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Widespread reproductive variation in North American turtles: temperature, egg size and optimality

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ABSTRACT

Theory predicts the existence of an optimal offspring size that balances the trade-off between offspring fitness and offspring number. However, in wild populations of many species, egg size can still vary from year to year for unknown reasons. Here, we hypothesize that among-year variation in population mean egg size of freshwater turtles is partly a consequence of their gonadal sensitivity to seasonal temperatures, a physiological mechanism which principally functions to synchronize reproduction with a favorable time of year. As part of this process, among-year variation in seasonal temperatures modifies the extent of egg follicle development, and this may translate into variation in mean egg size among years (both at the individual and population level). To test this hypothesis, we applied an information-theoretic approach to model relationships between mean egg mass and the temperatures experienced during discrete periods of follicular development in wild populations of three turtle species (*Chrysemys picta*, *Chelydra serpentina*, *Glyptemys insculpta*) over 12 consecutive years. Because follicular development occurs in the fall for *C. serpentina* and *G. insculpta*, whereas it occurs both in the fall and spring for *C. picta*, we expected only fall temperatures would explain egg size variation in *C. serpentina* and *G. insculpta*, whereas both fall and spring temperatures would correlate with egg size variation in *C. picta*. These predictions were upheld. We then compared among-year variation in within-female egg and clutch sizes of each species in order to evaluate whether such variation might still be consistent with some tenets of optimal egg size theory. In all three species, we found that clutch sizes vary more than egg sizes in spite of temperature-induced egg size variation, and this pattern of relatively high clutch-size variation matches theoretical predictions. Future work should explore the roles of direct and indirect (i.e., nutritional) influences of temperature on egg size in natural settings.

1. Introduction

Given that the energy available for reproduction is finite, a mother must trade off the number of offspring she produces against their size. Optimal egg size theory (OES) provides a mathematical framework for balancing this trade-off. As long as the relationship between offspring size and offspring fitness is positive and asymptotic, OES predicts that there will be a single offspring size that will maximize maternal fitness returns per reproductive episode in a given environment (Smith and Fretwell, 1974). Empirical tests have upheld some tenets of OES (Fox et al., 1997; Einum and Fleming, 2000), though many cases of inter- and intraspecific variation in egg size have been difficult to reconcile with theoretical predictions (reviewed in Bernardo, 1996).

In predictable environments, females may maximize fitness by adjusting offspring size and number to suit the anticipated selective environment of their offspring (i.e., to suit a particular, predictable offspring size–fitness function). For example, female seed beetles (*Stator limbatus*) produce only a few, well-provisioned eggs when laying on seeds with thick seed coats, but the same mothers produce many poorly provisioned offspring on seeds with thin seed coats. This is because larvae hatching from these eggs need to burrow through the seed coats before they can begin feeding, and offspring need more energy to burrow through thick seed coats (Fox et al., 1997). Such adaptive egg size plasticity requires a mother to have the ability to predict the selective environment of her offspring (McGinley et al., 1987; but see Kaplan and Cooper, 1984).

On the other hand, adaptive egg size plasticity is unlikely when hatching conditions cannot be readily predicted by the mother. Many turtle species, for example, lay clutches of eggs that must complete a prolonged incubation period before hatchlings emerge from the nest (Ernst and Lovich, 2009). Although properties of

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Table 1
A summary of studies that used inferential statistics to detect among-year variation in egg mass or egg width (a proxy of egg mass) in populations of North American turtles. The presence or absence of a significant ($P < 0.05$) among-year difference in egg size in at least one year of the study is identified as 'Yes' or 'No', respectively. All studies controlled for the effect of maternal body size.

	Location	Population	$^{\#}$Years	Egg mass	Egg width	References
Kinosternidae						
Sternotherus odoratus	Virginia	Grassy Lake	2	Yes	No	Mitchell (1985)
Sternotherus odoratus	S. Carolina	Ellenton Bay	13	–	Yes	Wilkinson and Gibbons (2005)
Kinosternon subrubrum	S. Carolina	Ellenton Bay	13	–	Yes	Wilkinson and Gibbons (2005)
Emydidae						
Chrysemys picta	Nebraska	Beem Lake	3	Yes	Yes	Rowe (1994a,b)
Chrysemys picta	Nebraska	Dobbin's Pond	3	No	No	Rowe (1994a,b)
Chrysemys picta	Nebraska	Swan Lake	3	No	No	Rowe (1994a,b)
Chrysemys picta	Nebraska	Hansen's Lake	3	No	No	Rowe (1994a,b)
Chrysemys picta	Nebraska	Gimlet Lake	4	No	No	Iverson and Smith (1993)
Chrysemys picta	Michigan	E.S.G. Reserve	4	–	No	Congdon and Tinkle (1982)
Chrysemys picta	Michigan	Beaver Island	6	Yes	–	Rowe et al. (2003)
Chrysemys picta	Illinois	Thomson	4	Yes	–	Bowden et al. (2011)
Chrysemys picta	Illinois	Thomson	7	Yes	–	Warner et al. (2010)
Chrysemys picta	Ontario	Wolf Howl Pond	3	Yes	Yes	Schwarzkopf and Brooks (1986)
Trachemys scripta	Virginia	Dismal Swamp	4	No	No	Mitchell and Pague (1990)
Trachemys scripta	Illinois	Pohlman Slough	3	Yes	–	Tucker et al. (1998)
Trachemys scripta	Illinois	Stump Lake	3	Yes	–	Tucker et al. (1998)
Trachemys scripta	Illinois	Swan Lake	3	Yes	–	Tucker et al. (1998)
Pseudemys floridana	S. Carolina	Ellenton Bay	13	–	No	Wilkinson and Gibbons (2005)
Clemmys guttata	Ontario	Georgian Bay	4	–	No	Litzgus and Brooks (1998)
Malaclemys terrapin	Maryland	Patuxent River	3–5	No	–	Roosenburg and Dunham (1997)
Chelydridae						
Chelydra serpentina	Nebraska	Gimlet Lake	2	No	–	Iverson et al. (1997)
Chelydra serpentina	Ontario	Sasajewun Lake	13–14	Yes	–	Brooks et al. (1991)
Testudinidae						
Gopherus agassizii	California	Fenner Valley	2	–	No	Wallis et al. (1999)

