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Sources and Significance of Among-Individual Reproductive Variation in a Northern Population of Painted Turtles (*Chrysemys picta*)

Njal Rollinson[1] and Ronald J. Brooks[1]

Painted Turtles (*Chrysemys picta*) are often used to test life-history theory. However, within populations, the factors that contribute to among-individual variation in egg size and clutch size are poorly understood, and an understanding of the biotic and abiotic parameters that contribute to this variation is important when framing patterns of maternal investment in a life-history context. We examined proximate sources of reproductive variation in a northern population of Painted Turtles, we attempted to frame these sources of variation in a life-history context, and we evaluate which optimality model most adequately explains patterns of reproductive allocation in populations of small-bodied turtles. We used multiple linear regression on data from 168 first clutches of marked females that nested at a long-term study site in Algonquin Park, Ontario, Canada, in 2004. We found that mean egg mass was positively related to maximum plastron length (MPL) and female age, and negatively related to clutch size and water temperature prior to oviposition. Clutch size was positively related to MPL and carapace height, and negatively related to mean egg mass, and the number of clutches laid in the season. Body size (MPL) was the most important predictor of each reproductive parameter, and residual analysis indicated that egg mass was more conserved than clutch size across the range of female body sizes sampled in this study. Thus, egg size may be optimized as a body size-specific function, and in light of this, we suggest that 'phenotype-habitat matching' may occur in *C. picta*. If a female's phenotype (e.g., body size) influences the selective environment of her eggs and hatchlings (e.g., if larger females generally nest farther away from water), then the optimal strategy of maternal investment should vary among maternal phenotypes. The positive correlation between egg mass and body size that was observed in the present study can be explained in adaptive terms under hypotheses based on the concept of phenotype–habitat matching.

RELATIVE reproductive success is the crux of natural selection, and as such, both size (quality) and number of offspring are of fundamental importance in evolution and ecology because of their direct consequences to parental fitness. The amount of energy allocated to each offspring will affect the probability of offspring survival, and it is commonly assumed that offspring fitness will increase with maternal investment per offspring at a decreasing rate (Smith and Fretwell, 1974). But because the energy available for reproduction is finite, individuals must decide how to apportion their resources among size and number of offspring, and natural selection should act on patterns of parental investment to strike a balance between offspring quality and quantity. However, selection may modify patterns of parental investment through a variety of pathways, and this can lead to complex interactions and tradeoffs that obscure the causative factors involved in allocation decisions (Stearns, 1992).

Optimality theories attempt to predict the strategy of maternal investment that will maximize maternal fitness within populations. Although several optimality models exist (Smith and Fretwell, 1974; Kaplan and Cooper, 1984; Parker and Begon, 1986), empirical data that lend clear support to any one model are relatively uncommon (Schwarzkopf and Brooks, 1986; Congdon and Gibbons, 1987; Roosenburg and Dunham, 1997). Presumably, inconsistencies between theory and observation owe in part to the complex nature of life-history traits (Stearns, 1992), where a failure to frame the proximate factors that contribute to variation in egg size and clutch size in the appropriate life-history context can lead to the erroneous rejection of a valid model (Tucker et al., 1998; Wilkinson and Gibbons, 2005). The selection of an appropriate model first requires an adequate understanding of the proximate factors that contribute to variation in reproductive measures within populations, and these proximate factors should then be framed in a life-history context so that ultimate reasons for allocation decisions may be elucidated (Fox et al., 1997; Einum and Fleming, 1999; Kaplan and Phillips, 2006).

Painted Turtles (*Chrysemys picta*) are relatively abundant in many parts of North America (Ernst et al., 1994), and reproductive data can be collected from this species with relative ease (Schwarzkopf and Brooks, 1986; Iverson and Smith, 1993; Congdon et al., 2003). The Painted Turtle has long served as a model organism to test life-history theory (Congdon and Tinkle, 1982; Schwarzkopf and Brooks, 1986; Congdon et al., 2003), and a number of studies have attempted to place patterns of maternal investment in this species into current theoretical framework; however, such studies have generally lent equivocal support to competing models (Schwarzkopf and Brooks, 1986; Congdon and Gibbons, 1987; Iverson and Smith, 1993; Roosenburg and Dunham, 1997; Clark et al., 2001; Wilkinson and Gibbons, 2005). In part, this ambiguity stems from the fact that among-individual variation in reproductive measures is pronounced in this species (Schwarzkopf and Brooks,

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1986; Iverson and Smith, 1993; Rowe, 1994), and the underlying sources of inter-individual variation are poorly understood (Rowe, 1994).

