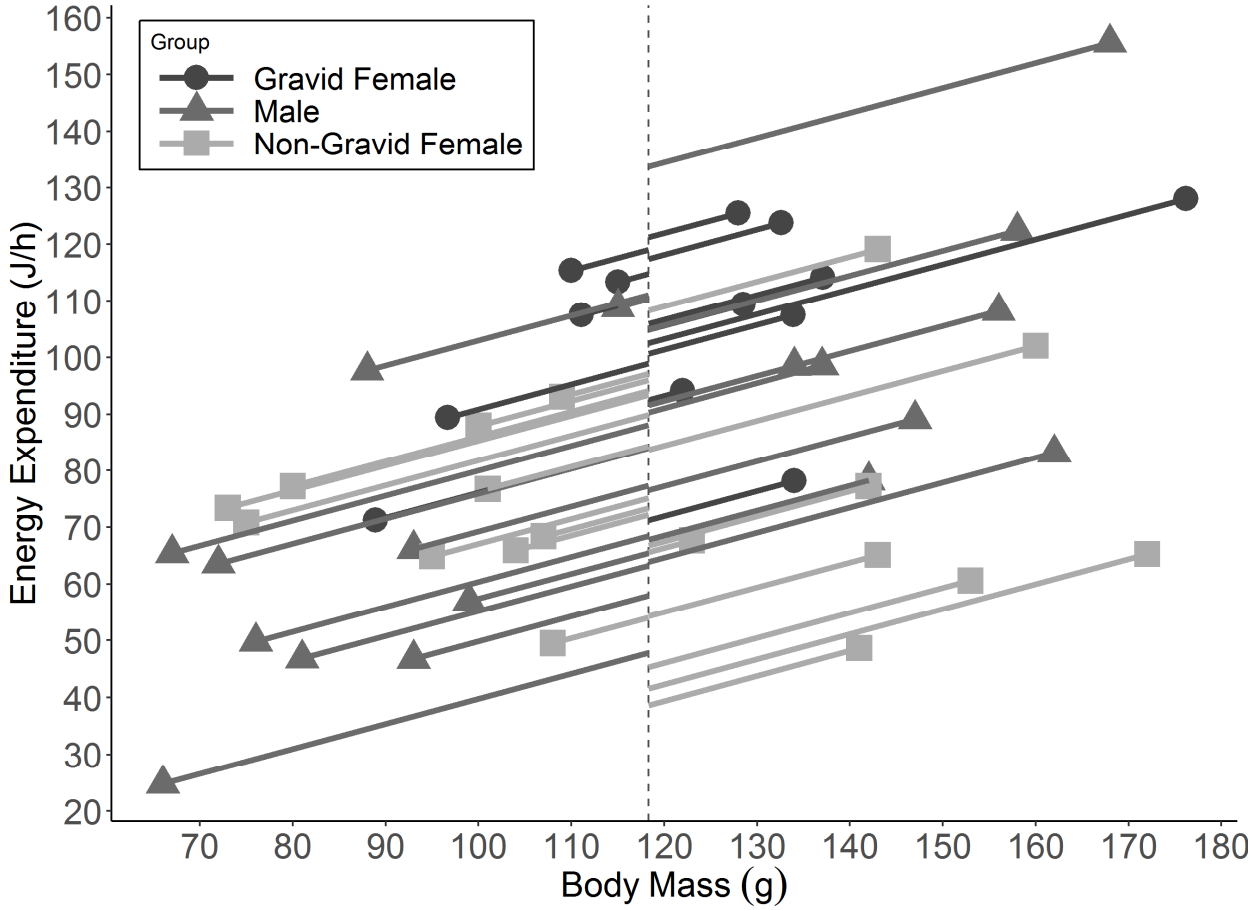

Figure 1: Adjusting each metabolic measurement (J h-1) to the mean gravid female body mass (g) along a common allometric slope for *Sternotherus odoratus*.