the maternal phenotype may result in predictable variation in the offspring nest environment (e.g., Hendry et al., 2001; Rollinson and Hutchings, 2011), it seems unlikely that female turtles can use environmental cues to predict the future selective environment of their offspring. The best maternal strategy in these cases is to assume a consistent offspring size–fitness function from environment to environment (e.g., year to year), which indicates a single optimal egg size for a given mother (McGinley et al., 1987; Einum and Fleming, 2004). However, among-year changes in population mean egg mass are commonly observed in freshwater turtles (Table 1). Whether among-year variation in egg size is inconsistent with OES or is within the range of error expected by theory demands further exploration. Understanding the mechanisms behind such variation could also provide insight into the various evolved – and possibly competing – mechanisms which control egg size in the first place.

Mechanistically, among-year variation in the egg size of freshwater turtles may be the result of variation in energy balance (Schwarzkopf and Brooks, 1986; Rowe, 1994a,b; Tucker et al., 1998). Here, egg sizes are expected to correlate positively with resource availability and processing rates. On the other hand, laboratory investigations have also shown that follicle development rates can be sensitive to temperature, independent of major nutritional influences (Ganzhorn and Licht, 1983; Mendonca, 1987; Weber et al., 2011; also see Sarkar et al., 1996); this latter sensitivity may act as a mechanism to synchronize the separate phases of follicular development with favorable times of the year (Lawrence and Soame, 2004). Both of these mechanisms could also be complementary, with temperature acting directly on follicular development via hormonal means (Olive, 1980; Licht and Porter, 1985; Bowden et al., 2004), and indirectly via nutritional means.

In either case, because the amount of variation in offspring size allowed under OES cannot be defined in absolute terms (McGinley et al., 1987; but see Tucker et al., 1998), in order for any such proximal variation to ultimately be consistent with OES, clutch size must vary more than egg size in a given environment. While this question has been evaluated in some wild populations of freshwater turtles (e.g., Iverson and Smith, 1993), the mechanistic role of temperature in inducing such egg size variation has not.

The present study attempts to determine the mechanistic role, if any, of temperature in determining egg size in a field setting, and later, we place annual egg size variation in the context of OES by comparing it to variation in clutch size. First, we hypothesize that among-year variation in egg size is partly an artifact of selection for gonadal regulation by temperature (Gould and Lewontin, 1979; Lawrence and Soame, 2004). Using egg size data from populations of three turtle species (painted turtles, *Chrysemys picta*; wood turtles, *Glyptemys insculpta*; snapping turtles, *Chelydra serpentina*) collected between 1991 and 2002 in Algonquin Park, Canada, we test whether among-year changes in egg mass are consistent with the predicted seasonal effects of temperature on follicular development, determined in laboratory experiments (Ganzhorn and Licht, 1983; Mendonca, 1987; Sarkar et al., 1996). We also compare the variability in egg sizes to variability in clutch sizes for each species, as OES predicts the latter to be more variable (Smith and Fretwell, 1974).

In order to accommodate their hibernating life histories, freshwater turtles in northeastern North America divide their egg development into four separate phases: the recrudescent (pre-hibernation), preparatory (post-hibernation), egg-laying and quiescent periods. Laboratory research predicts that the onset and termination of these follicular periods is determined largely by seasonal temperature cues (Ganzhorn and Licht, 1983; Mendonca, 1987; also see Mahmoud and Alkindi, 2008). Because the relative importance of each of these phases in terms of overall follicular development varies among species, the predictive power of temperatures from their approximate corresponding time periods in determining egg sizes should be similarly variable if a temperature–egg size relationship exists. If the predictive power of seasonal temperatures in determining egg sizes is consistent with variation in the egg-laying phenologies of separate species that nest in the same general environment, we argue that this provides good evidence for the role of temperature as an egg size-determining mechanism in nature.