In this study, we use multiple regression to test simultaneously for relationships among reproductive measures and a variety of biotic and abiotic factors in a northern population of Painted Turtles. Specifically, we test for the simultaneous effects of body size (Iverson and Smith, 1993; Rowe et al., 2003), body shape (Tucker et al., 1998), age (Iverson and Smith, 1993; Congdon et al., 2003; Bowden et al., 2004), clutch frequency, and water temperature prior to oviposition on reproductive measures of the first clutch. First, we test *a priori* hypotheses pertaining to the proximate factors that contribute to among-individual variation in reproductive measures. Second, we test *a priori* hypotheses relevant to tenets of classic egg size theory and extensions thereof (Smith and Fretwell, 1974; Parker and Begon, 1986). Finally, we discuss which models of investment are most likely to apply to *C. picta* and other species of small-bodied turtle.

Proximate sources of among-individual reproductive variation.—Painted Turtles lay one or two clutches in a season in our study population (Rollinson and Brooks, 2007), and there may be an energetic or volume-based tradeoff (i.e., based on packing constraints) between the first and second clutch. If the presence of a second clutch compromises the amount of energy that can be allocated to the first clutch, then females laying two clutches in a season will have a lower clutch mass (of the first clutch) compared to females laying only one clutch in a season. Second, in *C. picta* and *Sternotherus odoratus*, elevated temperatures in the final stages of the follicular cycle (i.e., just prior to the nesting season) may inhibit follicular development. This may result in fewer, smaller follicles for females experiencing relatively warm temperatures prior to oviposition (Ganzhorn and Licht, 1983; Mendonca, 1987). Accordingly, we tested for a negative relationship between the temperatures experienced prior to individual oviposition dates and each reproductive measure of the first clutch. Third, we tested whether female carapace morphology constrains clutch mass. We predicted that carapace height would be positively related to clutch mass, after controlling for plastron length. Fourth, we tested for negative correlations among clutch size and mean egg mass of the first clutch (Elgar and Heaphy, 1989; Iverson and Smith, 1993; Rowe, 1994). Although we generated no specific hypotheses for the latter tests, we assessed whether mean egg mass is an important predictor of clutch size (and vise versa). Finally, we included female age estimates in our regression models (see below), as age can affect reproductive parameters in *C. picta* (Iverson and Smith, 1993; Congdon et al., 2003).

Relative variability of egg mass versus clutch size.—Optimality theories operating under the assumption that offspring fitness will increase with maternal investment per offspring at a decreasing rate (Smith and Fretwell, 1974; Parker and Begon, 1986) generally predict that egg mass will be more conserved than clutch size. Accordingly, if females laying two clutches in a season have a lower first clutch mass than those producing only one clutch, then we predict that a reduction in the mass of the first clutch (for females laying two clutches in a season) will be realized through changes in clutch size, and not egg mass. If female carapace morphology constrains clutch mass, this variation should likewise be realized in terms of clutch size, but egg mass should not vary (Tucker et al., 1998). Finally, in turtles, the maximum amount of energy that can be invested in a clutch will increase with body size because of packing constraints; therefore, the effect of variation in resource acquisition should be more apparent in larger females, as these individuals are capable of investing relatively large amounts of energy in reproduction (Glazier, 2000). We hypothesized that if clutch size, but not egg mass, varies with resource acquisition, then after removing the linear effect of body size, there would be no relationship between the absolute value of egg mass residuals and body size, but the absolute value of clutch size and clutch mass residuals should be positively correlated with body size.