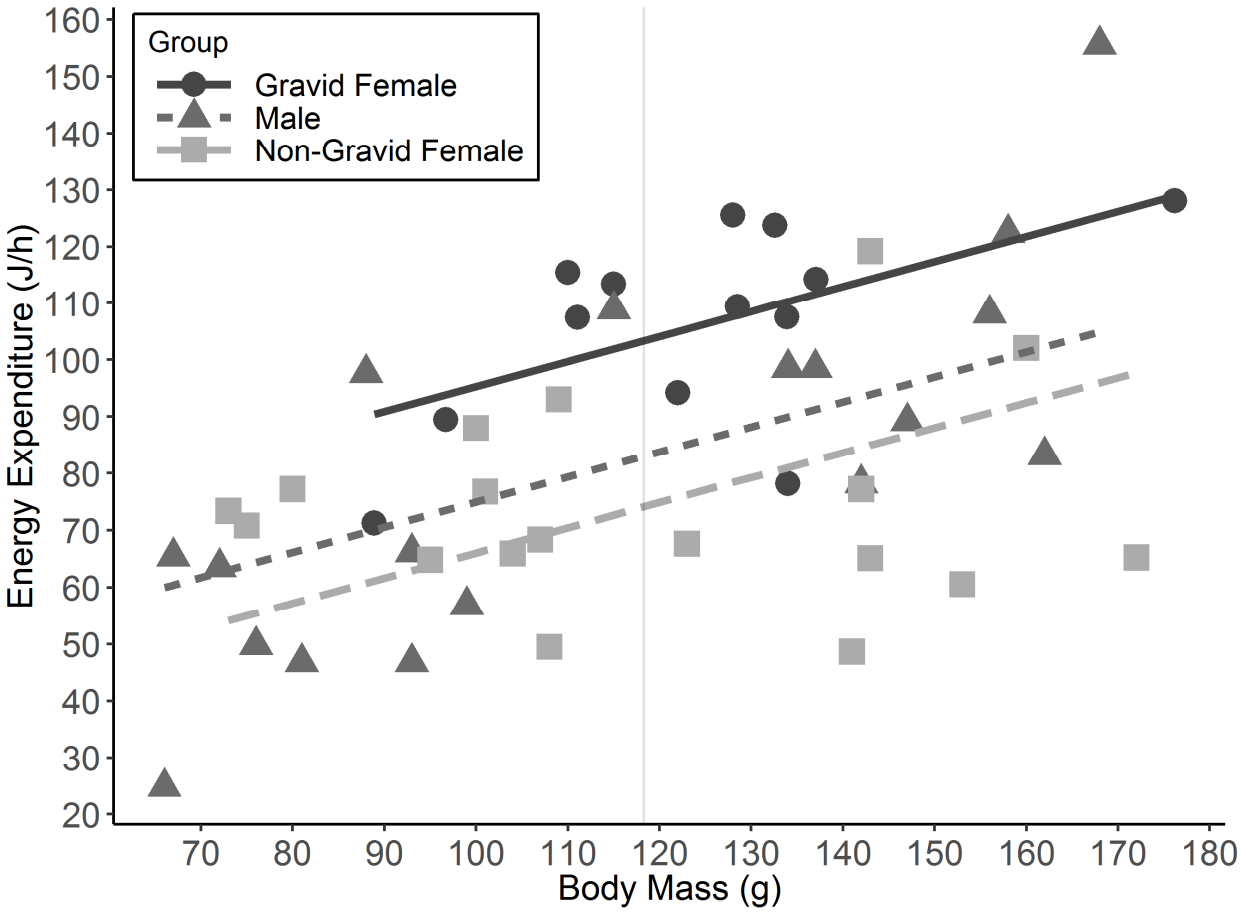

Figure 2: Metabolic rates (J h-1) and fitted relationships for gravid *Sternotherus odoratus* females (circles), males (triangle), and non-gravid females (squares). The vertical line at ~118 g represents the adjusted means for the three groups. Gravid female body mass was adjusted by subtracting the total clutch mass from the female's body mass.

