Relationships of maternal body size and morphology with egg and clutch size in the diamondback terrapin, Malaclemys terrapin (Testudines: Emydidae)

5 AUTHORS, INCLUDING:

Jacquelyn C Guzy
University of Arkansas
13 PUBLICATIONS **19** CITATIONS

SEE PROFILE

Jeff Lovich
United States Geological Survey
109 PUBLICATIONS **1,723** CITATIONS

SEE PROFILE

Michael E. Dorcas
Davidson College
104 PUBLICATIONS **1,399** CITATIONS

SEE PROFILE

Relationships of maternal body size and morphology with egg and clutch size in the diamondback terrapin, *Malaclemys terrapin* (Testudines: Emydidae)

MAXIMILIAN M. KERN[1]*, JACQUELYN C. GUZY[2], JEFFREY E. LOVICH[3], J. WHITFIELD GIBBONS[4] and MICHAEL E. DORCAS[5]

[1]*Graduate Group in Ecology, University of California Davis, Davis, CA, 95616, USA*
[2]*Department of Biological Sciences, University of Arkansas, Fayetteville, AR, 72701, USA*
[3]*U.S. Geological Survey, Southwest Biological Science Center, 2255 North Gemini Drive, Flagstaff, AZ, 86001-1637, USA*
[4]*Savannah River Ecology Laboratory, PO Drawer E, Aiken, SC, 29802, USA*
[5]*Department of Biology, Davidson College, Davidson, NC, 28035-7118, USA*

Because resources are finite, female animals face trade-offs between the size and number of offspring they are able to produce during a single reproductive event. Optimal egg size (OES) theory predicts that any increase in resources allocated to reproduction should increase clutch size with minimal effects on egg size. Variations of OES predict that egg size should be optimized, although not necessarily constant across a population, because optimality is contingent on maternal phenotypes, such as body size and morphology, and recent environmental conditions. We examined the relationships among body size variables (pelvic aperture width, caudal gap height, and plastron length), clutch size, and egg width of diamondback terrapins from separate but proximate populations at Kiawah Island and Edisto Island, South Carolina. We found that terrapins do not meet some of the predictions of OES theory. Both populations exhibited greater variation in egg size among clutches than within, suggesting an absence of optimization except as it may relate to phenotype/habitat matching. We found that egg size appeared to be constrained by more than just pelvic aperture width in Kiawah terrapins but not in the Edisto population. Terrapins at Edisto appeared to exhibit osteokinesis in the caudal region of their shells, which may aid in the oviposition of large eggs. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **00**, 000–000.

ADDITIONAL KEYWORDS: constraint – optimal egg size – reproduction – reptile – turtle.

INTRODUCTION

Natural selection should favour individuals that efficiently partition resources to maximize fitness. Resources allocated to growth, maintenance, and storage increase the parent's own chance of surviving to produce future generations in subsequent seasons. Conversely, allocating resources to reproduction favours higher offspring yield and/or offspring survival (Smith & Fretwell, 1974). Because energy available for reproduction is limited, organisms face trade-offs between the size or quality of offspring and the number of offspring produced (Smith & Fretwell, 1974; Brockelman, 1975). Producing more

offspring can increase the female parent's fitness because this increases the probability that some offspring will survive sufficiently long enough to contribute to future generations (Lack, 1954). However, by investing more energy (e.g. larger yolk reserves or embryo size) in fewer offspring, a mother can produce better quality offspring that are generally assumed to have higher survivorship and fitness (Bobyn & Brooks, 1994; Janzen & Warner, 2009; but see also Congdon *et al.*, 1999).

Optimal egg size (OES) theory predicts that egg size should be optimized within a single population by natural selection and that any increase in resources available for current reproduction should result in a larger clutch size (CS), or more clutches, but not larger egg size. The optimal size for eggs is

*Corresponding author. E-mail: maxkern@ucdavis.edu

predicted to occur at the point where increased fitness associated with producing larger eggs is equal to fitness reductions caused by producing fewer eggs (Smith & Fretwell, 1974; Brockelman, 1975). However, studies across various taxa have demonstrated how factors such as maternal phenotype and recent environmental conditions can cause deviation from OES theory (Wiklund, Karlsson & Forsberg, 1987; Bowden *et al.*, 2004; Lalonde, 2005). For example, maternal phenotype can constrain offspring size such that variation in egg size arises in a population when small-bodied females are unable to successfully oviposit an egg of the optimal size achieved by larger females as the result of some form of morphological constraint (Ryan & Lindeman, 2007). In such cases, egg size tends to be correlated with maternal body size and therefore not optimized. Additionally, if maternal phenotype influences the quality of habitat that eggs and offspring experience (e.g. nest location), variation in egg size within a single population would be expected, a phenomenon termed phenotype/ habitat matching (Roosenburg, 1996; Einum & Fleming, 2002; Rollinson & Brooks, 2008).

Many species of reptiles, especially turtles, display a correlation between maternal body size and egg size that contradicts the predictions of optimal egg size theory (Ryan & Lindeman, 2007). The egg sizes of some larger-bodied species of turtle such as yellow-bellied sliders (*Trachemys scripta* Schoepff 1792) and Florida river cooters (*Pseudemys floridana* LeConte 1830) appear to be morphologically unconstrained because pelvic aperture width (PAW; the distance between the two ilia where the egg passes through the pelvis) (Fig. 1A, B) increases at a faster rate than maximum egg width (EW) (Congdon & Gibbons, 1987; Wilkinson & Gibbons, 2005). However, some smaller-bodied species of turtles do not meet the prediction of optimal egg size theory; specifically, that CS rather than egg size should be increased when an increase in reproductive output becomes possible (Clark, Ewert & Nelson, 2001; Wilkinson & Gibbons, 2005; Rollinson & Brooks, 2008). For example, common map turtles (*Graptemys geographica* LeSueur 1817) and stripe-necked terrapins (*Mauremys leprosa* Schweigger 1812) were found to increase both CS and egg size with increasing body size (Ryan & Lindeman, 2007; Naimi *et al.*, 2012). Morphological constraints on egg size such as PAW and caudal gap height (CGH; the distance between the end of the carapace and the end of the plastron at the tail) (Fig. 1C) may account for some deviations from predictions of optimal egg size theory in turtles (Congdon & Gibbons, 1987; Clark *et al.*, 2001). In addition, the diversity of architectures exhibited by the turtle pelvis may also contribute to observed variation (Lovich *et al.*, 2012).

