Geographic Variation in Egg Size and Lipid Provisioning in the Diamondback Terrapin *Malaclemys terrapin*

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ABSTRACT

Understanding phenotypic differentiation among populations of wide-ranging species remains at the core of life-history research, because adaptation to local environmental conditions is expected. For example, when energy resources influence offspring fitness (as in oviparous ectotherms), the egg and hatchling environments are expected to influence selection by acting on the amount of energy allocated to offspring. Here we identify population variation in egg mass, length, width, and volume from diamondback terrapin *Malaclemys terrapin* eggs collected in Rhode Island (RI), Maryland (MD), and South Carolina (SC). Egg size (mean volume: 7.6, 8.1, and 9.1 cc in RI, MD, and SC, respectively) and clutch size (mean no. eggs: 16.1, 12.2, and 6.0 in RI, MD, and SC, respectively) differed among populations, which indicated that females produce larger clutches with smaller eggs at high latitudes and smaller clutches of larger eggs at lower latitudes. Lipid analyses indicated that eggs from SC contained yolks with a higher proportion of nonpolar lipids than did eggs from MD or RI (mean percentage of nonpolar lipids: 22.3%, 22.5%, and 31.8% in RI, MD, and SC, respectively). Thus, female terrapins in SC are laying larger eggs with increased lipid content to provide more energy for the developing embryo. Interestingly, total triacylglycerol (energetic lipid) was greater in southern populations but occurred in higher proportions in northern populations (total triacylglycerol: 88.0%, 85.4%, and 81.9% in RI, MD, and SC, respectively). This variation in triacylglycerol levels demonstrates the necessity for quantifying each lipid component. These data indicate a difference in reproductive strategy by which females in northern populations invest in higher fecundity with less energetic resources per offspring, whereas females in southern populations invest in larger eggs with considerably greater energy reserves.

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Introduction

Oviparous reptiles deposit eggs that must provide sufficient energy for the developing embryo to grow and meet its metabolic costs. The energy required to complete development can vary whenever environmental conditions influence embryonic development and metabolism (reviewed in Packard and Packard 1988). Therefore, broad-scale maternal investment in eggs can vary if environmental conditions alter the energetic requirements necessary to produce an embryo while simultaneously influencing the mother's fitness through offspring number (Smith and Fretwell 1974; Brockelman 1975; Messina and Fox 2001). Although the evolutionary significance of intraspecific variation in life-history traits such as offspring size and growth rates in wide-ranging species has been well studied (see reviews in Niewiarowski 1994; Morrison and Hero 2003), few studies have explored variation in maternal investment in eggs (Sinervo and Huey 1990; Du et al. 2010) on a similar scale.

Many species of reptiles, especially turtles and lizards, exhibit significant variation in clutch and egg sizes across their range (Sinervo et al. 1992; Iverson et al. 1993; Litzgus and Mousseau 2006). Iverson et al. (1993) demonstrated that clutch size increases and egg size decreases with increasing latitude in 11 turtle families representing 146 species. Although body size explains variation in egg size for many reptiles (Shine 1992; Du et al. 2005), habitat type, food availability, and breeding season also have explained such variation (Litzgus and Mousseau 2006; Haenel 2011). Egg size influences offspring fitness traits such as size (Sinervo and Huey 1990; Roosenburg and Kelley 1996; Du et al. 2010), swim speed (Miller et al. 1987; Miller 1993), and running speed (Sinervo and Huey 1990). Janzen et al. (2000) provided evidence that larger red-eared slider *Trachemys scripta* offspring have higher survivorship during migration after emergence. Alternatively, a long-term experiment indicated hatchling survivorship is not correlated with body size in snapping turtles *Chelydra serpentina* (Congdon et al. 1999). Thus, situations may exist in which there is no benefit afforded to larger offspring (Laurie and Brown 1990).

For oviparous reptiles, the egg represents the majority of the female's reproductive investment, and larger eggs typically represent a larger maternal investment per offspring (Bernardo 1996). With the exception of the energy used for choosing a

nest site, chelonian eggs contain virtually all the energy allocated by the female to each offspring. The yolk is composed primarily of protein and lipid materials and contains most of the material used by the developing embryo (Noble 1991; Thompson et al. 2001). Triacylglycerol, phospholipids, cholesterols, and cholesteryl esters are the major lipid classes in reptile yolks. Triacylglycerol comprises 70%–87% of the total lipids (Ballinger et al. 1992; Nagle et al. 1998) and serves as the primary energy source for the developing embryo (Congdon and Tinkle 1982).