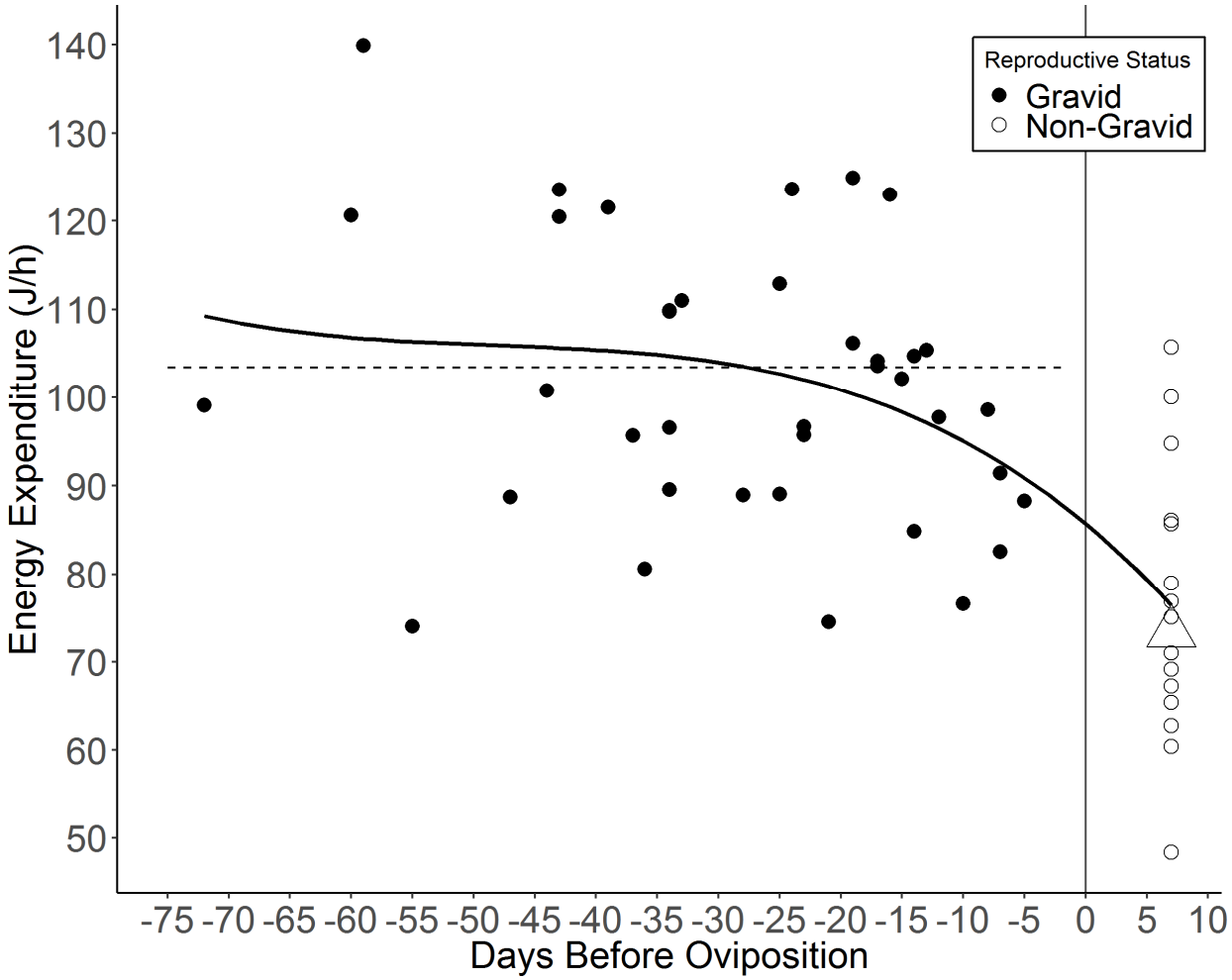

Figure 3: Mass adjusted metabolic rates (J h-1) for 15 gravid Sternotherus odoratus females repeatedly measured throughout the oviductal egg retention (GMR) period and post oviductal egg retention (POMR). The solid vertical line represents a break between metabolic rates taken for pre-oviposition and post-oviposition (day 0). The dashed horizontal line represents mean GMR pre-oviposition and the triangle represents mean NGMR post-oviposition.