Morphology and other factors affecting egg size can result in five different responses in turtles according to Lovich *et al.* (2012) (Fig. 2). The first of these potential responses is that OES theory is met and egg size is unconstrained and optimized. A second potential response is that egg size is not optimized because it is constrained by either PAW or CGH. A third possibility is that egg size is
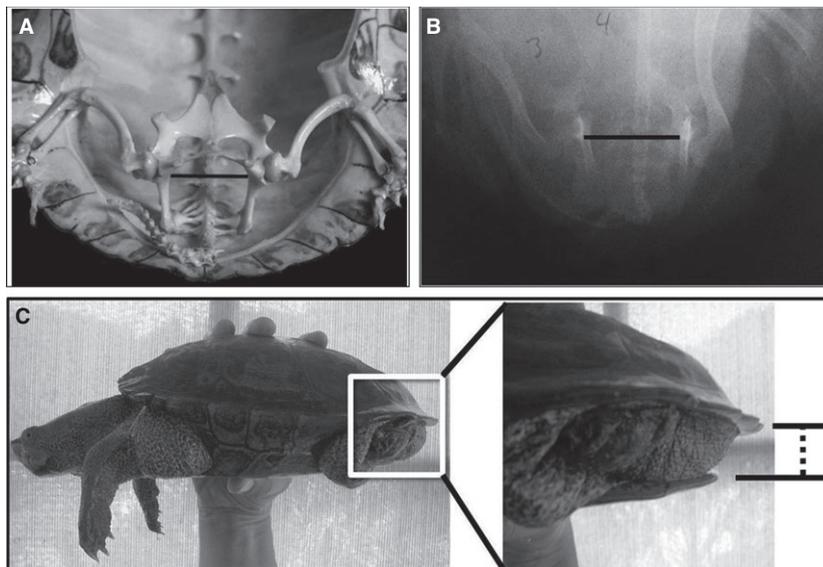
Figure 1. Pelvic aperture width and caudal gap height measurements in *Malaclemys terrapin*. A, pelvic aperture of a turtle skeleton represented by solid black line. B, pelvic aperture width as seen on an X-ray of a terrapin represented by solid black line. C, caudal gap height of a terrapin represented by dashed black line.
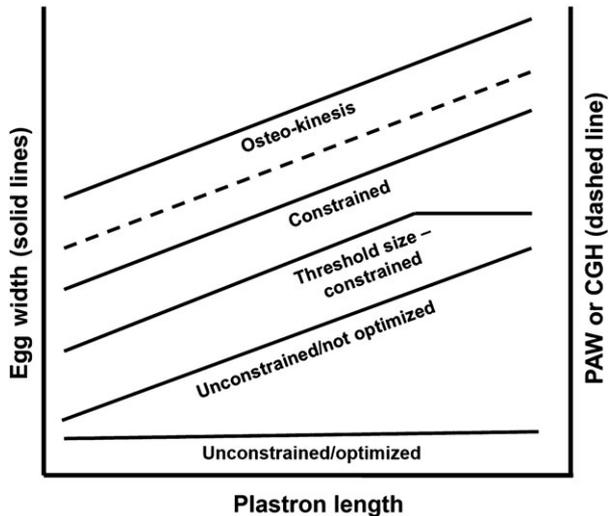
Figure 2. Potential responses of egg width to pelvic aperture width (PAW) or caudal gap height (CGH) in turtles. Labels refer to the line immediately above. The dashed line represents an expected relationship between PAW/CGH and plastron length. The unconstrained/optimized line is predicted by traditional optimal egg size theory. Adapted from Lovich *et al.* (2012).

morphologically constrained in all but the largest females. In this scenario, after a specific threshold body size, egg size no longer increases with increasing body size. A fourth possibility is that egg size may not be morphologically constrained, yet still not be optimized. Egg size can be constrained by several non-morphological factors, such as age (Clark *et al.*, 2001), genetics or other maternal effects (Rowe, 1994), however the specific mechanisms for this are not well-understood. For example, Rasmussen & Litzgus (2010) found that spotted turtles (*Clemmys guttata* Schneider 1792) in better body condition produced clutches with more eggs, but the eggs were shorter and weighed less than those of turtles that were lighter for their body length. Fifth, and finally, egg size may exceed the size of an aperture such that osteokinesis, the shifting of bone from its normal position, is necessary to allow the eggs to pass without breaking. Some turtles have been found to exhibit varying degrees of flexibility in different bones that make up the shell during oviposition so that larger eggs can be laid (e.g. *Gopherus berlandieri* Agassiz 1857, *Sternotherus odoratus* Latreille 1801, *Homopus signatus* Gmelin 1789; Rose & Judd, 1991; Clark *et al.*, 2001; Hofmeyr, Henen & Loehr, 2005).