Bobyn and Brooks (1994) showed that intermediate-sized snapping turtle hatchlings that had greater yolk reserves grew faster and survived longer in captivity compared with those with lesser yolk reserves. In a comparison of 12 turtle species, Congdon and Gibbons (1985) suggested turtle species whose hatchlings overwinter in the nest cavity allocate proportionally more lipid to the eggs than do species that emerge in the fall; however, Nagle et al. (1998) did not see this pattern in turtles of the family Kinosternidae. Water is another component in reptilian eggs, and Finkler et al. (2004) described a longitudinal gradient in water content in snapping turtle eggs. Such variation in egg size and components indicates that egg contents may reflect selection acting on egg size when environmental factors influence hatchling fitness.

The diamondback terrapin *Malaclemys terrapin* is a wide-ranging emydid turtle that has a continuous range from Massachusetts through much of the Texas coast. Seven subspecies are recognized based on morphological differences observed across the terrapin's range (Carr 1952). Hart (2005), however, recognized six genetically distinct management units, although three units from the eastern United States clustered together due to reduced variation relative to the remaining units.

Terrapins exhibit geographic clines in body size, egg size, and nesting season dates. Adult body size increases with increasing latitude (Seigel 1984; Roosenburg 1994), egg size decreases with increasing latitude (Seigel 1980), and nesting seasons begin later at higher latitudes (Seigel 1984; Roosenburg 1994). Mean clutch size increases with increasing latitude (Seigel 1980); however, Roosenburg and Dunham (1997) reported a mean clutch size in Maryland (MD) that was higher than those reported from New Jersey (Montevecchi and Burger 1975) and New York (Feinberg and Burke 2003). Interestingly, maximum clutch frequency seems consistent at three clutches per year among populations in southern Florida (Seigel 1980), MD (Roosenburg and Dunham 1997), and Rhode Island (RI; Mitro 2003). Terrapin egg size varies among clutches within a single population but varies little within a clutch (Roosenburg and Dunham 1997).

Herein, we describe clutch and egg size variation among three populations along a latitudinal gradient. We ask whether egg and lipid components vary among populations and, if so, whether the patterns are explained by variation in egg size alone or by differences in allocation strategies among populations. We also ask whether egg components vary throughout a complete nesting season in MD. Water content, nonpolar lipids (NPLs), and lean dry mass are known to increase with egg size in a single population (Roosenburg and Dennis 2005); however,

these components have not been compared among populations or throughout a complete nesting season.

Material and Methods

We located terrapin nests during the 2004 nesting season on Grice Island, South Carolina (SC; 32°47'N, 79°56'W), Patuxent River, MD (38°27'N, 76°39'W), and Nokum Hill, RI (41°45'N, 71°31'W). Nests that were less than 12 h old, determined by fresh tracks and the presence of unchalked eggs, were excavated to count, measure (length and width to the nearest millimeter), and weigh (to the nearest 0.1 g) each egg. We calculated egg volume using the ellipsoid formula [volume = $(\pi/6)$(length)(width^2)] (Iverson and Ewert 1991). For eggs from MD and RI, we froze ($-20°$C) two randomly selected eggs from a subset of clutches for lipid analysis. For eggs from SC, because of small clutch sizes, only one egg from a subset of clutches was collected and frozen ($-20°$C). Furthermore, because it was difficult to locate fresh nests in SC, we induced oviposition (1.5 mL kg^{-1} body mass) in females that were captured while ascending the nesting beach (Ewert and Legler 1978). Oxytocin is a less potent mammalian hormone that mimics the behavior of arginine vasotocin to induce oviposition (LaPointe 1977). Because oviposition was induced only in females that were already ascending the beach, this method would not impact the egg components that would have been delivered to the egg before ovulation, fertilization, shell production, and then nesting (Nagle et al. 1998).