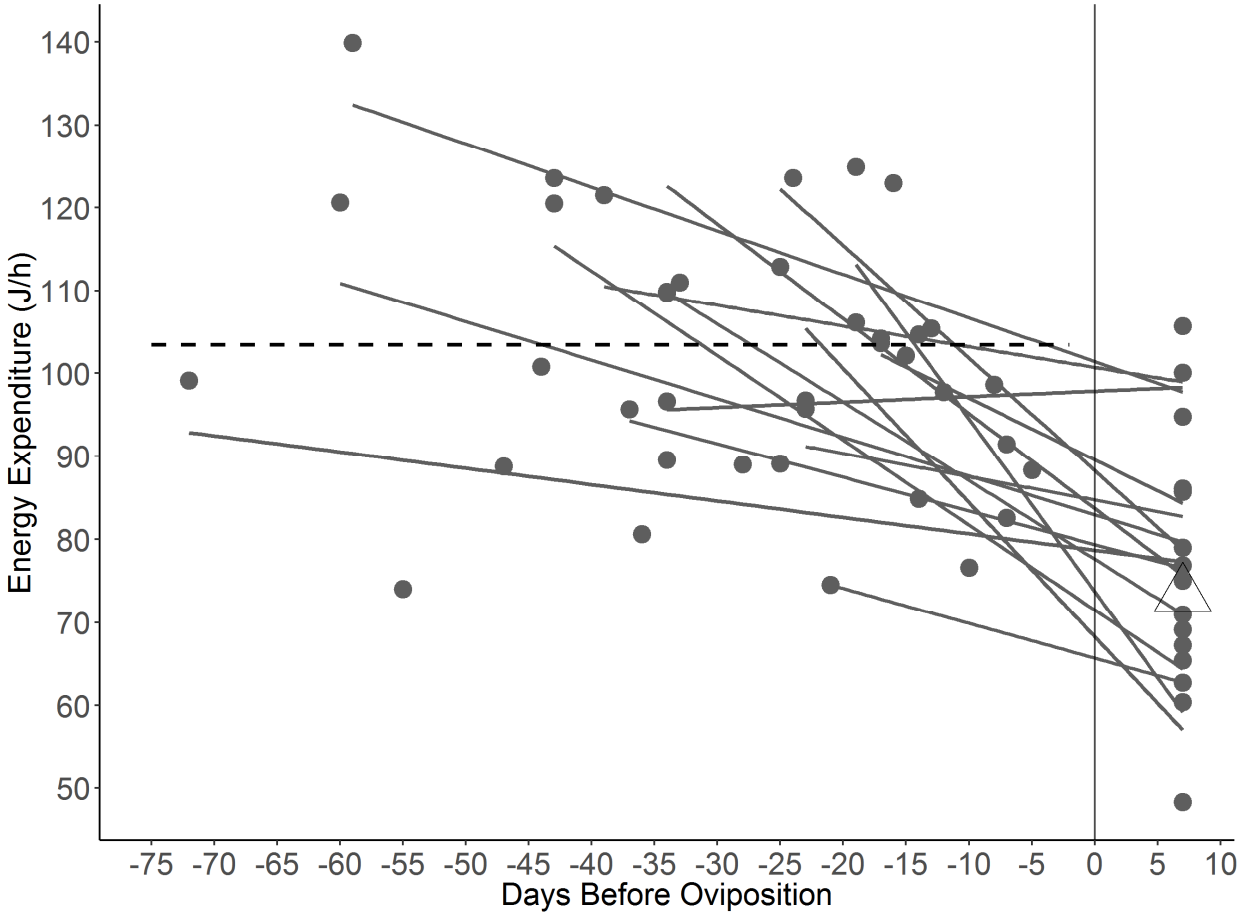

Figure 4: Whole animal metabolic rates (CO_2 production) for 15 gravid females throughout the oviductal egg retention (GMR) period and post-oviposition (POMR) in Eastern Musk Turtles. The solid vertical line at zero represents a break between metabolic rates taken for pre-oviposition and post-oviposition. The dashed line represents mean GMR pre-oviposition and the triangle represents mean (POMR) post-oviposition.