MATERIALS AND METHODS

Site description.—This study is part of a larger life-history study that was initiated in Algonquin Provincial Park (Ontario, Canada) in 1977. There are two ponds in which the vast majority of turtles reside. Wolf Howl Pond (WHP: 45°34′N, 78°41′W) is a 1.70 ha black spruce (*Picea mariana*) bog that is predominantly 1.3 m deep and is bisected into an east and west side by a 5 m high railway embankment. The railway embankment is the only known nesting site for these turtles. Partially submerged stumps and logs riddle WHP, and there are numerous floating mats of *Sphagnum*, leatherleaf (*Chamaedaphne calyculata*) and sedge (*Rynchnospora alba* and *Eleocharis smallii*). It is surrounded primarily by a balsam fir forest (*Abies balsamifera*) to the north, south, and west. West Rose Lake (WRL) is located approximately 500 m southeast of WHP; it is larger in total surface area, and the railway embankment runs along its westernmost shore. WRL is nearly identical to WHP in terms of habitat parameters and is of comparable depth.

Clutch information.—Turtles were captured with dipnets between late April and early June of 2004. Individuals were brought back to a nearby research station, where turtles were permanently marked by filing notches in their marginal scutes (Cagle, 1939), and where alphanumeric aluminum tags were wired through the rear marginal scutes. To avoid recapturing individuals and to facilitate identification during nesting season, each individual's unique alphanumeric code was painted on their carapace. Nesting patrols were initiated on 2 June when females were detected as gravid using palpation. Three or four researchers were involved in these patrols, and patrolling consisted of walking back and forth along the railway embankment (encompassing both ponds) every 30–40 minutes from approx. 1500 h until at least 2200 h. Each researcher recorded detailed information on all turtle observations. All turtles observed nesting were allowed to nest, and clutches were excavated within five hours and brought back to the research station where egg mass (to the nearest 0.1 g) was measured with an electronic scale. The mean egg mass (g) of each clutch was then calculated and used in subsequent analyses. Clutches were reburied in their original locations at the nesting site.

About 20% of females in our population lay two clutches in a season; the second clutch is usually deposited 15 to 21 days after the first clutch (Samson, 2003). Egg mass and clutch size of the second clutch are generally smaller than in

Table 1. **Relationships between Mean Egg Mass, Clutch Size, Clutch Mass, and Their Respective Predictor Variables (Multiple Linear Regression, Backwards Elimination) for Female Painted Turtles (*Chrysemys picta*) Nesting in 2004 in Algonquin Park, ON, Canada ($n = 168$).** MPL is maximum plastron length. The regression equation for mean egg mass is Y = 0.294 (MPL) − 0.146 (clutch size) + 0.163 (age class) − 0.167 (water temperature) + 5.28. For clutch size, the equation is Y = 0.640 (MPL) − 0.788 (mean egg mass) + 1.28 (carapace height) − 0.399 (clutch frequency) − 4.01, and the equation for clutch mass is Y = 4.83 (MPL) + 8.17 (carapace height) − 3.55 (clutch frequency) − 1.87 (water temperature) − 40.9.

Dependent variable	Predictors	Cumulative model r^2	Standardized coefficient	t	P
Mean egg mass (g)	MPL (cm)	0.271	0.429	4.12	<0.001
	Clutch size	0.371	−0.328	−4.96	<0.001
	Age class	0.415	0.284	2.88	0.005
	Water temperature (°C)	0.449	−0.189	−3.15	0.002
Clutch size	MPL (cm)	0.218	0.414	3.72	<0.001
	Mean egg mass (g)	0.326	−0.350	−4.66	<0.001
	Carapace height (cm)	0.357	0.292	2.79	0.006
	Clutch frequency	0.370	−0.118	−1.84	0.068
Clutch mass (g)	MPL (cm)	0.420	0.429	4.46	<0.001
	Carapace height (cm)	0.447	0.256	2.65	0.009
	Clutch frequency	0.456	−0.144	−2.31	0.022
	Water temperature (°C)	0.469	−0.128	−2.04	0.043

the first clutch (Iverson and Smith, 1993; Samson, 2003), and because of this, we used data that were obtained only from first clutches in our study. Although the railway embankment is the only known nesting site for turtles in this population, we still miss a number of nests in each year. Unfortunately, this means that second clutches are occasionally recorded as first clutches in our permanent data records. To minimize clutch uncertainty, we reviewed the nesting observations that were recorded in field books for the 2004 season. This method is quite effective because turtles often have several failed nest attempts (often spread over several days) before they successfully deposit a clutch. We inferred that a female's first clutch was missed if she was seen twice on the embankment more than eight days apart and only one clutch (from the latter period) was collected. Only confirmed first clutches were included in our analyses.