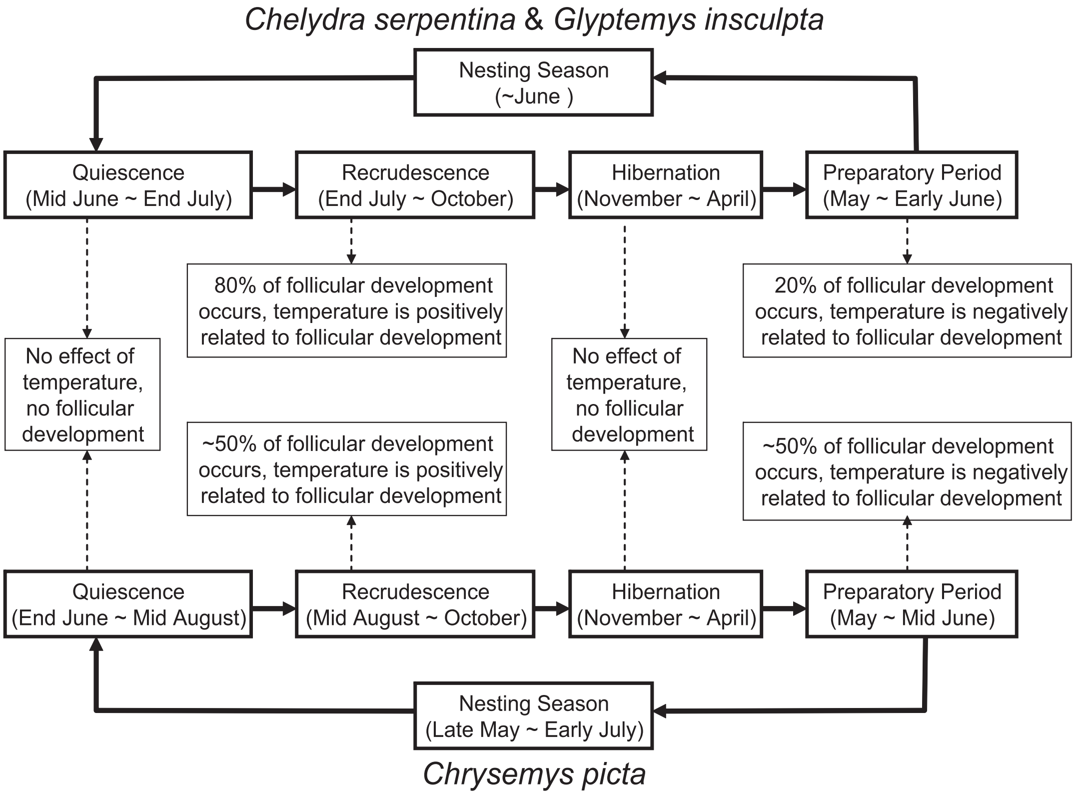

Fig. 1. Estimated timing of the follicular cycle of *Chrysemys picta*, *Chelydra serpentina*, and *Glyptemys insculpta*. Solid arrows and bold-framed boxes show the progression of the gonadal cycle in relation to time of year. Dashed arrows and light-framed boxes refer to the direct effect of temperature on follicular development during the relevant phase of the gonadal cycle. *G. insculpta* and *C. picta* both belong to the family Emydidae (Gaffney and Meylan, 1988), but these two species differ in their seasonal patterns of follicular development (Powell, 1967). *C. serpentina* (Chelydridae) is a distant relative of both *C. picta* and *G. insculpta* (Gaffney and Meylan, 1988), but the gonadal cycle of *C. serpentina* is identical to that of *G. insculpta* (Powell, 1967; White and Murphy, 1973; Mahmoud and Alkindi, 2008).

To parameterize a set of models evaluating the mechanistic role of temperature in determining egg size, we first recognized that in *C. picta*, about half of the energy allocated to follicles that will be ovulated in the following spring occurs in the recrudescent period (Callard et al., 1978; Congdon and Tinkle, 1982; Fig. 1). We estimated that this period occurs from mid August through October in our study population; warm temperatures during this period stimulate follicular growth both in *C. picta* (Ganzhorn and Licht, 1983) and in other species (e.g., *Sternotherus odoratus*, a kinosternid turtle; Mendonca, 1987). The remaining energy is allocated to follicles in the preparatory period (Callard et al., 1978; Congdon and Tinkle, 1982), which comprises the time between emergence from hibernation and the nesting season (between late April ~ early May to late May ~ early June in our population). Interestingly, the effect of temperature on gonadal development is reversed in *C. picta* during this period (Ganzhorn and Licht, 1983; Mendonca, 1987), as warm temperatures now have an inhibitory effect on follicular growth (Rollinson and Brooks, 2008a), presumably via a hormonal pathway (e.g., Bowden et al., 2004). Hence, all else being equal, we expect a negative relationship between temperature and egg size during the preparatory period for *C. picta*. Overall, we expect that mean egg mass of *C. picta* will be best predicted by a model incorporating both recrudescent and preparatory temperatures, as follicular growth occurs during both these periods (Table 2; Fig. 1).

In *C. serpentina*, by contrast, most or all the energy of the following year's clutch (Fig. 1) is suspected to be allocated to developing follicles during the recrudescent period (White and Murphy, 1973; Mahmoud and Licht, 1997; Mahmoud and Alkindi, 2008), and we estimate that recrudescence occurs between mid July and October in our study population (White and Murphy, 1973; Mahmoud and Licht, 1997). As little or no follicular growth occurs during the preparatory period in *C. serpentina* (Fig. 1), we expect that egg mass

Table 2
Estimated timing of the follicular cycle of *Chrysemys picta*, *Chelydra serpentina* and *Glyptemys insculpta* from Algonquin Park. 'Prediction' is our a priori prediction for the direction of the relationship between standardized egg mass and mean temperature of the corresponding month. 'Observation' is the observed direction of the relationship between temperature and standardized egg mass in the model that best predicted variation in egg mass. When 95% confidence intervals overlap 0, the observed relationship (i.e., 'observation') is 'none'. See Table 4 for parameter estimates and standard errors.

Species	Expected and observed results	August (recrudescence)	September (recrudescence)	October (recrudescence)	Spring (preparatory)
C. picta[a]	Prediction	+	+	+	–
	Observation	–	+	+	–
C. serpentina[b]	Prediction	+	+	+	None
	Observation	+	+	+	None
G. insculpta[b]	Prediction	+	+	+	None
	Observation	None	+	+	None

[a] We predict the full model will best predict variation in standardized egg mass.

[b] We predict the recrudescent model will best predict variation in standardized egg mass.

will be best predicted by a model incorporating only the temperatures experienced during the recrudescent period. In this model, we expect a positive relationship between temperature of the recrudescent period and egg mass.

The timing of the follicular cycle of *G. insculpta* is poorly studied, but it likely mirrors that of *C. serpentina* (Powell, 1967; White and Murphy, 1973). Similarities in the reproductive ecology of these two species also support this contention (Ernst and Lovich, 2009). As such, our estimates of the timing of follicular development and predictions for *G. insculpta* mirror those of *C. serpentina* (Table 2).

To place these mechanistic drivers into the ultimate context of OES, we finally tested whether the coefficients of variation (CVs) in clutch size across years were greater than the CVs in egg size across years for each species. If an optimal egg size exists, or if natural selection is at least an important agent in determining patterns of offspring size variation, theory predicts that clutch size will vary more than egg size among years (Smith and Fretwell, 1974; Orzack and Sober, 1994).