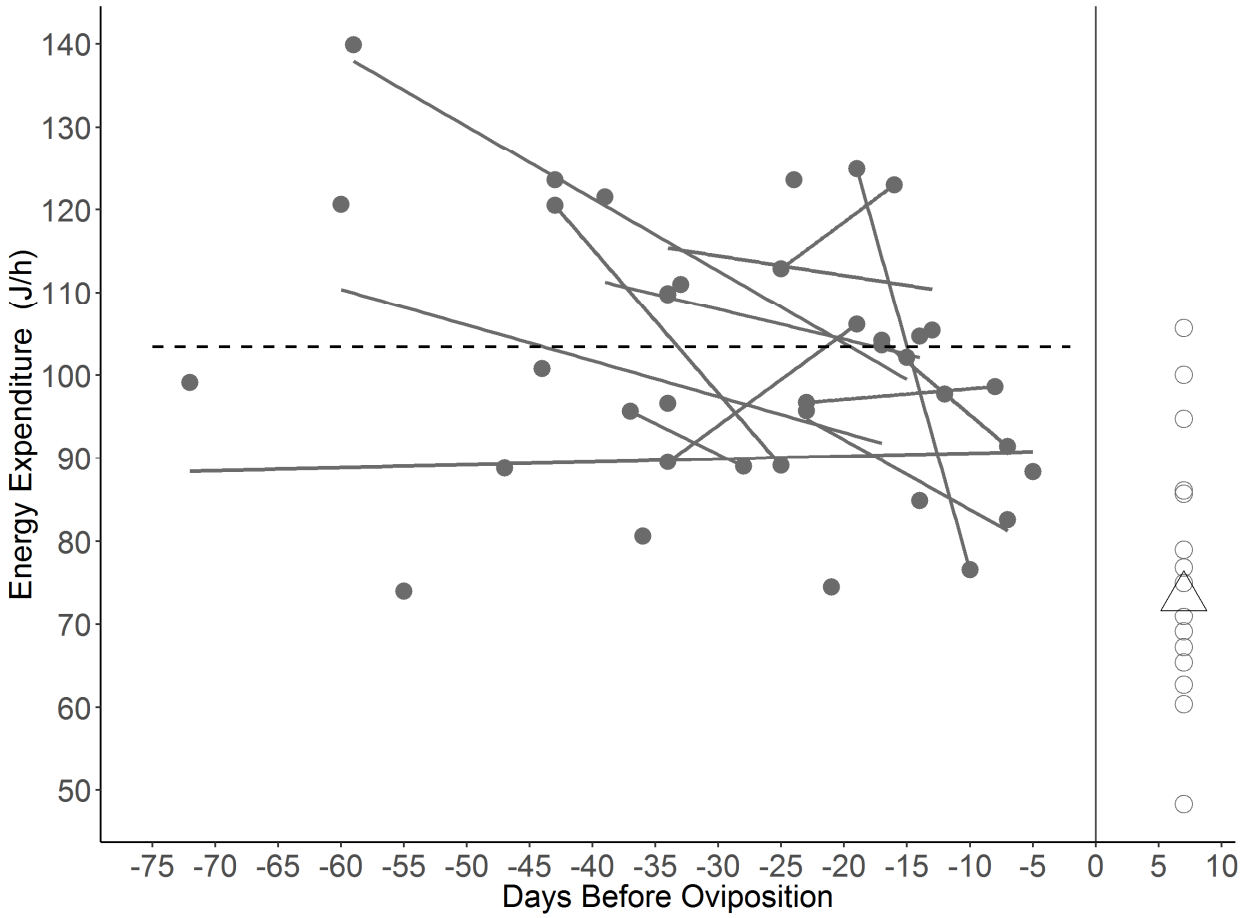

Figure 5: Whole animal metabolic rates (CO2 production) for 15 gravid females throughout the oviductal egg retention (GMR) period in Eastern Musk Turtles. The solid vertical line at zero represents a break between metabolic rates taken for pre-oviposition and post-oviposition. The dashed line represents mean GMR pre-oviposition and the triangle represents mean (POMR) post-oviposition.