Female age and body size.—We related maximum plastron length (MPL, cm) and carapace height (cm) to mean egg mass, clutch size, and clutch mass of the first clutch. Carapace height and MPL were measured to the nearest 0.01 cm in the spring of 2004 using calipers. Because age affects reproductive parameters in Painted Turtles (Congdon et al., 2003), we included age estimates in our analyses. Although most turtles that hatched later than 1985 are of known age, most age estimates for adult individuals in this population are approximations based on the Von Bertalanffy growth equation (Samson, 2003). We included the age parameter into our regression models by creating four age classes where age class 1 = primiparous (~12–15 yrs)–24 yrs ($n = 51$); age class 2 = 25–34 yrs ($n = 23$); age class 3 = 35–44 yrs ($n = 32$); and age class 4 > 45 yrs ($n = 62$).

Clutch frequency.—We tested whether mean egg mass, clutch size, and clutch mass of the first clutch were related to the number of clutches laid by individuals in 2004. Clutch frequency (i.e., one or two clutches) was included as a categorical variable in our analyses.

Water temperature.—Between 21 April and 8 July, 2004, five temperature data-loggers (Thermochron iButtons, Dallas Semi-Conductor, Sunnyvale, CA) recorded water temperature (T_W) every 25 minutes in WHP (approximate depths = 0.1–1.3 m). Data-loggers were separated by a minimum of approx. 30 m, and they were placed in areas of the pond where turtle abundance was highest. Data-loggers were suspended in the water column using string, bobbers, and small anchors. Among years, variation in the temperature that occurs 25 days prior to the onset of nesting season is inversely related to mean egg mass, clutch size, and clutch mass (Rollinson and Brooks, unpubl.). However, in any given year, oviposition dates may vary among individuals by up to 30 days, so the temperature experienced in the 25 days prior to oviposition by females nesting early in the nesting season differs from that experienced by females nesting later in the season; therefore, there is potential for these differences in temperature to influence allocation patterns (Ganzhorn and Licht, 1983; Mendonca, 1987). It took an average of six days from the beginning to the completion of egg shelling in 2004, so we assumed that egg shelling began six days before a female's first nesting attempt (Rollinson, 2006). We calculated the mean water temperature for the 25-day period prior to the estimated date that egg shelling began for each female, and we included this variable (T_W) in our analyses.

Statistical analyses.—We used stepwise linear regression (backwards elimination) to test for relationships between reproductive measures of the first clutch and the aforementioned parameters. Colinearity among independent variables was deemed acceptable if tolerance values were greater than 0.1 (Quinn and Keough, 2002). Regressions were performed in Statistical Package for the Social Sciences (v. 12.0, SPSS Inc., Statistical Package for the Social Sciences, Chicago, IL, 2003).

RESULTS

Proximate sources of among-individual reproductive variation.—About 45% of the variation in mean egg mass was explained by (ordered in terms of standardized coefficients) MPL (27.1%), clutch size (10.0%), age class (4.4%), and T_W (3.4%; Table 1). Clutch frequency and carapace height were not related to mean egg mass. About 37% of the variation in clutch size was explained by MPL (21.8%), mean egg mass (10.8%), carapace height (3.1%), and clutch frequency (1.3%), though clutch frequency was marginally insignificantly related to clutch size (Table 1). Age class and T_W were not significant predictors of clutch size. Finally, about 42% percent of the variation in clutch mass was explained by variation in maternal MPL; an additional 4.9% of this variation (totaling 46.9%) was explained by carapace height (2.7%), clutch frequency (0.9%), and T_W (1.3%; Table 1). Variation in age class was not associated with variation in clutch mass. Minimum tolerance values in all initial (≥ 0.320) and final models (≥ 0.353) exceeded 0.1, which indicates that the degree of colinearity among independent variables was acceptable.

Relative variability of egg mass versus clutch size.—Residuals of mean egg mass over MPL, of clutch size over MPL, and of clutch mass over MPL were obtained by means of linear regression (Fig. 1). Residuals of all regressions were transformed into absolute values and subsequently regressed (independently) against MPL using Spearman Rank Correlations. Clutch size and clutch mass residuals were positively correlated with MPL, but egg mass residuals were not (Fig. 2).