2. Materials and methods

Reproductive data for *C. picta* were obtained from females inhabiting two neighboring spruce (*Picea mariana*) bogs in Algonquin Park, Ontario, Canada (for details see Rollinson et al., 2008). Reproductive data for *C. serpentina* were obtained from females nesting on a gravel dam (or in its vicinity) on Lake Sasajewun, a mesotrophic lake in Algonquin Park, Ontario, Canada (see Brooks et al., 1991). Data for *G. insculpta* were obtained from nest sites in a nearby deciduous forest. The mean distance among all turtle populations was 46 ± 21 km (mean ± SD).

Between late May and early July, nesting patrols were conducted for each population by one to three people who walked the length of known nesting locations every 30–45 min. Females were generally captured immediately after nest completion, and maximum plastron length (Mpl) or maximum carapace length (Mcl) was measured with calipers to the nearest 0.01 cm for *C. picta* and *G. insculpta*, respectively. Body mass of *C. serpentina* was measured to the nearest 100 g using a Pesola scale (Pesola AG, Baar, Switzerland). All turtles were permanently marked by filing notches in their marginal scutes (Cagle, 1939). Clutches were excavated within 5 h of nest completion. Egg mass was determined to the nearest 0.1 g and averaged for each clutch. Eggs were then reburied at the original nesting site.

In our population, almost all *C. picta* reproduce every year (Samson, 2003; see also Congdon et al., 2003), and some individuals lay two clutches in a season; the second clutch is usually deposited 10–20 days after the first. Since mean egg mass and clutch size of the second clutch are generally smaller than those of the first clutch (Iverson and Smith, 1993; Samson, 2003), we did not include any known second clutches in our analysis. Although we keep track of the turtles that have already laid a first clutch in a given year, sometimes we miss an individual's first clutch, and then we collect its second clutch and assume it is a first clutch. We therefore minimized the number of 'false' first clutches in our data set by considering only clutches from the first 15 days of a nesting season. The resulting restricted data set for *C. picta* represented 56.6% of all "first" nests measured between 1991 and 2002. An analysis including all "first clutch" data, regardless of lay date, appears in Table S1 (see supplementary data in Appendix A).

Air temperature data were obtained from Environment Canada weather stations less than 50 km from the nesting sites. Consistent with the approach of Environment Canada, monthly temperatures were calculated from the mean of the daily maximum and daily minimum temperatures for August, September and October of each year. These values were used in our analyses as estimates of the temperatures experienced during the recrudescent period (Fig. 1). Dates of emergence from hibernation (mid April to early May) and nesting onset (late May to mid June) vary among years, so there is temporal variation in the timing of the preparatory period among years (see also Schwanz and Janzen, 2008). To correct for among-year variation in spring reproductive phenology, we again used weather data provided by Environment Canada, but here we calculated the temperature experienced in the 30 days prior to nesting season onset for each species for each year of the study. The average temperature for this 30-day period ("spring" temperature) is an estimate of the temperatures experienced during the preparatory period (Fig. 1).

We fit three linear mixed models for each species (3 models per species; 9 models in total) using the *lme4* package (Bates and Maechler, 2010) in R 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria). All models used a maximum likelihood parameter estimation technique. Each model predicted standardized egg mass as a function of a specific set of temperatures. Then these models were ranked for each species based on differences in Akaike's information criterion adjusted for small sample sizes (ΔAIC_c) using the software package *AICcmodavg* (Mazerolle, 2010). Finally, we evaluated whether the "best model" for each species, and the directionality of its coefficients, matched our a priori expectations (Table 2). All clutches came from females of known identity, so all models included a mean-zero, normally distributed random intercept term which accounted for differences in mean egg size among mothers (Gelman and Hill, 2007). We also corrected for the effect of female body size (Mpl for *C. picta*; Mcl for *G. insculpta*; kg for *C. serpentina*), which has an asymptotic relationship with egg size for *C. picta* (Rollinson and Brooks, 2008b), *C. serpentina* and *G. insculpta* (N. Rollinson and R.J. Brooks, unpublished data).

Mean egg mass per clutch was standardized in each species dataset:

$$\frac{(x_i - \bar{x})}{\sigma_x},$$

where σ_x is the standard deviation. Standardized egg mass was used as the dependent variable in each model to allow for graphical comparability of model predictions among species. For each species, female body size was also standardized by first averaging all body size data within females (i.e., to obtain one body size data point per female); then we used these means to calculate the grand mean and standard deviation. We also included standardized clutch size as a linear covariate (Rowe, 1994a; Rollinson and Brooks, 2008a,b), because the rate at which vitellogenin is deposited on developing follicles should decrease with an increasing number of follicles that are concomitantly undergoing development (Congdon and Tinkle, 1982; Sinervo and Licht, 1991). We then included "year" as a mean-centered (i.e., $x_i - \bar{x}$), linear covariate of egg mass in every model to control for possible systematic increases or decreases in egg mass over time (e.g., a gradual increase in water level over several years, resulting in a change in the relationship between air temperature and water temperature). We verified that year was not collinear ($r < 0.75$) with any temperature predictors. Hence, all models (see below) were based on the null formulation model

$$Y_{ij} = \beta_0 + \beta_1 \times BS_{ij} + \beta_2 \times BS_{ij}^2 + \beta_3 \times CS_{ij} + \beta_4 \times year_j + u_i + e_{ij},$$

where Y_{ij} is the mean egg mass for turtle i in year j, β_0 is the intercept, BS is standardized body size, CS is standardized clutch size, $year$ is the mean-centered value of the year in which reproduction occurred, u_i is the random effect of female i, e_{ij} is residual error and β_{1-4} are the parameters to be estimated.

We fit three different models for each species. First, we fit a "recrudescent model", which incorporated the mean temperatures of the months corresponding to the estimated recrudescent period

(August, September and October; Table 2). This model took the form

$$Y_{ij} = \beta_0 + \beta_1 \times BS_{ij} + \beta_2 + BS^2_{ij} + \beta_3 \times CS_{ij} + \beta_4 \times year_j + \beta_5 \times August_{j-1} + \beta_6 \times September_{j-1} + \beta_7 \times October_{j-1} + u_i + e_{ij},$$

where $August_{j-1}$, $September_{j-1}$ and $October_{j-1}$ are the mean temperatures of August, September and October of the year prior to reproduction, respectively.