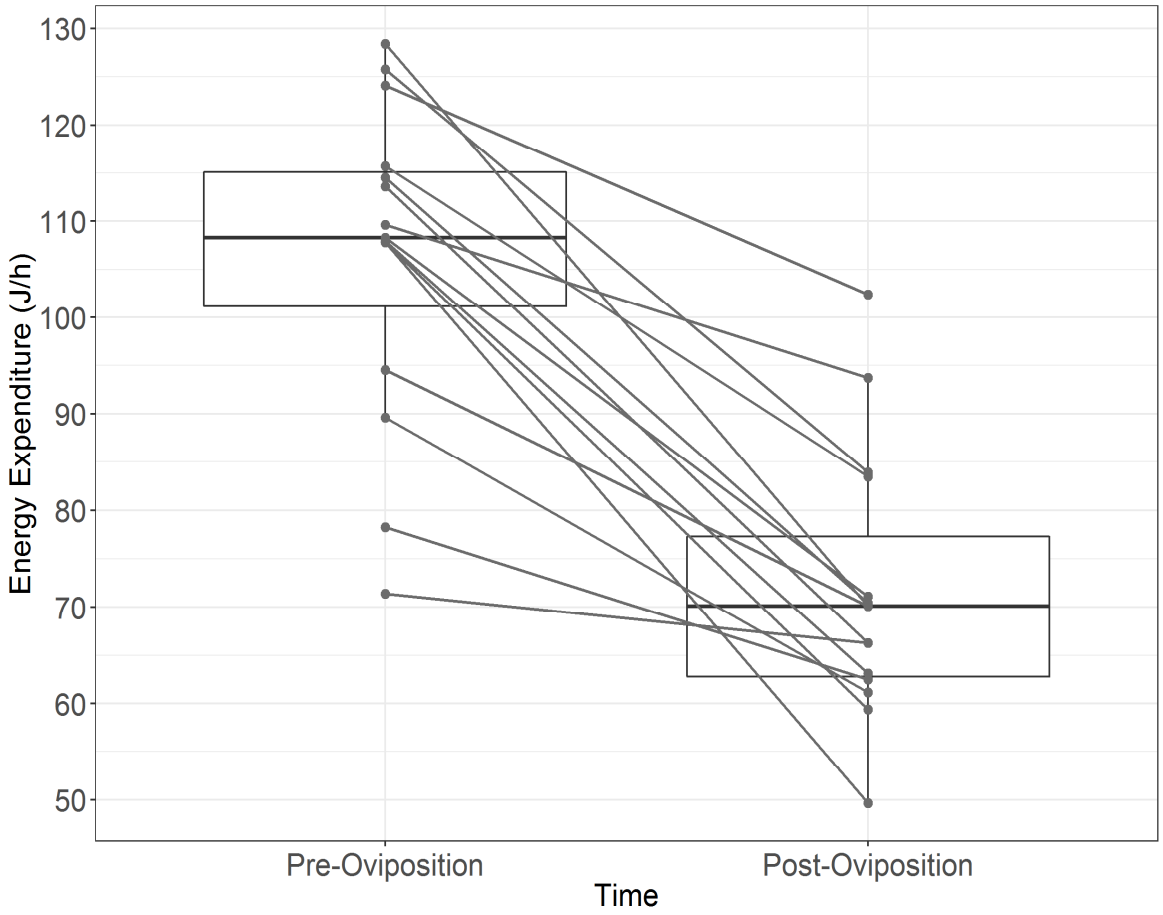

Figure 6: Whole metabolic rates pre and post oviposition for 15 gravid females.

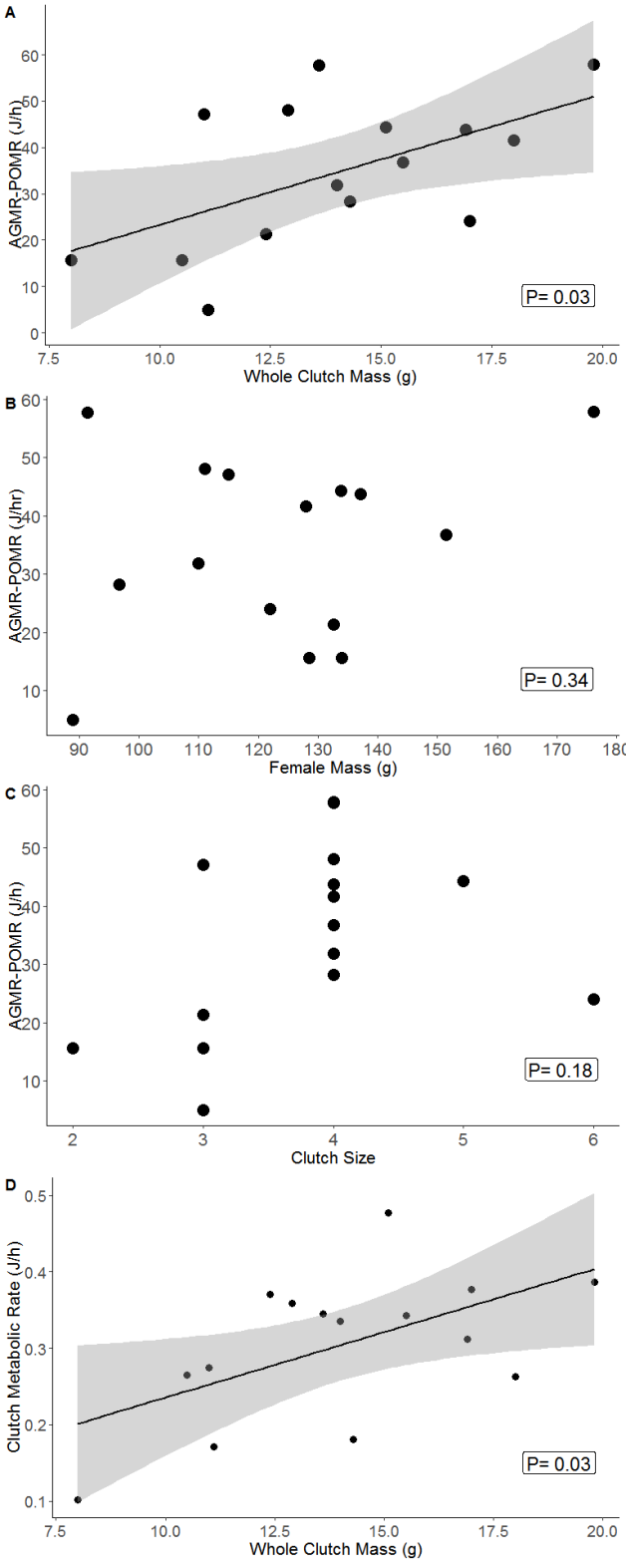
A
AGMR-POMR (J/h)
Whole Clutch Mass (g)
P= 0.03
B
AGMR-POMR (J/hr)
Female Mass (g)
P= 0.34
C
AGMR-POMR (J/h)
Clutch Size
P= 0.18
D
Clutch Metabolic Rate (J/h)
Whole Clutch Mass (g)
P= 0.03