All eggs were collected within a 2-wk period at the beginning of nesting season for each population (RI: June 20; MD: June 1; SC: May 20). Thus, a different female produced each clutch that we collected, and each nest most likely represents the first clutch of the season for that female. Because most eggs were collected after oviposition, we could obtain female body size only from oxytocined females from SC. Female body size increases with latitude in terrapins (reviewed in Brennessel 2006), so variation in egg size across populations may be partially explained by differences in the females' body size (Iverson et al. 1993; Litzgus and Mousseau 2006).

We also collected three eggs from freshly deposited nests during the beginning (June 1), middle (June 25), and end (July 20) of the nesting season on the Patuxent River, MD. Collecting eggs throughout the nesting season in 2003 allowed us to look at within-nesting-season variation in egg size and energy content among clutches. Earlier work suggests that NPL mass does not vary within a clutch (Roosenburg and Dennis 2005) in this population, so we were confident that the collected eggs were representative of the entire clutch.

We transported frozen eggs to Ohio University and thawed them before separating the yolk from the shell and albumen. The components were dried separately at 60°C to a constant mass (to the nearest 0.0001 g). We ground the dried yolk to an even consistency using a mortar and pestle. A SoxTech HT2 1045 extraction unit refluxed petroleum ether over the sample for 90 min at 100°C in a new preweighed Whatman cellulose thimble (33 mm × 80 mm). We rinsed samples for 45 min,

and the remaining solvent evaporated for an additional 15 min. We then dried the extracted lipids in a drying oven at 60°C for 60 min before weighing to the nearest 0.0001 g. The calculated sample recoveries after transferring to the extraction thimbles ranged from 96% to 99%. Previous work indicates that this method results in the extraction of 100% of the NPL within the sample (Roosenburg and Dennis 2005). We extracted six empty extraction thimbles to serve as a blank control with no change in their mass. The lean dry yolk mass was calculated as the dry yolk mass minus the lipid dry mass.

We used thin-layer chromatography (TLC) and flame-ionization detection (FID) to quantify the yolk lipid components (Institute of Marine and Environmental Technology, Baltimore, MD). A 0.1-g subsample of yolk was dissolved in 4 mL of 2 : 1 ratio of methylene chloride and methanol to extract the total lipids. We homogenized the material for 3 min and then centrifuged the sample at 2,000 rpm for 15 min. This procedure was repeated to ensure high extraction efficiency (89%–94%). The extracted lipids were washed with 0.88% KCl in water and then allowed to dry overnight.

We reconstituted the dry lipids in methylene chloride at a concentration of 10 $\mu g/\mu L$. After solvent focusing (Ackman et al. 1990), samples were spotted on Chromarod silica-gel-coated glass rods in triplicates. The samples were focused in a 1 : 1 ratio of methanol and chloroform and then developed for 45 min in an 85 : 15 : 0.1 ratio of hexane, diethyl ether, and formic acid to separate sterol esters, triacylglycerols, and cholesterol. Then, we scanned the rods in an Iatroscan TH-10 TLC/FID analyzer using a hydrogen flame with a gas flow rate of 150 mL/min. Each rod was scanned for 30 s to produce the component lipid peaks. We ran a series of standards, in triplicate, for esters, triacylglycerols, and cholesterol to produce standard curves for analysis ($r^2 > 0.98$ for all curves). The final component values for each sample were calculated as the mean of each triplicate set. Component values obtained from the Iatroscan were also multiplied by total wet yolk mass to determine the total content value for each yolk.

We analyzed data using SAS for PC, version 9.1.3. Egg size and lipid composition data were log transformed, as appropriate, to meet the assumption of normality for all parametric analyses. A multivariate ANOVA (MANOVA) was used to test for differences in egg size, and then a nested ANOVA was used to partition the variation of each egg measurement among locations (fixed effect) and clutch (nested effect). Linear regression analysis determined the relationship between egg size

and lipid composition. After revealing a strong clutch effect, egg and lipid data were analyzed using an ANCOVA with clutch mean values as treatment effects and lean dry yolk mass as the covariate. A Tukey-Kramer (TK) post hoc analysis was used for pairwise comparisons of all significant results. We also tested for homogeneity of slopes by testing for a significant population by covariate interactions.