Next, we fit a "preparatory model", which incorporated only the mean temperature of the estimated preparatory period (spring temperature; Table 2). This model took the form

$$Y_{ij} = \beta_0 + \beta_1 \times BS_{ij} + \beta_2 \times BS^2_{ij} + \beta_3 \times CS_{ij} + \beta_4 \times Year_j + \beta_5 \times spring_j + u_i + e_{ij},$$

where *spring* is the mean temperature experienced in the 30 days prior to the onset of the nesting season of the relevant species.

Finally, we fit a "full model", which took the form

$$Y_{ij} = \beta_0 + \beta_1 \times BS_{ij} + \beta_2 \times BS^2_{ij} + \beta_3 \times CS_{ij} + \beta_4 \times year_j + \beta_5 \times August_{j-1} + \beta_6 \times September_{j-1} + \beta_7 \times October_{j-1} + \beta_8 \times spring_j + u_i + e_{ij},$$

where mean egg mass is predicted by both the recrudescent and preparatory temperatures. The three models were ranked based on AIC_c values, which is appropriate when the ratio of samples to predictors is lower than 40 (Burnham and Anderson, 2002), as was the case for two of our three datasets. We confirmed that model fits were appropriate by inspecting residual vs. fitted plots for any systematic patterns; none were found.

We then tested whether among-year variation in egg mass violated a prediction of OES by comparing among-year variation in egg mass of each species to among-year variation in clutch size. For each female in the datasets delineated above, we calculated the coefficient of variation ($\text{CV} = (\sigma/\bar{x}) \times 100$) for egg mass across all years and compared it to the CV of clutch size across those same years. The coefficients of variation were compared using a paired-samples *t*-test, where values for mean egg mass were paired with values for clutch size within females. Two *C. picta* and seven *G. insculpta* were excluded from this analysis, as these individuals had only one year of reproductive data.

3. Results

In total, our sample for *C. picta* consisted of 136 females and 745 clutches. For *C. picta*, we predicted that the full model would have the lowest AIC_c and that parameter estimates would be positive for August, September and October temperatures, and negative for spring temperature. These predictions were largely upheld: the full model was indeed the best model for *C. picta* (Tables 2 and 3), and it included both a positive effect of September and October temperatures and a negative effect of spring temperature (Table 4; Fig. 2). The positive relationship between recrudescent (September, October) temperatures and standardized egg mass was similar in magnitude to the negative effect of preparatory temperatures, which corresponds to the estimated 50% of energetic allocation to developing follicles occurring during the recrudescent and preparatory periods, respectively (Tables 2 and 4; Fig. 1). The relationship between standardized egg mass and August temperature was negative, however, which does not correspond to our predictions (Tables 2 and 4).

Table 3
Models predicting variation in standardized egg mass are ranked for each species (see Section 2 for a full description of models). Changes in AIC_c values between competing models are identified by ΔAIC_c. Models with the most support are shown in bold; note that for *G. insculpta*, because the recrudescent and full models differ by only a single term, and because the ΔAIC_c is less than 2, the recrudescent model (with one fewer term) is deemed superior (Burnham and Anderson, 2002, p. 131).

Species	Model	ΔAIC_c
C. picta	Recrudescent	40.3
	Full	**0**
	Spring	87.4
C. serpentina	**Recrudescent**	**0**
	Full	1.74
	Spring	48.6
G. insculpta	**Recrudescent**	**0.98**
	Full	0
	Spring	15.9

For *C. serpentina* our sample comprised 195 clutches from 30 females, and for *G. insculpta* it comprised 204 clutches from 46 females. We predicted that the recrudescent model would best predict variation in standardized egg mass of *G. insculpta* and *C. serpentina*, and that parameter estimates would be positive for August, September and October temperatures (Table 2). These predictions were upheld (Tables 3 and 4; Fig. 2). While our data appear to suggest that, contrary to our predictions, the recrudescent model is not strongly supported in *C. serpentina* or *G. insculpta* (Table 3), the recrudescent model is, in fact, supported in both species. This is because the competing full models differ by a single parameter from the recrudescent model, they have functionally equivalent AICc values ($\Delta\text{AICc} \leq 2$), and they also share relatively consistent parameter values (Table 4). For these three reasons, it would be inappropriate to consider ΔAIC_c values in isolation in this case, as the small changes in ΔAIC_c in the full models can be attributed to the presence of the additional variable (spring temperature) that improves model fit only due to overfitting, rather than by making a substantial contribution of information. As such, the extra term is essentially unimportant (see Burnham and Anderson, 2002, p. 131). Patterns of egg size variation with temperature were, therefore, consistent with our hypothesis, as egg size variation was similar between *G. insculpta* and *C. serpentina* (Tables 2 and 4). This is further evidenced by synchronous among-year egg size variation in these two species (Fig. 3B), whereas the patterns of among-year variation for *C. picta* were unique (Fig. 3A). Annual data for each species are summarized in Table S2 (see supplementary data in Appendix A).

Finally, variation in egg mass across years was less pronounced than variation in clutch size for females of all three species. Coefficients of variation in egg mass were, in fact, very similar for each species (~6%), whereas the coefficient of variation for clutch size always exceeded 10% (Table 5).

4. Discussion

Among-year variation in egg size of North American turtles has been documented in at least five species from three different families (Table 1). We suspect that it may be present in the vast majority of North American populations and species, as studies of short duration or those which measure proxies of egg size, such as egg width, are less likely to detect this phenomenon (e.g., Mitchell, 1985; Rowe, 1994b). The present study endeavors to understand why such widespread, among-year variation in egg size occurs. Specifically, we tested whether in situ relationships between mean egg mass and temperature in three turtle species matched the relationships between follicular development and temperature that have been observed under laboratory conditions (Ganzhorn and Licht, 1983; Mendonca, 1987; Sarkar et al., 1996). We used an information-theoretic approach to test our hypothesis, and our a priori predictions were upheld: we predicted the correct relationship between egg size and temperature in 10 out of 12

Table 4
Best-supported models predicting standardized egg mass for each species. Note that '*t*' is the ratio of 1 standard error (SE) to the slope estimate (β).