The present study focused on the relationships of maternal body size and morphology with egg size and CS in diamondback terrapins (*Malaclemys terrapin* Schoepff 1793). Terrapins are medium-sized

emydid turtles that exclusively inhabit brackish waters of the Atlantic and Gulf Coasts of the USA, from Cape Cod to Texas (Ernst & Lovich, 2009). Terrapins fit some predictions of OES theory but not others, given that considerable plasticity in both egg size and CS has been documented (Roosenburg & Dunham, 1997). Egg mass differs little within an individual female's clutch during a single reproductive event; however, substantial variation in mean egg mass can exist among clutches within females from the same population (Roosenburg & Dunham, 1997). Also consistent with OES theory is the greater variation in CS and mass compared to that of egg size and mass. However, because both egg size and CS vary substantially within individual females, both within and between years, terrapins do not conform neatly to the predictions of OES theory (Roosenburg & Dunham, 1997). A previous study which used plastron length (PL) as a surrogate for metrics that are potentially constraining, namely PAW and CGH, reported that egg size may not be constrained in diamondback terrapins (Roosenburg & Dunham, 1997). Studies of diamondback terrapin reproductive biology have not directly measured CGH to determine whether it acts as a constraint to egg size. The objectives of the present study were to: (1) identify deviations from OES in diamondback terrapins by measuring reproductive output (clutch and egg size) and (2) determine whether such deviations can be explained by maternal body size and/or morphology.

MATERIAL AND METHODS

Two separate populations of diamondback terrapins were studied at different times and with slightly different techniques. The first group of terrapins was sampled from 1984 to 1988 in two tidal creek tributaries of the Kiawah River in Charleston County, South Carolina, as part of a larger ongoing study of the species (Lovich & Gibbons, 1990; Gibbons *et al.*, 2001). A detailed description and map of the study site is provided in Dorcas, Willson & Gibbons (2007). The second group of terrapins consisted of individuals captured in Townsend Creek on nearby (< 10 km) Edisto Island, Charleston County, South Carolina, in 2012 between May and July. Gibbons *et al.* (2001) found that terrapins in our area exhibit high site fidelity, making it unlikely that there would be any mixing between populations.

Sampling of the Kiawah group was irregular, although each creek was sampled at least once every year (1984–1988). We conducted sampling at low tide using seines and trammel nets (Lovich & Gibbons, 1990), with a few captures being made by hand or

with dipnets. Females of reproductive age were taken to the Savannah River Ecology Laboratory in Aiken County, South Carolina, and X-radiographed (MinXray 903 Type B-85 set to 80 kv for 1.0 s) to obtain clutch data (Gibbons & Greene, 1979; Hinton *et al.*, 1997), before being released at the point of capture.

Sampling of the Edisto group was conducted nearly every day between late May and the end of July 2012. Terrapins were captured at low tide using seines and trammel nets. All females were X-radiographed using a digital X-ray machine (Standard Vet VetRay by Sedecal set to 85 kVp, 8 mAs, 320 mA) at a veterinary office in Charleston before release at the point of capture.

From the X-radiographs, we measured PAW, which we define as the widest distance between the ilia (Naimi *et al.*, 2012) (Fig. 1A, B). We determined the mean EW of clutches from each gravid female by measuring the maximum width of each egg in the clutch and dividing the sum of all EWs by CS. X-rays for Kiawah terrapins were measured using digital calipers on the original film X-rays. Graham & Peto-kas (1989) found that turtle eggs on X-rays are enlarged and result in some degree of magnification; thus, all X-ray measurements for the Kiawah population in our study are reported as X-ray EW (XREW) and X-ray PAW (XRPAW) without correction for magnification. X-ray measurements from the Edisto population are reported as corrected (cXREW and cXRPAW) because the digital X-ray images of Edisto terrapins were analyzed using K-PACS (IMAGE Information Systems Ltd) with a penny coin included in the images to calibrate measurements. CS was recorded as the number of shelled eggs visible in each X-ray. Curved PL was measured directly with the turtle in hand and not from the X-rays for both Kiawah and Edisto turtles. For the Edisto terrapins, we also took measurements of CGH (Fig. 1C) in hand using digital calipers. No CGH measurements were taken during data collection on the Kiawah turtles in the 1980s.

STATISTICAL ANALYSIS

For the Kiawah population, 27 X-rays were used in initial analyses; however, only a subset of 20 allowed accurate measurement of XRPAW because, in seven of the images, the terrapin moved when the image was captured causing blurring. These 20 X-rays were used in regression analyses and all 27 were used to determine the variation in EW within and among clutches. In total, 22 gravid females were captured from the Edisto population and X-rayed and all animals were used for each analysis performed on this population. We also calculated the clearance between mean EW per clutch and both CGH and PAW by subtracting the former from the latter two variables. We did not combine samples for Kiawah and Edisto islands because, although the populations are proximate, sampling took place in two different time periods and X-ray measurements were corrected for magnification error in the latter but not in the former.

Data were analyzed after $\log_{10}$ transformation to improve linearity and enable comparison with other studies (King, 2000) for maternal body size, aperture size, and EW. Major axis regressions were performed in PAST, version 2.17 (Hammer, Harper & Ryan, 2001) to test for isometry between PL and PAW in both populations and between PL and CGH for the Edisto terrapins (Ryan & Lindeman, 2007; Naimi *et al.*, 2012). To analyze potential morphological constraint on optimal egg size, the relationships of PAW, CGH, and mean EW with PL were examined using a linear regression after $\log_{10}$ transformation (Congdon & Gibbons, 1987). Spearman rank correlation values were calculated to examine relationships between PL and both CS and mean EW. Analysis of covariance (ANCOVA) was used to examine the variation in EW within and between clutches, with PL as the covariate for both populations; Shapiro–Wilk tests indicated that measurements were distributed normally for these variables. Coefficients of variation (i.e. the ratio of the SD to the mean) for EW and CS were compared because CS is predicted to be more variable than egg size by traditional OES theory. Correlations and ANOVAs were performed with STATISTICA, version 7.1 (StatSoft, Inc.) .

RESULTS

The curved PL of gravid females at Kiawah Island varied from 135 to 175 mm, with a mean ± SD of 153.07 ± 8.86 mm (Table 1). Measurements of XRPAW varied from 23.02 to 29.98 mm, with a mean ± SD of 26.54 ± 1.77 mm and XREW varied from 20.52 to 25.89 mm, with a mean ± SD of 23.67 ± 1.16 mm. The CS varied from two to seven eggs, with a mean ± SD of 5.0 ± 1.2 eggs per clutch. CS and mean EW were not correlated with PL [Spearman rho (ρ) = 0.291, $P = 0.303$; and Spearman rho (ρ) = 0.354, $P = 0.090$, respectively]. Variation in XREW among clutches was significantly greater than variation within clutches (ANCOVA; $F = 26.845$, d.f. = 25, $P < 0.001$). The coefficient of variation for the log of CS was 0.183, whereas that for the log of XREW was 0.016 (over one order of magnitude difference).