DISCUSSION

We used multiple regression to test for cumulative effects of several biotic and abiotic parameters on reproductive measures of Painted Turtles. Body size explained the largest proportion of variation in each reproductive measure, and other parameters explained an additional 1% to 10% of this variation. However, even though four independent variables were included in each final model (Table 1), R^2 values never exceeded 50%, so over half of the inter-individual variation in egg mass, clutch size, and clutch mass was not related to factors measured in this study. Genetic factors and body condition may account some of this unexplained variation (Rowe, 1994; Rollinson and Brooks, 2008). Below, we first attempt to frame each source of reproductive variation in the life-history context of *C. picta*. Second, we discuss some of the optimality models that have been applied previously to populations of freshwater turtles, and finally, we attempt to identify the most promising model of maternal investment for small-bodied turtles.

Proximate sources of among-individual reproductive variation.—The mass of the first clutch was smaller in females laying two clutches in a season, compared to those laying only one clutch (Table 1), and this is consistent with our first hypothesis: there is an energetic or volume-based tradeoff between the first and second clutch. Unfortunately, we cannot be certain of the underlying mechanism involved; that is, the observed relationship would be expected if there is less room in the abdominal cavity for a first clutch if a second clutch is concomitantly completing vitellogenesis (Congdon and Tinkle, 1982), or if the energy required to support the development of a second clutch compromises the amount of energy that can be allocated to a first clutch. We do, however, suspect that the former explanation has more merit. The vast majority of females in our population lay at least one clutch in a season (Samson, 2003), and reproductive output in this population does not appear to be limited by resource acquisition (Rollinson and Brooks, 2007). Thus, the apparent trade-off between clutch frequency and energy allocation to the first clutch is likely due to space limitations, not energy shortages. However, Rowe et al. (2003) report that many females in a population on Beaver Island, Michigan, do not reproduce in every year, so the conclusion that females in our population are not 'energy-limited' may not apply to all northern populations.