Species	Model	ΔAIC_c	Parameters	β	SE	t
C. picta			β_0	0.297	0.531	0.56
	Full	–	Mpl	0.420	0.057	7.3
			Mpl^2	−0.164	0.036	−4.5
			Year	−0.007	0.008	−0.82
			Cs	−0.235	0.027	−8.7
			Spring	−0.173	0.026	−6.6
			Aug	−0.048	0.02	−2.4
			Sept	0.168	0.019	8.6
			Oct	0.101	0.026	3.9
C. serpentina			β_0	−6.76	1.05	−6.4
	Recrudescent	0	Kg	0.677	0.107	6.3
			Kg^2	−0.230	0.066	−3.5
			Year	−0.008	0.015	−0.54
			Cs	−0.244	0.070	−3.5
			Aug	0.184	0.044	4.1
			Sept	0.220	0.044	5.0
			Oct	0.180	0.04	4.5
			β_0	−6.48	1.12	−5.8
	Full	1.74	Kg	0.680	0.107	6.4
			Kg^2	−0.226	0.066	−3.4
			Year	−0.011	0.016	−0.69
			Cs	−0.245	0.070	−3.5
			Spring	−0.040	0.056	−0.72
			Aug	0.202	0.051	4.0
			Sept	0.213	0.045	4.8
			Oct	0.183	0.04	4.6
G. insculpta			β_0	−3.634	0.923	−3.9
	Recrudescent	0.98	Mcl	0.387	0.103	3.7
			Mcl^2	−0.150	0.072	−2.0
			Year	−0.016	0.020	−0.83
			Cs	−0.141	0.048	−2.9
			Aug	0.077	0.040	1.9
			Sept	0.128	0.044	2.9
			Oct	0.105	0.042	2.5
			β_0	−2.74	1.04	−2.6
	Full	0	Mcl	0.381	0.103	3.7
			Mcl^2	−0.142	0.071	−2.0
			Year	−0.016	0.019	−0.82
			Cs	−0.141	0.048	−3.0
			Spring	−0.066	0.037	−1.8
			Aug	0.067	0.040	1.7
			Sept	0.129	0.043	3.0
			Oct	0.116	0.042	2.8

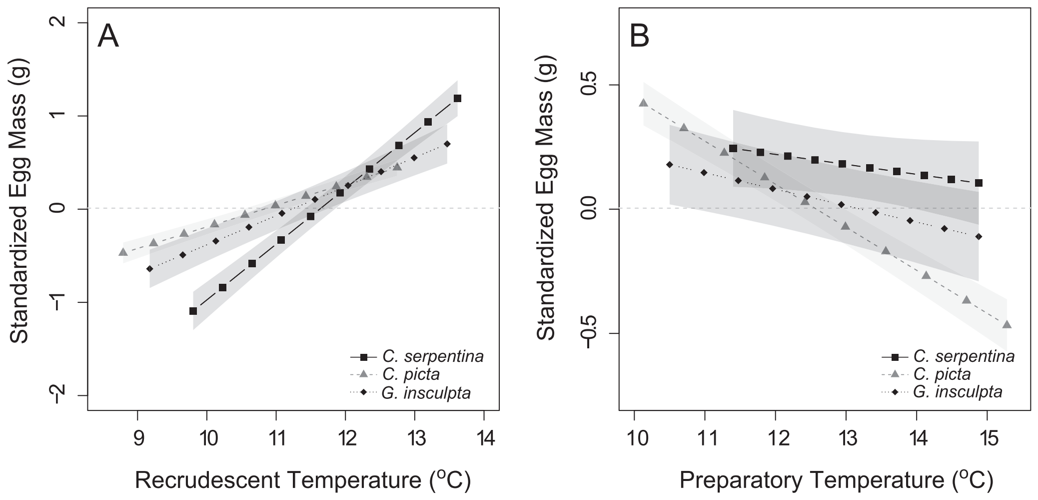

Fig. 2. Standardized mean egg mass predicted from our models as a function of variation in temperature, all else being equal. (A) "Recrudescent temperature" is the mean temperature (°C) of ordered sequences of realistic August, September and October values (derived from field minimum and maximum values for each species), weighted by the absolute values of the parameter estimates of each species' relevant full model (see Table 4 for parameter estimates). (B) "Preparatory temperature" is spring temperature (°C), and the range of temperature presented reflects the range observed in the field. Shaded areas around predictive lines are ±1 SE of estimates. Models were built with standardized dependent variables, so the intercepts and the slopes of the relationships between egg mass and temperature can be compared among species.