The curved PL of gravid females from Edisto varied from 148 to 177 mm, with a mean ± SD of

Table 1. Summary of results regarding body size, aperture size, and reproductive output variables for Kiawah and Edisto populations

	Sample size	PL (mm)		PAW (mm)		CGH (mm)		EW (mm)		CS (# eggs)	
		Variation	Mean	Variation	Mean	Variation	Mean	Variation	Mean	Variation	Mean
Kiawah	27	135–175	153.07	23.02–29.98	26.54	–	–	20.52–25.89	23.67	2–7	5
Edisto	22	148–177	160.14	27.7–34.9	32.06	19.93–31.85	24.13	21.0–26.9	24.41	5–10	7.23

Measurements of pelvic aperture width (PAW) and egg width (EW) were corrected for magnification error in the Edisto population, but not in the Kiawah population.
PL, plastron length; CGH, caudal gap height; CS, clutch size.

160.14 ± 7.05 mm (Table 1). Measurements of cXRPAW varied from 27.7 to 34.9 mm, with a mean ± SD of 32.06 ± 2.24 mm. CGH varied from 19.93 to 31.85, with a mean ± SD of 24.13 ± 2.65 mm. Values of cXREW varied from 21.0 to 26.9 mm, with a mean ± SD of 24.41 ± 1.02 mm; this value is higher than that of Kiawah Island (23.67 ± 1.16 mm), even without correcting for magnification error for Kiawah terrapins. CS at Edisto varied from five to 10 eggs, with a mean ± SD of 7.23 ± 1.23 eggs per clutch. Neither CS, nor mean cXREW was correlated with PL [Spearman rho (ρ) = 0.224, P = 0.316; and Spearman rho (ρ) = 0.246, P = 0.270, respectively]. Variation in cXREW among clutches was significantly greater than the variation in cXREW within clutches (ANCOVA; F = 12.146, d.f. = 20, $P < 0.001$). The coefficient of variation for the log of CS was 0.089, whereas that for the log of cXREW was 0.011.

Mean CS was greater in Edisto than in Kiawah (Kiawah mean = 5.00, Edisto mean = 7.23, t = −6.076, d.f. = 44, $P < 0.01$). The difference in mean CS between the populations remained significant when using PL as a covariate (ANCOVA; F = 17.64, d.f. = 1, $P < 0.01$).

A significant positive isometric relationship existed between XRPAW and maternal PL for the Kiawah population [slope = 1.55, confidence interval (CI) = 0.06–3.03] (Fig. 3). Ninety-five percent CIs around the $\log_{10}$ transformed linear regressions of XRPAW and mean XREW on PL showed the slopes of the two equations to be different (PAW slope = 1.223, PAW r^2 = 0.712, PAW $P < 0.001$; mean EW slope = 0.445, mean EW r^2 = 0.268, mean EW P = 0.010) (Fig. 4A). Additionally, XRPAW increased more with increasing maternal body size than mean XREW (Fig. 4A). The mean egg clearance through the XRPAW (i.e. the difference between XRPAW and mean XREW) increased significantly with increasing PL (r^2 = 0.436, P = 0.002) (Fig. 4B).

For the population of terrapins from Edisto, a significant positive isometric relationship was found between both CGH (slope = 1.40, CI = 1.32–1.48) (Fig. 5A) and cXRPAW (slope = 1.47, CI = 0.05–2.88) (Fig. 5B) with PL. Ninety-five percent CIs around the $\log_{10}$ transformed linear regressions of cXRPAW and mean cXREW on PL showed the slopes of the lines to be different (PAW slope = 0.577, PAW r^2 = 0.128, PAW P = 0.103; mean EW slope = 0.223, mean EW r^2 = 0.076, mean EW P = 0.214) (Fig. 6A). The same analysis for CGH resulted in overlapping 95% confidence intervals and the slope of the line of CGH on PL did not appear significantly different from the slope of the line of mean cXREW on PL (CGH slope = 0.132, CGH r^2 = 0.054, CGH P = 0.811; mean EW slope = 0.503, mean EW r^2 = 0.235, mean EW P = 0.031) (Fig. 6B). The mean egg clearance through cXRPAW and through CGH showed no relationship with PL (r^2 = 0.055, P = 0.292; r^2 = 0.003, P = 0.816, respectively) (Fig. 6C). However, slightly more than half of the females from the Edisto population had eggs within their clutch that were wider than their CGH.

DISCUSSION

To our knowledge, the present study is the first to investigate and observe small landscape-level variation in OES for turtles. We found that egg size appeared to be constrained by more than just PAW in the smallest Kiawah terrapins because the mean egg clearance through the pelvic aperture increased with maternal body size, indicating that some factor other than morphology may act to constrain egg size even in larger Kiawah terrapins. However, we found that egg size did not appear to be constrained in the Edisto population. In addition, terrapins may exhibit osteokinesis in the caudal region of their shells (as suggested by our Edisto data), which may aid in the oviposition of large eggs. Both populations exhibited greater variation in egg size among clutches than within, suggesting an absence of optimization. This absence of optimization could be attributed to a lack
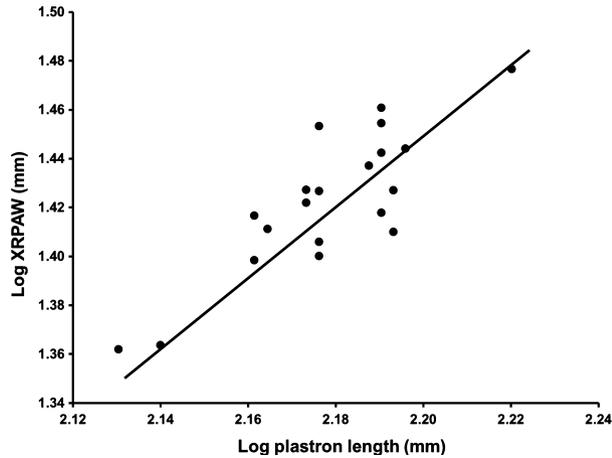
Figure 3. Relationship between plastron length (PL) and the size of X-ray pelvic aperture width (XRPAW) for the population from Kiawah. The slope obtained from the regression equation was compared to the expected isometric slope of 1 with 95% confidence intervals (CI) (regression equation log XRPAW = −1.94 + (1.55 × log PL), r^2 = 0.71, CI = 0.06–3.03).