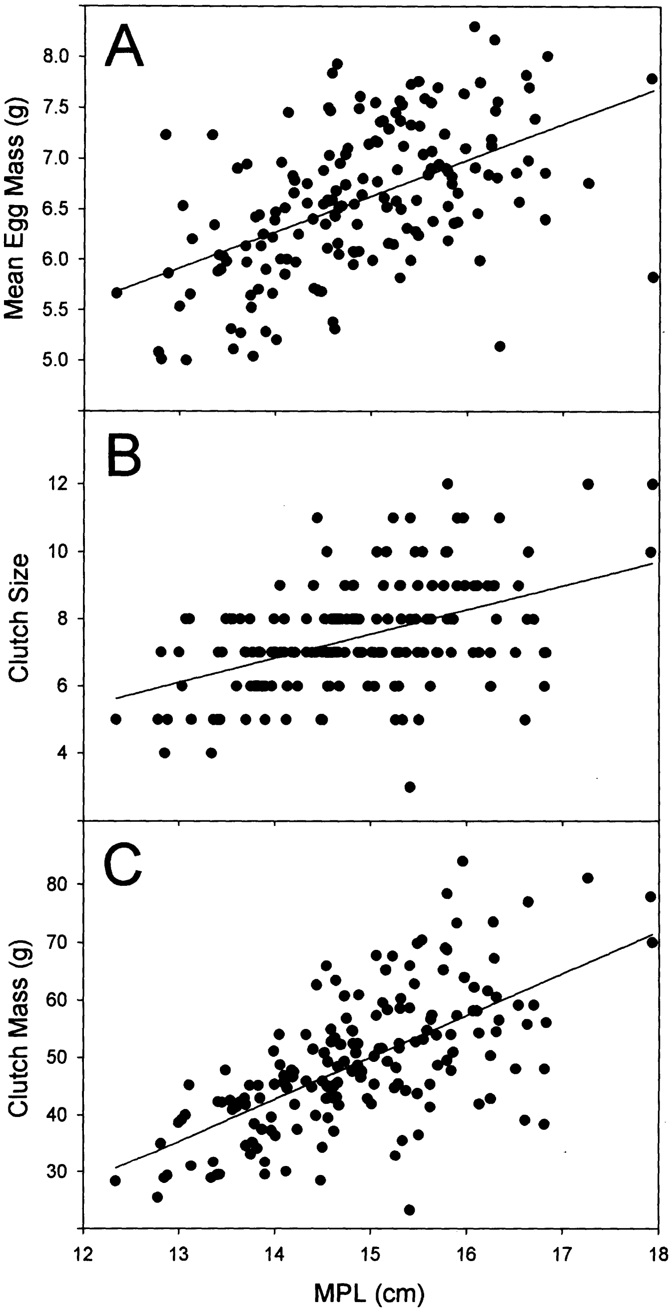

Fig. 1. Linear relationships between maximum plastron length (MPL, cm) and (A) mean egg mass ($R^2 = 0.271$, $t = 7.85$, $P < 0.001$, $y = 0.357x + 1.28$), (B) clutch size ($R^2 = 0.218$, $t = 6.81$, $P < 0.001$, $y = 0.722x - 3.28$), and (C) clutch mass ($R^2 = 0.420$, $t = 11.0$, $P < 0.001$, $y = 7.29x - 59.3$) for Painted Turtles (*Chrysemys picta*) nesting in Algonquin Park, ON, Canada.

In the present study, temperature variation prior to and within the nesting season was inversely related to variation in clutch mass and mean egg mass. Our findings are consistent with previous laboratory studies which have shown that elevated spring temperature acts directly on patterns of vitellogenin deposition and inhibits follicular