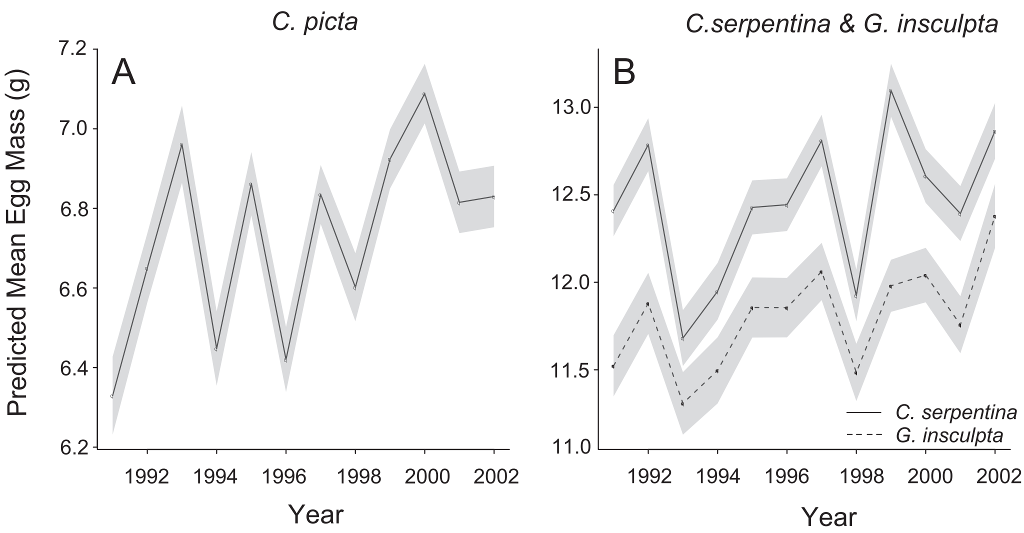

Fig. 3. Predicted mean egg mass by year for (A) *C. picta*, (B) *C. serpentina* and *G. insculpta*. For each species, unstandardized egg mass values are derived from (best) model predictions (Table 3), assuming a constant (mean) body size and clutch size across years.

cases (Table 2, see below), and based on a priori knowledge of species-specific gonadal cycles (Fig. 1), we predicted the best model in all three species (Tables 2 and 3). Hence, our data support the hypothesis that egg size variation – independent of variation in maternal size and clutch size – occurs because annual variation in temperature alters the regulation of gonadotropic hormones during follicular development, which in turn affects the rate of vitellogenin deposition on developing follicles (Gillet and Quetin, 2006).

However, among-year variation in temperature may also drive among-year variation in the energy balance of turtles (e.g., Kepenis and McManus, 1974; Avery et al., 1993). We emphasize that our analyses cannot discriminate between a direct, regulatory effect of temperature on follicular development, and an indirect effect of temperature on egg size acting via variation in resource harvesting and processing rates. Hence, in the present study, we propose an attractive complementary hypothesis which can account for among-year changes in egg size, but we do not reject the traditional view that egg size variation can also be the result of variation in energy balance, which then affects offspring provisioning.

The traditional view indicates that the positive relationship between egg mass and the temperature of August, September and October (Fig. 2A) is indicative of a positive energy balance, which ultimately results in a greater per-offspring investment (e.g., Schwarzkopf and Brooks, 1986; Rowe, 1994a; Tucker et al., 1998). Indeed, energy allocation to developing follicles may come primarily from harvested resources during recrudescence, as has been evidenced by a concomitant increase in follicular size and stored lipids during the recrudescent period (Congdon and Tinkle, 1982). Negative energy balances may occur when energetic demands exceed the energy acquired through resource acquisition (Berrigan and Charnov, 1994; Perrin, 1995), and this may explain why *C. picta* exhibited a negative relationship between egg mass and spring temperature (Fig. 2B). In *C. picta*, half the energy allocated to reproduction is provisioned during the preparatory period (Fig. 1), and there is apparently very little feeding during this time (Congdon, 1989; N. Rollinson, pers. obs.). Direct evidence indicates that females rely entirely on stored lipids to provision developing follicles during the preparatory period (Congdon and Tinkle, 1982; Congdon, 1989). Hence, for *C. picta*, negative energy balances are likely common during the preparatory period, and warm springs may result in more pronounced negative energy balances while follicles complete development. A negative energy balance may lead to less energy for offspring provisioning, and hence a smaller egg size (Berrigan and Charnov, 1994; Perrin, 1995). This effect is, however, expected to be much weaker in *G. insculpta* and *C. serpentina*, as follicles have nearly completed development by the end of recrudescence in these species (Fig. 1), so the energy balance during the preparatory period can at best have only a small effect on per-offspring investment. The patterns observed in the present study are consistent with this mechanism, where strong negative effects of spring temperature were observed in *C. picta*, but not in *G. insculpta* or *C. serpentina* (Fig. 2B).

On the other hand, egg size variation may not be related to variation in harvested resources in our study populations. For example, *C. picta* generally harbors extensive lipid stores that are used for reproduction when environmental conditions are poor (Congdon and Tinkle, 1982; Congdon, 1989), and studies of emydid turtles have found, at best, limited evidence of income breeding (Congdon and Tinkle, 1982; Rollinson and Brooks, 2007; Litzgus et al., 2008). Therefore, it is not clear why a negative energy balance in the spring should compromise per-offspring investment (i.e., lead to a smaller mean egg size), as lipid stores could buffer egg size against environmentally induced variation. Hence, a temperature sensitivity of gonadal developmental rate also seems to be a

Table 5

Egg mass (EM in g) and clutch size (CS) were averaged across years within females, then across all females (A). Paired-samples *t*-tests were used to compare among-year variation in EM to among-year variation in CS within females, separately, for each species of turtle. *T*-tests were performed only on CVs, and not on EM and CS means.

Species	EM (SE)	CS (SE)	CV EM (SE)	CV CS (SE)	*n*	*P*
C. picta	6.65 (0.051)	7.32 (0.11)	6.16 (0.26)	14.3 (0.70)	134	<0.001
C. serpentina	12.1 (0.18)	35.9 (1.08)	6.04 (0.43)	10.9 (0.81)	30	<0.001
G. insculpta	11.5 (0.18)	9.61 (0.22)	6.13 (0.43)	18.7 (1.73)	39	<0.001

CV, mean coefficient of variation ($(\sigma/\bar{x}) \times 100$) in EM and in CS within females, among years.
n, number of females (i.e., paired data points) used in each analysis.
SE, standard error.

reasonable explanation for the population-wide effect we observed here.