Results

Egg size (MANOVA, Wilks's $\lambda = 0.55$, $F_{6,457} = 53.1$, $P < 0.001$) and clutch size (ANOVA, $F_{2,40} = 119$, $P < 0.0001$) differed among all populations (TK), indicating that larger clutches with smaller eggs occur at high latitudes and smaller clutches with larger eggs at low latitudes (table 1). Egg mass (ANOVA, $F_{2,423} = 13.4$, $P < 0.0001$), egg length (ANOVA, $F_{2,423} = 18.1$, $P < 0.0001$), and egg volume (ANOVA, $F_{2,423} = 8.3$, $P = 0.0001$) differed among populations. There was also a clutch effect on egg mass (ANOVA, $F_{40,423} = 55.5$, $P < 0.0001$), egg length (ANOVA, $F_{40,423} = 26.7$, $P < 0.0001$), and egg volume (ANOVA, $F_{40,423} = 44.1$, $P < 0.0001$). Although egg width differed significantly among clutches (ANOVA, $F_{40,423} = 41.1$, $P < 0.0001$), egg width did not vary among populations (ANOVA, $F_{2,423} = 3.0$, $P = 0.06$). Because of the strong clutch effects, all further analyses were conducted using clutch mean values as independent measurements. Additionally, egg size in MD did not vary among eggs collected at the beginning, middle, and end of the nesting season (egg mass: ANOVA, $F_{2,25} = 1.9$, $P > 0.05$; egg length: ANOVA, $F_{2,25} = 0.12$, $P > 0.05$).

Changes in egg size were accompanied by changes in both egg length and egg width in all populations (fig. 1). Clutch mean egg length (RI: $r = 0.94$, $P < 0.0001$; MD: $r = 0.53$, $P < 0.04$; SC: $r = 0.46$, $P < 0.05$) and clutch mean egg width (RI: $r = 0.97$, $P < 0.0001$; MD: $r = 0.90$, $P < 0.0001$; SC: $r = 0.90$, $P < 0.0001$) are correlated with clutch mean egg mass among all populations. The slopes of the regression lines were similar among the three populations for mean egg length ($F_{2,37} = 0.95$, $P = 0.3943$) and mean egg width ($F_{2,37} = 1.47$, $P = 0.2423$), which suggests that the relationship in egg length and width to egg mass is similar for all populations (fig. 1).

Egg energy content varied across populations such that eggs in SC contained greater energy stores than did eggs in the more northern populations. The mean wet yolk mass increased from RI to MD and then to SC (ANCOVA, $F_{2,32} = 4.5$, $P = 0.0182$; table 2), and thus NPL mass per unit lean dry mass

Table 1: Egg size measurements from *Malaclemys terrapin* eggs collected in Rhode Island, Maryland, and South Carolina

State	No. clutches/ no. eggs	Clutch size	Clutch mass (g)	Egg mass (g)	Egg length (mm)	Egg width (mm)	Egg volume (cc)
				Mean ± SE			
Rhode Island	12/193	16.1[A] ± .65	135.6[A] ± 7.1	8.4[A] ± .08	32.9[A] ± .13	20.9 ± .08	7.6[A] ± .08
Maryland	14/171	12.2[B] ± .42	121.2[B] ± 4.4	9.9[B] ± .09	34.3[B] ± .13	21.2 ± .09	8.1[B] ± .08
South Carolina	17/102	6.0[C] ± .38	62.4[C] ± 4.2	10.4[B] ± .08	36.3[C] ± .12	21.8 ± .08	9.1[C] ± .08

Note. Means of significant variables (shown in bold) followed by a unique letter indicate significant pairwise comparisons ($P < 0.05$ by Tukey test).
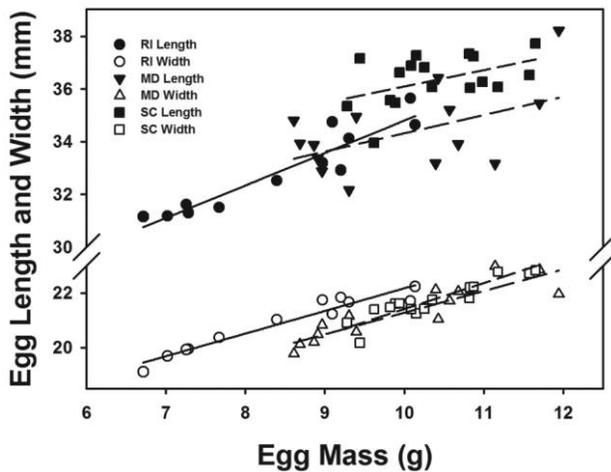
Figure 1. Relationship between egg width (open symbols) and egg length (filled symbols) to egg mass in *Malaclemys terrapin* eggs collected from Rhode Island, Maryland, and South Carolina.