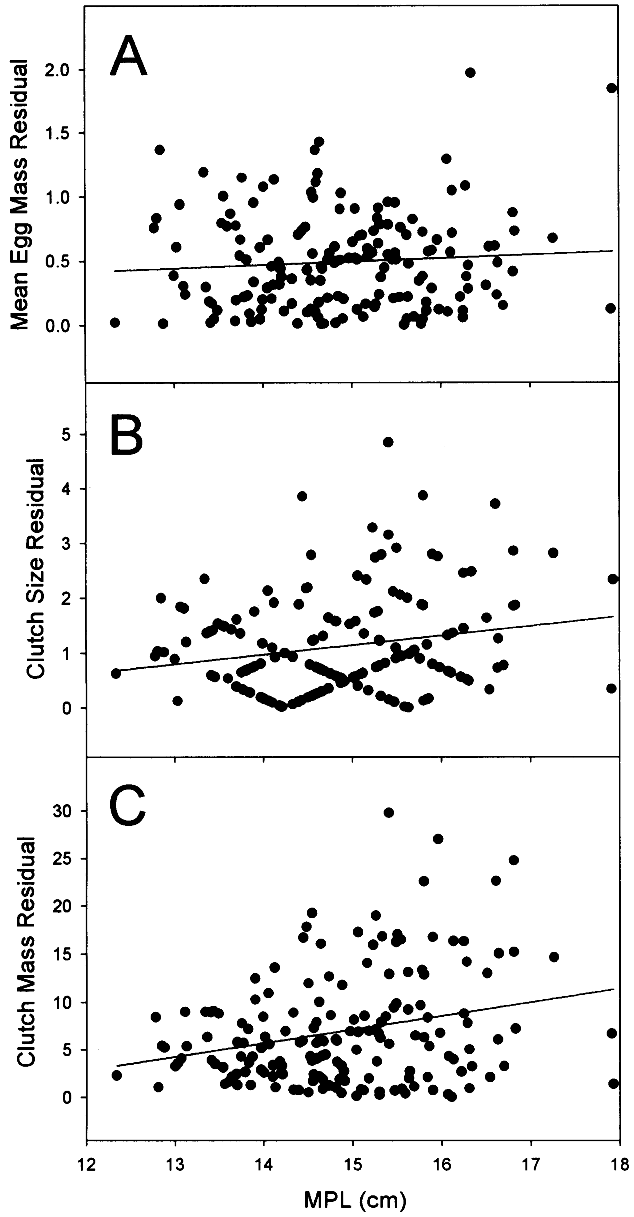

Fig. 2. Residuals of linear relationships between maximum plastron length (MPL, cm) and (A) mean egg mass, (B) clutch size, and (C) clutch mass of the first clutch were obtained (Fig. 1) for Painted Turtles (*Chrysemys picta*) that nested in Algonquin Park, ON, Canada. Residuals were then transformed into absolute values and regressed against MPL using Spearman Rank Correlations (R_S). The non-significant correlation observed for (A) mean egg mass ($R_S = 0.046$, $P = 0.56$) indicates that among-individual variation in mean egg mass did not increase with body size. The positive correlations observed for (B) clutch size ($R_S = 0.164$, $P = 0.034$) and for (C) clutch mass ($R_S = 0.185$, $P = 0.016$) indicate that among-individual variation in both clutch size and clutch mass increased with body size. Linear functions were added to illustrate the prevailing trends.

development (Ganzhorn and Licht, 1983; Mendonca, 1987). Temperature is known to play a key role in gonadal regulation of *C. picta* (Ganzhorn and Licht, 1983) and in other turtles (Mendonca, 1987; Sarkar et al., 1996). In general, peak summer temperatures are associated with follicular quiescence, when large follicles that were not ovulated are resorbed in preparation for the recrudescent phase, and no follicular growth occurs (Moll, 1973; Mendonca, 1987; Sarkar et al., 1996). A seasonal drop in temperature initially stimulates follicular development during the recrudescent phase (mid August–late October in northern populations of *C. picta*), but prolonged exposure to warm temperatures and/or exposure to warm temperature just prior to ovulation inhibits follicular development (Ganzhorn and Licht, 1983; Mendonca, 1987; Sarkar et al., 1996). Ultimately, it is possible that the inhibitory effect of warm temperature on follicular development ensures that the timing of the reproductive cycle is more or less synchronous among years, and within populations, it is likely that selection for this trait is more important than selection against small variations in egg and clutch size. That reproductive parameters were inversely related to temperature in the present study may therefore represent a vestige of selection for a temperature-moderated gonadal cycle.

In populations of *C. picta* that lay multiple clutches in a season, egg mass, clutch size, and (accordingly) clutch mass tend to decrease in successive clutches (Iverson and Smith, 1993; Samson, 2003). It is tempting to suggest that a decrease in egg mass and clutch size with clutch number is a temperature-moderated effect, where increases in temperature throughout the nesting season fully account for decreases in egg mass and clutch size in successive (later) clutches. We deem this unlikely, however, at least for northern populations that lay a maximum of two clutches in a season. This is because follicles that contribute to the second clutch complete vitellogenesis shortly after those of the first clutch are ovulated (Congdon and Tinkle, 1982), and therefore it is likely that the follicles of a second clutch experience temperatures that are similar to those experienced by the first clutch. But it would be interesting to examine the relationship between increases in seasonal temperature and reproductive measures for populations laying three or more clutches in a season (Iverson and Smith, 1993), as the follicles that contribute to third and fourth clutches may experience temperatures that are different from earlier clutches.

That clutch size did not increase with female age (independent of body size) supports the findings of Iverson and Smith (1993) and Congdon et al. (2003). Egg mass did, however, increase with female age (independent of body size), and this finding is likewise supported by previous studies (Iverson and Smith, 1993; Congdon et al., 2003; Bowden et al., 2004). Bowden et al. (2004) demonstrated that younger *C. picta* lay eggs that are about 20% lighter than those of older individuals of the same body size. They argue that testosterone levels may be responsible for the observed disparity in egg size (Callard et al., 1978; Staub and De Beer, 1997), but because younger individuals are presumed to be growing more quickly than the older individuals, the authors suggested that this phenomenon was a tradeoff between growth and reproduction (Barnes, 1962; Warner, 1984). Congdon et al. (2003) likewise found that egg size increased with age independent of body size in a Michigan population of *C. picta*. Interestingly, a careful inspection of the data provided by Congdon et al. (2003) suggests that the relationship between age and egg size is driven entirely by smaller females that produce small eggs for their body size.