of strong selective pressures on egg size, which would drive its canalization around an optimum value. However, consistent with OES theory, both populations of diamondback terrapins showed no significant correlation between CS or egg size with body size, which is a surprising result given that other terrapin studies have demonstrated significant correlations between CS and maternal body size (Montevecchi & Burger, 1975; Roosenburg & Dunham, 1997). Additionally, in conformance with predictions of OES theory, CS varied more than egg size in both populations. Within the Kiawah population, the coefficients of variation for the two variables exhibited a difference greater than one order of magnitude. Overall, OES in the Kiawah population appears to represent a combination of scenario 3 (egg size is morphologically constrained until a threshold body size is reached) and scenario 4 (egg size is not morphologically constrained but also not optimized) described in Lovich *et al.* (2012) because smaller females show evidence of morphological constraint, but larger females do not appear to exhibit canalization around an optimal egg size despite a lack of obvious morphological constraint. OES in the Edisto population appears to best match scenario 4 (egg size is not morphologically constrained but also not optimized) because we did not find evidence for constraint by either aperture, although the variation in egg size could suggest that egg size has not experienced selection pressures sufficiently strong enough to canalize the trait around an optimal value.
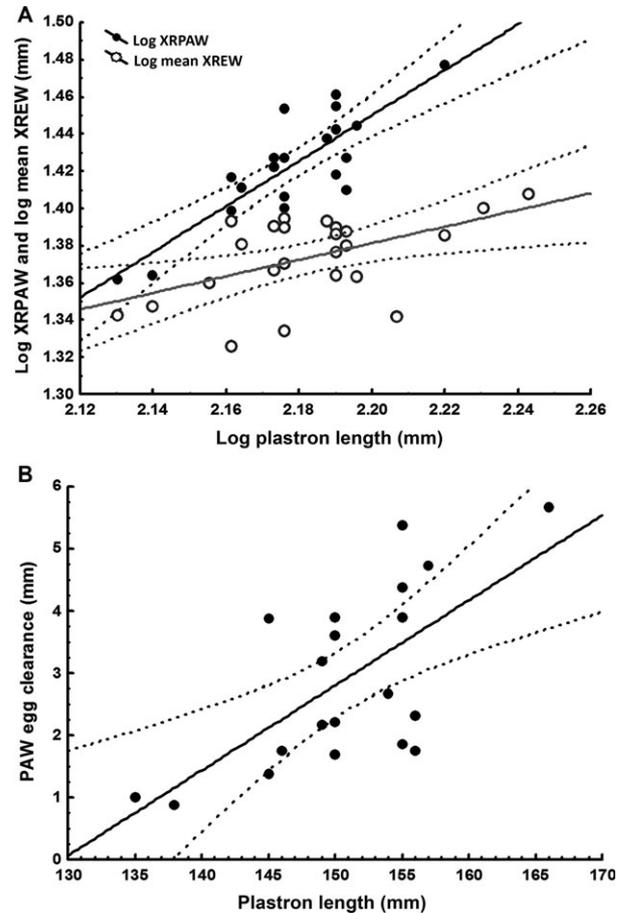
Figure 4. Analyses of constraint for the Kiawah population. A, linear regressions of log-transformed X-ray pelvic aperture width (XRPAW) and mean X-ray egg width (XREW) on plastron length (PL). B, linear regression of the clearance of mean egg width through the corresponding individual's PAW on PL. The PAWs belonging to the two smallest individuals (smallest PL lengths) are smaller than many of the egg widths of other terrapins, suggesting that optimal egg size may be constrained in the species at least at some body sizes.