increased similarly among the three populations (ANCOVA, $F_{2,32} = 12.3$, $P = 0.001$). The NPL mass increased from 0.384 g in RI to 0.668 g in SC and contributed from 22.3% of the dry yolk mass in RI to 31.8% in SC. Additionally, water (ANCOVA, $F_{2,9} = 1.5$, $P = 0.2727$) and NPL (ANCOVA, $F_{2,9} = 0.6$, $P = 0.5648$) content were similar in MD eggs collected through the nesting season. For all populations, the NPL mass was positively correlated with yolk lean dry mass (RI: $\rho = 0.86$, $P = 0.0003$; MD: $\rho = 0.74$, $P = 0.022$; SC: $\rho = 0.66$, $P = 0.0077$; fig. 2). Egg yolk from SC and MD had similar mean lean mass (1.5 g) but was significantly larger than lean mass in RI eggs (1.37 g; $F_{2,32} = 3.5$, $P = 0.042$). The slopes of the relationship of lean dry mass with water mass ($F_{2,30} = 0.35$, $P = 0.7095$) and lean dry mass with NPL mass ($F_{2,30} = 0.43$, $P = 0.6522$) were similar for all three populations.

The major component of the yolk lipids was triacylglycerol (energetic lipids) for all populations. The total triacylglycerol in eggs increased with decreasing latitude (ANCOVA, $F_{2,19} = 31.2$, $P < 0.0001$) as a function of larger eggs with more yolk. However, the proportion of triacylglycerol to total extracted lipid actually increased (table 3) with increasing latitude. Cholesterols and phospholipids (both structural lipids) made up a smaller percentage of the total yolk dry mass for all pop-

ulations but decreased in mass with increasing latitude (cholesterol: $F_{2,19} = 5.12$, $P = 0.0167$; phospholipid: $F_{2,19} = 14.93$, $P < 0.0001$).

Triacylglycerol (RI: $\rho = 0.78$, $P = 0.0214$; MD: $\rho = 0.92$, $P = 0.0036$; SC: $\rho = 0.64$, $P = 0.0404$) and cholesterol (RI: $\rho = 0.41$, $P = 0.0310$; MD: $\rho = 0.92$, $P = 0.0039$; SC: $\rho = 0.73$, $P = 0.0379$) were correlated with lean dry yolk mass among all populations (fig. 3). Phospholipid mass was correlated with lean yolk mass only in MD (RI: $\rho = 0.01$, $P = 0.9951$; MD: $\rho = 0.96$, $P = 0.0007$; SC: $\rho = 0.09$, $P = 0.8389$). The slopes of the fit lines for the relationship of triacylglycerol and cholesterol with lean yolk mass did not differ among populations (triacylglycerol: $F_{2,17} = 0.075$, $P = 0.9276$; cholesterol: $F_{2,17} = 2.06$, $P = 0.1582$), but the relationship between phospholipids and lean yolk mass did vary with population ($F_{2,17} = 3.685$, $P = 0.0449$).

Discussion

We identify a trend among three populations of *Malaclemys terrapin* that suggests a geographic cline in which clutch size increases and egg mass, volume, and length decrease with increasing latitude. Clutch size and mass in RI are more than twice what they are in SC. Larger eggs within and among populations contain more energetic lipids than do smaller eggs, and the energetic content of smaller eggs from RI is approximately 57% of the energetic content of the larger eggs from SC. Interestingly, RI eggs contained a higher percentage of triacylglycerol (88%) than did other populations; however, the total energetic content of MD and SC eggs was still greater than that of RI eggs. This indicates a shift in reproductive strategy in which females in northern populations invest in larger clutches of smaller eggs and females in southern populations invest in larger but fewer eggs. Female body size may partially explain this variation (Congdon and Gibbons 1985), but recent studies indicate that ambient temperature can influence reproductive allocation in ectotherms (Du et al. 2010; Telemeco et al. 2010; Starostová et al. 2012).