We also identified one line of evidence which suggests that a direct action of temperature on follicular development is contributing to among-year variation in egg mass. For *C. picta*, we found a weak negative effect of August temperatures on mean egg mass (Tables 2 and 4), whereas the effect of August temperatures on egg size of *C. serpentina* and *G. insculpta* was positive. This is interesting, because compared to *C. serpentina* and *G. insculpta*, the period of follicular quiescence (the period during which no follicular growth occurs, and which precedes the late-summer onset of follicular recrudescence) in *C. picta* is relatively long and likely extends into early August (Congdon and Tinkle, 1982). Moreover, the duration of follicular quiescence across the geographic range of *C. picta* correlates with the duration of high summer temperatures (Ganzhorn and Licht, 1983). Hence, if the follicles are responding directly to temperature-based (vs. nutritionally based) cues – i.e., beginning their recrudescent period once the temperature drops appreciably in late summer – an especially warm August may induce a prolonged period of follicular quiescence in our population of *C. picta*, resulting in a shorter window for follicular recrudescence before hibernation, and hence a smaller egg size. We would not expect this particular effect to be present in *C. serpentina* and *G. insculpta*, as the period of follicular quiescence appears to be shorter in these species (Powell, 1967; Mahmoud and Licht, 1997; Mahmoud and Alkindi, 2008), and hence, their follicular recrudescence lasts longer. Consequently, the mean egg size of *C. serpentina* and *G. insculpta* should be less sensitive to small changes in the timing of the onset of recrudescence. Our results are consistent with this line of reasoning. Furthermore, at our North American study site, warmer August temperatures likely correlate with improved feeding opportunities; hence, a negative relationship between summer temperatures and mean egg size would not be expected if egg size was influenced solely by nutritional factors.

More broadly, we found that despite clear among-year variation in egg mass in the present study (Fig. 3; Table S2 in Appendix A), clutch size was far more variable than egg mass in all species (e.g., standardized CS coefficients < 0.25 in Table 4; also see Table 5). This is consistent with predictions under OES, where variation in reproductive output should be realized primarily in terms of clutch size, and secondarily in terms of egg size (Smith and Fretwell, 1974). While our results are in concordance with studies which have shown that egg size varies less than clutch size in turtles (e.g., Iverson and Smith, 1993), it is difficult to speculate on the ecological and demographic implications of temperature-induced egg size plasticity. This is because we still lack a general understanding of the ecological and evolutionary importance of egg size variation in most turtle species. For example, in the present study, the substantial support ($|t| \geq 1.99$) for negative quadratic relationships between body size and egg mass in all species models (Table 4) may be indicative of genetic correlations among total reproductive effort and egg size (e.g., Caley et al., 2001; Czesak and Fox, 2003); it could also point to pelvic aperture constraints (Congdon and Gibbons, 1987; but see Iverson and Smith, 1993; Janzen and Warner, 2009); or it could even indicate a positive correlation between optimal egg size and maternal body size (Rollinson and Brooks, 2008a,b). Similarly, in *C. picta*, why is egg size in the second clutch smaller than that of the first clutch (e.g., Iverson and Smith, 1993)? It may be because offspring from the first clutch spend more time in the nest cavity and need more energy to overwinter successfully in the nest (Rollinson and Brooks, 2008a). In other words, the function relating offspring size to offspring fitness may differ among the first and second clutches. An alternative explanation is that this phenomenon also represents a genetic correlation between egg size and reproductive effort, as second clutch mass is often relatively small (e.g., Iverson and Smith, 1993; Samson, 2003). These questions and many others cannot be resolved without a better understanding of the relationship between offspring fitness and investment per offspring, and how this function differs among environments and species. This fitness function is the crux of the issue in the vast majority of studies that investigate size–number strategies, but few studies have endeavored to estimate it in turtles (Janzen, 1993; Congdon et al., 1999; Janzen et al., 2000a,b; see also Warner et al., 2010). While laboratory studies have provided a framework in which to undertake field investigations of offspring size–number strategies (e.g., Packard et al., 1987; Janzen and Warner, 2009), and while descriptive field studies have demonstrated the breadth of offspring size–number variation both within and among turtle species (e.g., Iverson and Smith, 1993; Wilkinson and Gibbons, 2005), manipulative experiments that are performed during the hatchling stage are now necessary to estimate the offspring size-fitness function (and hence optimal egg size) in natural populations (see Janzen, 1993; Congdon et al., 1999; Janzen et al., 2000a,b). Without such studies, we will never understand the ecological and evolutionary significance of egg size variation in turtles.

Our assumptions regarding the direction of the relationship between temperature and follicular development do, however, need further validation. Our current predictions rely on two studies performed on two species of freshwater turtles that tested follicular growth at two or three different temperatures across a reproductive season (Ganzhorn and Licht, 1983; Mendonca, 1987; see also Sarkar et al., 1996). Although the findings were consistent among species and studies, we do not know the shape of the relationship between follicular development and temperature, so our predictions are based on the assumption that there is a linear or near-linear relationship between follicular development and the temperatures tested by Ganzhorn and Licht (1983) and Mendonca (1987). Hence, laboratory tests should expand on these findings before our hypothesis can be validated. Should laboratory tests confirm these original findings over a broader range of temperatures, then we expect patterns of egg size variation in the field to mirror patterns of follicular development found in the laboratory in many other turtle species.

Finally, we note that air temperature is likely a more accurate predictor of body temperature for *C. picta*, as this species basks frequently (Krawchuk and Brooks, 1998), and for *G. insculpta*, which spend much of their time basking and foraging in forests and open areas (Dubois et al., 2009). *C. serpentina*, on the other hand, spend the vast majority of their time submerged in lakes and rivers (Brown et al., 1990), so air temperature is likely a less accurate predictor of body temperature in this species. However, these interspecific differences in behavior appear to be unimportant in terms of the ability of air temperature to reflect relative differences in the overall thermal environment. In our study, we found that in spite of basking ecology, variations in air temperature alone predicted significant variations in egg size, consistent with theoretical expectations. In fact, the phylogeny, basking behavior and general ecology of *C. serpentina* and *G. insculpta* are divergent (Gaffney and Meylan, 1988; Ernst and Lovich, 2009), but patterns of egg size variation with temperature are identical in these species (Tables 2 and 4).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.zool.2011.10.005.

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