MATERNAL BODY SIZE

The discrepancies between the present study and other previous studies that have investigated the relationship of maternal body size and reproductive output in diamondback terrapins may be the result of multiple factors. Our inability to detect a significant correlation in CS and egg size with maternal body size may be a result of our relatively small sample sizes (Kiawah, $N = 24$; Edisto, $N = 22$). In a study of yellow-bellied sliders, Gibbons, Greene & Patterson (1982) found a highly significant positive correlation of CS with maternal body size when large sample sizes included individuals from two populations, whereas they detected a lower correlation when each population was analyzed separately.
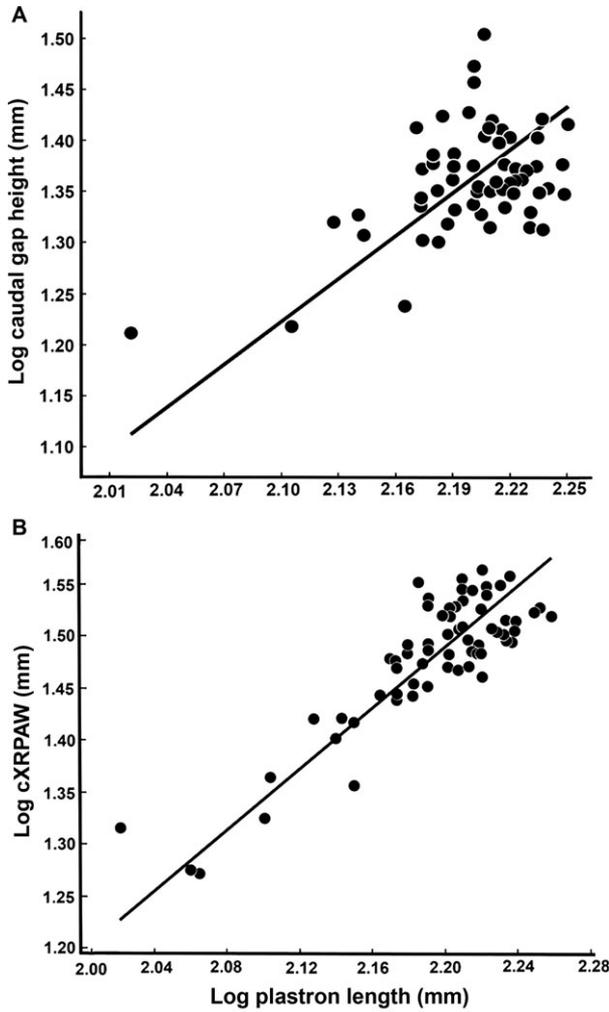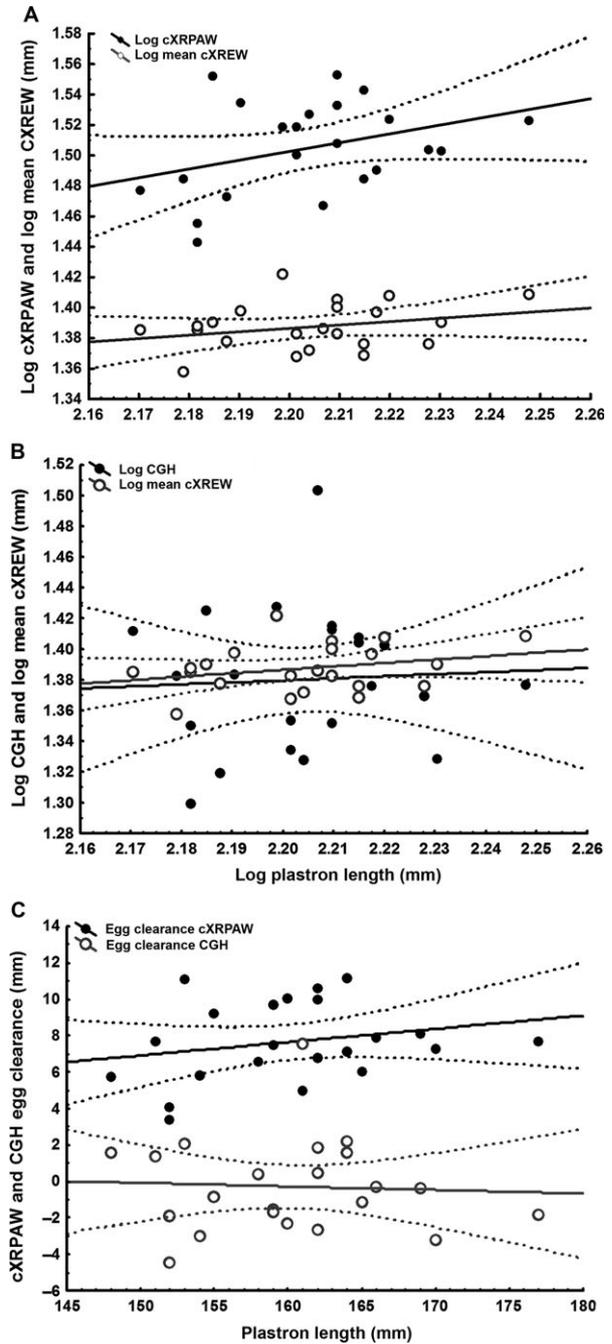
Figure 5. Relationship between plastron length (PL) and (A) the size of caudal gap height (CGH) and (B) the size of corrected X-ray pelvic aperture width (cXRPAW) for the Edisto population. For CGH, the slope obtained from the regression equation was compared to the expected isometric slope of 1 with 95% confidence intervals (CIs) (regression equation log cXRPAW = −1.71 + (1.40 × log PL), $r^2 = 0.26$, CI = 1.32–1.48). For cXRPAW, the slope obtained from the regression equation was compared with the expected isometric slope of 1 with 95% confidence intervals [regression equation log cXRPAW = −1.74 + (1.47 × log PL), $r^2 = 0.76$, CI = 0.05–2.88].

However, it is also possible that such correlations between the chosen metrics of maternal body size and reproductive output do not exist within the study populations.

POTENTIAL MORPHOLOGICAL CONSTRAINT

In addition to examining the relationships of PAW and CGH with body size, we also directly examined

Figure 6. Analyses of constraint for the Edisto population. A, linear regressions of log-transformed corrected X-ray pelvic aperture width (cXRPAW) and mean corrected X-ray egg width (cXREW) on plastron length (PL); the separation of the 95% confidence intervals suggests that egg size is unconstrained by PAW. B, linear regressions of log-transformed caudal gap height (CGH) and mean EW on PL; the substantial degree of overlap between the 95% confidence intervals (CIs) is most likely the result of an unexpected amount of osteokinesis. C, linear regression of the clearance of mean egg width through the corresponding individual's PAW and CGH on PL.