The chelonian egg typically contains energy in excess of what is required to complete embryogenesis. Residual yolk and hatchling fat bodies are used for maintenance and dispersal after hatching (Congdon 1989). The prevalence of this excess energy within and among turtle species suggests that it plays an important role in energy levels after hatching. Because most

Table 2: Egg yolk components in *Malaclemys terrapin* eggs collected in Rhode Island, Maryland, and South Carolina

State	No. clutches/ no. eggs	Mean ± SE					
		Wet yolk mass (g)	NPL mass (g)	Lean mass (g)	% Water (wet yolk)	% NPL (wet yolk)	% NPL (dry yolk)
Rhode Island	11/22	3.95[A] ± .15	.384[A] ± .15	1.37[A] ± .05	53.2 ± 2.81	9.9 ± .76	22.3 ± .86
Maryland	9/18	4.19[B] ± .14	.471[B] ± .02	1.53[B] ± .05	53.2 ± 2.74	10.3 ± .90	22.5 ± .45
South Carolina	15/15	4.59[C] ± .16	.668[C] ± .02	1.55[B] ± .05	51.5 ± 2.70	14.8 ± .83	31.8 ± .64

Note. Means of significant variables (shown in bold) followed by a unique letter indicate significant pairwise comparisons ($P < 0.05$ by Tukey test). NPL = nonpolar lipid.
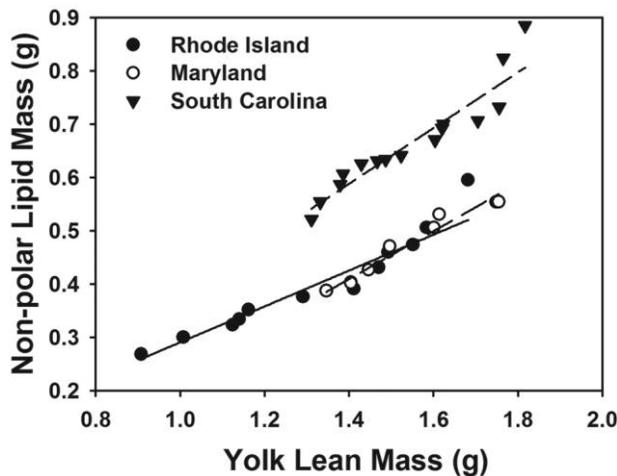
Figure 2. Relationship between nonpolar lipid mass and yolk lean mass in *Malaclemys terrapin* eggs collected from Rhode Island ($r^2 = 0.93$, $y = 0.37x - 0.086$), Maryland ($r^2 = 0.96$, $y = 0.44x - 0.204$, and South Carolina ($r^2 = 0.89$, $y = 0.55x - 0.178$).

turtle hatchlings emerge at the end of the resource-rich growing season or overwinter in their nest, energetic lipids in the residual yolk may provide enough energy to survive the negative energy balance experienced until resources again become abundant (Nagle et al. 1998). The amount of energy that remains can vary when incubation conditions (e.g., higher temperatures) require more energy to complete embryogenesis (Congdon and Gibbons 1990; Lance and Morafka 2001). If warmer incubation temperatures in SC require more energy to complete an embryo, then selection may be increasing egg size as a mechanism to increase energy for embryogenesis.

Incubation temperature and soil moisture can influence the rate of development and possibly the rate of energy utilization during embryogenesis (Gutzke and Packard 1987; Janzen et al. 1990). For example, turtle species with hatchlings that overwinter in the nest chamber are provided with more energy (Congdon et al. 1983; Rowe et al. 1995). Species with extended incubation periods may require a larger energetic investment to complete embryogenesis (Ewert 1985); thus, eggs from these species, but not necessarily the hatchlings, will have a higher proportion of energetic lipids (Nagle et al. 1998). Nagle et al. (1998) reported that egg lipid levels varied among species, but these differences were not reflected in the hatchling lipid levels.