Figure 7: Energetic costs (J h-1) of oviductal egg retention (AGMR-POMR/POMR) for 15 gravid Sternotherus odoratus females in relation to a) whole-clutch mass (g), b) whole-clutch size, c) gravid female body mass(g), and d) whole clutch metabolic rate (J h-1) in relation to whole clutch mass (g)

APPENDIX B: TABLES

Table 1**:** Mean whole animal mean metabolic rates, standard error (SE), and number of samples (N) of *Sternotherus odoratus* for gravid females(GMR), post-oviposition females (POMR), adjusted gravid females(AGMR), male (MMR), non-gravid (NGMR) females, and whole-clutch metabolism

Group	**Energy Expenditure** $(J\ h^{-1})$	**SE**	**N**
Gravid Females (GMR)	103.4	5.6	15
Egg Clutch*	0.298	0.03	15
Gravid Females- Adjusted (AGMR)	103.1	4.9	15
Post-Oviposition Females (POMR)	73.3	3.7	15
Non-Gravid Female (NGMR)	74.1	4.7	18
AGMR-NGMR	32.7	4.4	15
Males (MMR)	83.0	4.8	17

***** Mean clutch size was 4 eggs. All eggs from a clutch were weighed and measured for metabolic rate 24 h post oviposition. The metabolic rates for the clutches were averaged.

Table 2: Pairwise comparisons of group-specific mean metabolic rates with standard errors (SE) of *Sternotherus odoratus* turtles. Estimates are allometrically-adjusted to the mean body mass of all individuals (118 g).

Contrast	Estimate ($J\ h^{-1}$)	SE	*t*	P-value
Gravid Female- Male	20.80	6.06	3.430	0.0030
Gravid Female- Non-Gravid Female	29.73	6.01	4.950	0.001
Male- Non-Gravid Female	8.93	7.06	1.260	0.4200

Table 3: Model selection table for generalized linear mixed models with ΔAIC (AIC is the Akaike information criterion) values < 2 and AIC weights (wt) were used determine effects on gravid metabolic rate (GMR) during oviductal egg retention in *Sternotherus odoratus*. Turtle ID was considered a random effect. Clutch size and clutch mass for each individual gravid female was not included due to high collinearity with clutch metabolism.

Model	**AICc**	**ΔAICc**	**AICcWt**	**Cum.Wt**
GMR~ Female Body Mass	375.2	0	0.21	0.21
GMR ~ Days to Oviposition+ Female Body Mass	375.3	0.01	0.21	0.42
GMR~ Clutch Size+ Egg Metabolic Rate	375.6	0.31	0.18	0.60
GMR~ Days To Oviposition+ Egg Metabolic Rate	375.6	0.37	0.18	0.78
GMR~ Egg Metabolic Rate	376.8	1.56	0.10	0.88
GMR~Clutch Size+ Egg Metabolic Rate	376.9	1.65	0.09	0.97

APPENDIX C: GLOSSARY OF ACRONYMS

Acronym	Description
GMR	Gravid Female Metabolic Rate
AGMR	Adjusted Gravid Female Metabolic Rate. This is calculated by taking the gravid female metabolic rate and subtracting it by the whole clutch metabolic rate.
POMR	Post Oviposition Metabolic Rate for gravid females. This rate was taken after female had nested.
NGMR	Non-Gravid Female Metabolic Rate. Females were considered non-gravid after being x-rayed.
MMR	Male Metabolic Rate.