the relationship between both aperture sizes and EW. We found that, in the Kiawah population, egg size may be constrained given that EW increased with maternal body size, a trend not observed in the Edisto population. However, the lack of optimization in the Kiawah population does not appear to be imposed solely by morphological constraints because egg size only appears to be constrained by PAW in the smallest Kiawah females (scenario 3 in Lovich *et al.*, 2012) (Fig. 4A), whereas the degree of variation in egg size in the larger, non-morphologically constrained females also suggests a lack of optimization (scenario 4 in Lovich *et al.*, 2012). The difference in apparent constraint of egg size of the two geographically close populations is somewhat unexpected, although it may be a result of small samples sizes or the fact that Kiawah terrapins are gravid at slightly smaller body sizes (gravid Kiawah females PL 135–175 mm, mean ± SD = 153.07 ± 8.86 mm; gravid Edisto females PL = 148–177 mm, mean ± SD = 160.14 ± 7.05 mm). If the smaller size of gravid terrapins found in Kiawah reflects the fact that they start reproducing at a younger age than Edisto terrapins, then the difference in constraint between the two populations may be driven by the younger reproductively active Kiawah terrapins (Macip-Ríos *et al.*, 2012). For example, egg size can be constrained physiologically by an elevated testosterone level in younger female turtles (Bowden *et al.*, 2004). However, although age and body size are generally positively correlated in reptiles, the relationship is often fairly weak (Halliday & Verrell, 1988; Congdon *et al.*, 2013). Conversely, the smaller Kiawah turtles may not be younger than the larger Edisto turtles, and the difference in size may actually be the result of some other factor not investigated as part of the present study (e.g. environmental, genetics, variation of environment conditions during our 30-year study).

POTENTIAL OSTEOKINESIS

Our investigations of CGH as a factor with the potential to constrain egg size yielded a surprising finding within the Edisto population. Just over 50% of individuals had eggs within their clutch that were wider than their CGH. Certain species of turtle such as the speckled padloper (*H. signatus*), common musk turtle (*S. odoratus*), and Texas tortoise (*G. berlandieri*) have been found to lay eggs larger than would normally pass through the caudal gap, although these animals are able to oviposit intact eggs via osteokinesis (Rose & Judd, 1991; Clark *et al.*, 2001; Hofmeyr *et al.*, 2005). The leathery-shelled eggs laid by diamondback terrapins (Montevecchi & Burger, 1975) may be able to withstand a

certain amount of deformation when passing through the caudal gap. Such flexibility in this region of the shell of the diamondback terrapin has not been documented in any literature concerning this species and suggests that the mothers may undergo some form of osteokinesis during oviposition to accommodate the size of her eggs.

CONFORMANCE TO PREDICTIONS OF OES THEORY

Based on the results of our investigations, neither population of diamondback terrapins appears to conform perfectly to the predictions of OES theory, although this failure to conform completely does not appear to be the result of morphological constraint on egg size, except in the smallest reproductive females from Kiawah. The extent of variability in CS and mean EW relative to the mean demonstrated that CS varies more in both populations than mean EW, which is consistent with the predictions of OES theory. However, both populations exhibited more variation in EW among clutches than within clutches. The traditional OES model predicts that there should be almost no variation in egg size within the clutch of a single individual or across a population within the same type of habitat (i.e. egg size should be centered on a single optimal size; Smith & Fretwell, 1974). The relative lack of variation in egg size within clutches matches this prediction but, because egg size was not constant across each population, these two populations deviate from traditional OES theory. Future research should examine the nesting behavior of females producing eggs of different sizes to determine whether a phenotype/habitat matching strategy might be adaptive in the context of selection that appears too weak to canalize the egg sizes of the two populations around an optimum value. For example, Roosenburg (1996) found evidence suggesting that terrapins laying larger eggs select nest sites that are more open and presumably warmer. Conversely, terrapins laying smaller eggs were found to select nest sites shaded by vegetation. Terrapins exhibit temperature-dependent sex determination such that higher nest site temperatures produce females. Female terrapins are larger than males and take longer to reach reproductive maturity; thus, by selecting warmer habitats in which to oviposit larger eggs, mothers may be providing their female offspring with the selective advantage of hatching at a larger size and reaching reproductive maturity at a younger age (Roosenburg, 1996).

The population of diamondback terrapins from Edisto appears to conform to the predictions of OES theory better than that from Kiawah, although we still detected a significant amount of variation in mean EW among clutches of individuals in Edisto.

Population level variation in optimization and constraint of egg size in turtles has previously been documented in a kinosternid species in Mexico and this difference has been hypothesized to be the result of differential environmental stability and human impact on the habitats occupied by each distinct population (Macip-Ríos *et al.*, 2009; Macip-Ríos, Sustaita-Rodríguez & Casas-Andreu, 2013). The results of the present study demonstrate how some of the myriad factors that could potentially constrain the optimization of a trait, such as egg size, can differ even between close populations. Additionally, our results highlight the potential difficulties and complexities in determining whether a trait might be optimized, constrained or simply not under selection sufficiently strong enough to canalize the trait around a single optimal value.

ACKNOWLEDGEMENTS

REFERENCES

Bobyn ML, Brooks RJ. 1994. Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (*Chelydra serpentina*). *Journal of Zoology, London* **233:** 233–257.

Bowden RM, Harms HK, Paitz RT, Janzen FJ. 2004. Does optimal egg size vary with demographic stage because of a physiological constraint? *Functional Ecology* **18:** 522–529.

Brockelman W. 1975. Competition, fitness of offspring, and optimal clutch size. *American Naturalist* **109:** 677–699.

Clark P, Ewert M, Nelson C. 2001. Physical apertures as constraints on egg size and shape in the common musk turtle, *Sternotherus odoratus*. *Functional Ecology* **15:** 70–77.

Congdon JD, Gibbons JW. 1987. Morphological constraint on egg size: a challenge to optimal egg size theory? *Proceedings of the National Academy of Sciences of the United States of America* **84:** 4145–4147.

Congdon JD, Nagle R, Dunham AE, Beck C, Kinney O, Yeomans S. 1999. The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): an evaluation of the 'bigger is better' hypothesis. *Oecologia* **121:** 224–235.

Congdon JD, Gibbons JW, Brooks RJ, Rollinson N, Tsaliagos RN. 2013. Indeterminate growth in long-lived freshwater turtles as a component of individual fitness. *Evolutionary Ecology* **27:** 445–459.

Dorcas ME, Willson JD, Gibbons JW. 2007. Crab trapping causes population decline and demographic changes in diamondback terrapins over two decades. *Biological Conservation* **137:** 334–340.