Interestingly, we find that eggs in RI, where overwintering is common, are smaller and have less energy than eggs in SC, where hatchlings emerge immediately. Given that female turtles in RI, compared with female turtles in SC, allocate a smaller proportion of NPL to the eggs, this species demonstrates a pattern of increasing energy allocation in populations with immediate emergence. During winter months, the northern populations are exposed to cooler temperatures and shorter days that result in hibernation (Yearicks et al. 1981) and most likely in reduced metabolic rates and energy expenditures (Reese et al. 2002). Therefore, it is likely that hatchlings from southern populations that emerge immediately after hatching and that do not hibernate will have higher energy expenditure after hatching than will hatchlings from northern populations. This variation in energy demand after hatching may explain the variation in egg size and lipid levels described in our study.

We found no difference in egg mass, egg length, or egg width among eggs collected through a single nesting season in MD. Additionally, there were no differences in lipid content among the eggs, which suggests that, on average, female investment in reproduction is similar throughout the nesting season. Follicle enlargement typically begins in the fall (Ernst 1971; Congdon and Tinkle 1982), with final follicle maturation in the spring (Congdon and Tinkle 1982), but for species such as *Chrysemys picta*, *Trachemys scripta*, and *M. terrapin* that deposit two or more clutches in a season, follicles for several clutches typically develop simultaneously (Congdon and Gibbons 1990). In contrast with our findings, second clutches in *T. scripta* have proportionally more energy than first clutches, but this extra energy is most likely to derive from harvested energy and not from stored reserves (Congdon and Gibbons 1990).

Similar to previous studies with terrapins (Roosenburg and Dennis 2005), egg mass correlated more strongly with egg width than with egg length. However, there were no differences in mean egg width among the populations, which suggests that within-population variation in egg mass is influenced by egg length and width but among-population variation in egg mass is influenced primarily by egg length. These different patterns may reflect a constraint in egg morphology in southern populations, because females are smaller in southern populations (Seigel 1984) yet produce larger eggs. Although female body size was not measured in this study, adult female terrapins in southern populations are generally smaller than adult females in northern populations. To enlarge egg size, females in southern populations may have to increase egg length more than width because of a relatively smaller pelvic aperture size in these

Table 3: Yolk lipid components in *Malaclemys terrapin* eggs collected in Rhode Island, Maryland, and South Carolina

State	No. eggs	Triacylglycerol (g)	Triacylglycerol (% of total lipids)	Cholesterol (g)	Cholesterol (% of total lipids)	Phospholipids (g)	Phospholipids (% of total lipids)
Rhode Island	8	.37[A] ± .04	88.0 ± 1.3	.0062[A] ± .001	1.5 ± .05	.044[A] ± .04	10.4 ± 1.18
Maryland	7	.44[B] ± .04	85.4 ± 1.8	.0083[A] ± .01	1.5 ± .11	.073[B] ± .01	13.0 ± 1.69
South Carolina	8	.65[C] ± .03	81.9 ± 1.1	.0120[B] ± .001	1.5 ± .13	.134[C] ± .01	16.6 ± 1.05