Einum S, Fleming IA. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values? *American Naturalist* **160:** 756–765.

Ernst CH, Lovich JE. 2009. *Turtles of the United States and Canada*. Baltimore, MD: Johns Hopkins University Press.

Gibbons JW, Greene JL. 1979. X-ray photography: a technique to determine reproductive patterns of freshwater turtles. *Herpetologica* **35:** 86–89.

Gibbons JW, Greene JL, Patterson KK. 1982. Variation in reproductive characteristics of aquatic turtles. *Copeia* **1982:** 776–784.

Gibbons JW, Lovich JE, Tucker AD, Fitzsimmons NN, Greene JL. 2001. Demographic and ecological factors affecting conservation and management of the diamondback terrapin (*Malaclemys terrapin*) in South Carolina. *Chelonian Conservation Biology* **4:** 66–74.

Graham TE, Petokas PJ. 1989. Correcting for magnification when taking measurements directly from radiographs. *Herpetological Review* **20:** 46–47.

Halliday TR, Verrell PA. 1988. Body size and age in amphibians and reptiles. *Journal of Herpetology* **22:** 253–265.

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: palaeontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4:** 1–9.

Hinton TG, Fedderman PD, Lovich JE, Congdon JD, Gibbons JW. 1997. Radiographic determination of fecundity: is the technique safe for developing turtle embryos? *Chelonian Conservation Biology* **2:** 409–414.

Hofmeyr MD, Henen BT, Loehr VJT. 2005. Overcoming environmental and morphological constraints: egg size and pelvic kinesis in the smallest tortoise, *Homopus signatus*. *Canadian Journal of Zoology* **83:** 1343–1352.

Janzen FJ, Warner DA. 2009. Parent-offspring conflict and selection on egg size in turtles. *Journal of Evolutionary Biology* **22:** 2222–2230.

King RB. 2000. Analyzing the relationship between clutch size and female body size in reptiles. *Journal of Herpetology* **34:** 148–150.

Lack D. 1954. *The natural regulation of animal numbers*. Oxford: Oxford University Press.

Lalonde RG. 2005. Egg size variation does not affect offspring performance under intraspecific competition in *Nasonia vitripennis*, a gregarious parasitoid. *Journal of Animal Ecology* **74:** 630–635.

Lovich JE, Gibbons JW. 1990. Age at maturity influences adult sex ratio in the turtle *Malaclemys terrapin*. *Oikos* **59:** 126–134.

Lovich JE, Madrak SV, Drost CA, Monatesti AJ, Casper D, Znari M. 2012. Optimal egg size in a suboptimal environment: reproductive ecology of female Sonora mud turtles (*Kinosternon sonoriense*) in central Arizona, USA. *Amphibia-Reptilia* **33:** 161–170.

Macip-Ríos R, de Lourdes Arias Cisneros M, Aguilar-Miguel XS, Casas-Andreu G. 2009. Population ecology and reproduction of the Mexican mud turtle (*Kinosternon integrum*) in Tonatico, Estado de México. *Western North American Naturalist* **69.4:** 501–510.

Macip-Ríos R, Brauer-Robleda P, Casas-Andreu G, de Lourdes Arias-Cisneros M, Sustaita-Rodríguez VH. 2012. Evidence for the morphological constraint hypothesis and optimal offspring size theory in the Mexican mud turtle (*Kinosternon integrum*). *Zoological Science* **29:** 60–65.

Macip-Ríos R, Sustaita-Rodríguez VH, Casas-Andreu G. 2013. Evidence of pelvic and nonpelvic constraint on egg size in two species of *Kinosternon* from Mexico. *Chelonian Conservation and Biology* **12:** 218–226.

Montevecchi W, Burger J. 1975. Aspects of the reproductive biology of the Northern diamondback terrapin *Malaclemys terrapin terrapin*. *American Midland Naturalist* **94:** 166–178.

Naimi M, Znari M, Lovich JE, Feddadi Y, Ait Baamrane MA. 2012. Clutch and egg allometry of the turtle *Mauremys leprosa* (Chelonia: Geoemydidae) from a polluted peri-urban river in west-central Morocco. *Herpetological Journal* **22:** 43–49.

Rasmussen ML, Litzgus JD. 2010. Patterns of maternal investment in spotted turtles (*Clemmys guttata*): implications of trade-offs, scales of analyses, and incubation substrates. *Ecoscience* **17:** 47–58.

Rollinson N, Brooks RJ. 2008. Optimal offspring provisioning when egg size is 'constrained': a case study with the painted turtle *Chrysemys picta*. *Oikos* **117:** 144–151.

Roosenburg WM. 1996. Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination? *American Zoologist* **36:** 157–168.

Roosenburg WM, Dunham AE. 1997. Allocation of reproductive output: egg- and clutch-size variation in the diamondback terrapin. *Copeia* **1997:** 290–297.

Rose FL, Judd FW. 1991. Egg size versus carapace-xiphiplastron aperture size in *Gopherus berlandieri*. *Journal of Herpetology* **25:** 248–250.

Rowe JW. 1994. Egg size and shape variation within and among Nebraskan painted turtle (*Chrysemys picta bellii*) populations: relationships to clutch and maternal body size. *Copeia* **1994:** 1034–1040.

Ryan KM, Lindeman PV. 2007. Reproductive allometry in the common map turtle, *Graptemys geographica*. *American Midland Naturalist* **158:** 49–59.

Smith C, Fretwell S. 1974. Optimal balance between size and number of offspring. *American Naturalist* **108:** 499–506.

Wiklund C, Karlsson B, Forsberg J. 1987. Adaptive versus constraint explanations for egg-to-body size relationships in two butterfly families. *American Naturalist* **130:** 828–838.

Wilkinson L, Gibbons JW. 2005. Patterns of reproductive allocation: clutch and egg size variation in three freshwater turtles. *Copeia* **2005:** 868–879.