Note. Means of significant variables (shown in bold) followed by a unique letter indicate significant pairwise comparisons ($P < 0.05$ by Tukey test).
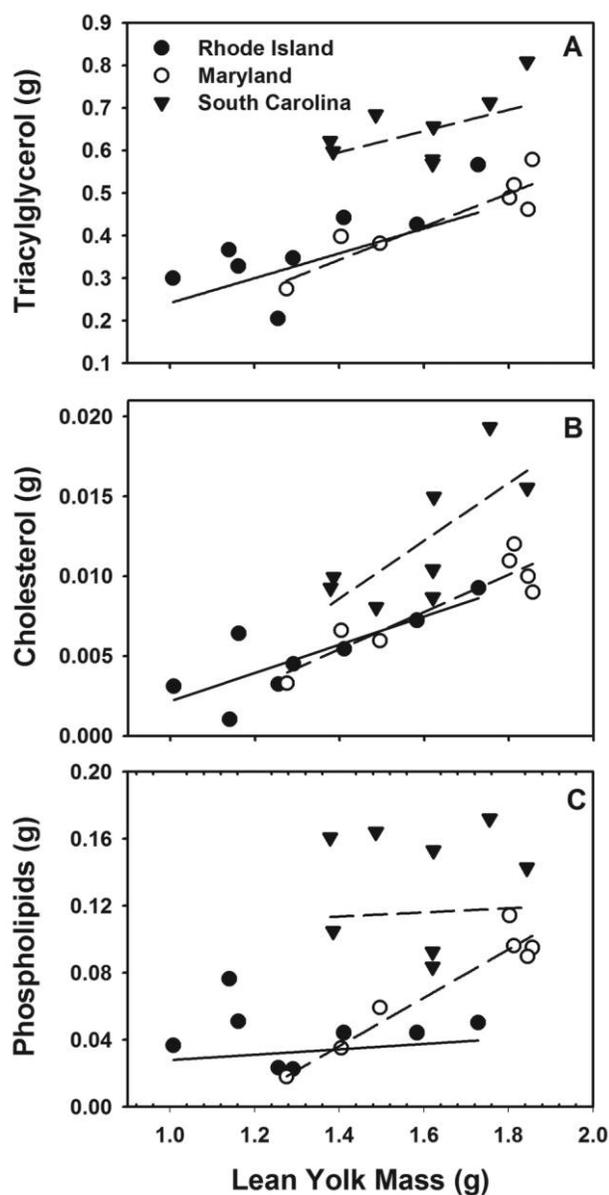
Figure 3. Relationships of triacylglycerol, cholesterol, and phospholipids from *Malaclemys terrapin* eggs collected in Rhode Island, Maryland, and South Carolina.

populations (Tucker et al. 1978; Congdon and Gibbons 1987; Clark et al. 2001). However, within a given population, egg size may increase through increased length and width if there is a strong correlation between egg size and female body size. Such a correlation has been documented in other species of turtles (Congdon et al. 1987; Iverson and Moler 1997; Bowden et al. 2004), but additional research is needed to resolve this issue.

The positive relationship among NPL mass and yolk lean mass was not different among populations; thus, the change in lean mass was proportional to the change in NPL mass. However, total NPL mass and water content were greater in eggs from SC, and the proportions of NPL to wet yolk mass

(14.8%) and dry yolk mass (31.8%) were higher in eggs from SC than in eggs from MD or RI. Females in southern populations are not only building larger eggs but also providing the egg with more NPL. Rowe (1994) described egg size variation in populations of *C. picta* but did not see differences in the proportion of NPL (Rowe et al. 1995).

Triacylglycerol is the primary component of egg lipids in all three populations (82%–88%). These values are comparable to *Kinosternon bauri* (86%), *Kinosturnon subrubrum* (85%), *Sternotherus odoratus* (84%), and *Apalone mutica* (82%; Nagle et al. 1998, 2003) but are higher than those reported for *Chelydra serpentina* (68%), *C. picta* (72%), and *Emydoidea blandingii* (70%; Rowe et al. 1995). Interestingly, there was a general increase in the proportion of triacylglycerol to total lipids with increasing latitude. The total lipids in eggs from RI contained a higher proportion of triacylglycerol (88%) than did the total lipids in eggs from SC. However, the SC eggs contained a larger amount of total triacylglycerol in the egg. This change is associated with a decrease in the percentage of phospholipids allocated to the total lipids. Phospholipids function primarily as structural components of cell membranes, so providing a higher proportion of triacylglycerol at the expense of phospholipids may influence the relationship between offspring size and egg size among populations.

Cholesterol is a structural lipid that is often extracted in nonpolar solvents and may influence the accuracy of using NPL measurements as an index of available energy stores (Nagle et al. 1998). This study found that cholesterol is a minor component of the total lipids (1.5%) and that the proportion does not vary among populations. The variation in the energetic NPL (triacylglycerol) was complemented by differences in quantities of polar lipids, which suggests that NPL measurements alone should not be used as an accurate measure of the available energy stores in eggs of this species.

The population variation described here indicates egg size and yolk energy allocation may be tightly coupled to the incubation and hatchling environment through the energetic demand of the developing embryo and hatchlings. This may lead to local adaptation that optimizes maternal investment in offspring and offspring number. Comparisons of embryological energetics and hatchling energy utilization, growth, and survivorship need to be described among different populations to further test this hypothesis.

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