In accordance with this possibility, we conducted a supplementary analysis to test whether the relationship between age class and mean egg mass observed in the present study is the result of younger females producing relatively small eggs for their body size. After removing the linear effect of body size, age class is a significant predictor of mean egg mass ($R^2 = 0.031$, $P = 0.023$). However, when only age class 1 is omitted from the analysis, age class is not a significant predictor of variation in mean egg mass ($R^2 < 0.01$, $P = 0.41$), though age remains a significant predictor when we omit only age class 2 ($R^2 = 0.042$, $P = 0.013$), age class 3 ($R^2 = 0.028$, $P = 0.049$), or age class 4 ($R^2 = 0.055$, $P = 0.017$). Thus, the relationship between age and egg mass observed in our study is detectable only because the youngest females produce smaller eggs for a given body size.

Although egg size does not appear to vary as a function of age in *Emydoidea blandingii* (Congdon et al., 2001), it is of interest that younger female *Sternotherus odoratus* and *Kinosternon subrubrum* produce eggs with smaller widths than older females of a similar body size (Clark et al., 2001; Wilkinson and Gibbons, 2005). At this time, however, it remains unclear whether the relationship between age and egg width is entirely driven by the youngest females of these two species, as appears to be the case with *C. picta*. Regardless, this age-related trait may be widespread among turtles, and it may be of great ecological and evolutionary significance. For example, Paitz et al. (2007) showed that maternal age influences offspring survivorship in a population of Painted Turtles (Illinois), though the underlying mechanism(s) is not fully understood (Bowden et al., 2004; Harms et al., 2005).

Finally, it is worth noting that the relationship between age and egg size is realized independent of temperature. Because younger females may nest later in the nesting season (Gibbons and Greene, 1990; Bowden et al., 2004), and given that mean temperature toward the end of the nesting season is greater than mean temperature that occurs prior to, and at the beginning of the nesting season, the relationship between female age and egg mass could be an artifact of temporal increases in temperature, coupled with later nesting dates for younger females. However, we have shown that this is not the case; even though younger females tended to nest later in the nesting season in the present study (partial correlation between age class and Julian nest date, controlling for female body size, $r = -0.188$, $P = 0.015$), the relationship between age class and egg mass is realized over and above of the effect of temperature on egg mass (Table 1).

Relative variability of egg mass versus clutch size.—Previous studies have found that egg mass is generally more conserved than clutch size for a variety of freshwater turtle species (Elgar and Heaphy, 1989; Iverson and Smith, 1993; Roosenburg and Dunham, 1997; Clark et al., 2001). Three lines of evidence from the present study also support this notion. First, clutch size residuals were correlated with MPL, but mean egg mass residuals were not (Figs. 1, 2). This suggests that variation in resource acquisition, which is easier to detect at larger female body sizes (Glazier, 2000), is more strongly related to clutch size than to egg mass. Second, some of the variation in the mass of the first clutch was attributable to clutch frequency (Table 1), but this variation was realized in terms of clutch size, and not mean egg mass, so egg mass again appeared to be conserved. Finally, clutch mass also varied with carapace height, but this variation in clutch mass was attributable to variation in clutch size and not mean egg mass. Our findings suggest that egg mass is relatively conserved at a given female body size, even though it increases with body size.

Choosing an appropriate optimality model.—There are two models of maternal investment that we deem inappropriate for populations of *C. picta* and other turtle species, and these models have received some attention in the turtle literature (Roosenburg and Dunham, 1997; Rowe et al., 2003). First, Kaplan and Cooper's (1984) model of egg size plasticity shows how inter and/or intra-clutch variation in egg size can enhance maternal fitness in environments where the maternal investment per offspring–offspring fitness function is not predictable. However, turtles generally have a long reproductive lifespan, and iteroparity greatly attenuates the increases in maternal fitness that could be accomplished through egg size variation (McGinley et al., 1987; Schultz, 1991). For this reason, we feel that future research should be directed away from the Kaplan–Cooper model. Second, if the environment experienced by a maternal female predicts the environment that will be experienced by her offspring, phenotypic plasticity in egg size and clutch size can enhance maternal fitness (Perrin, 1988; Yampolski and Scheiner, 1996; Fox et al., 1997). Temperature-moderated variation in egg mass (Table 1), for example, may therefore be adaptive if Painted Turtles are using temperature as an indicator of future environmental quality (Rowe et al., 2003). However, the egg incubation period can exceed 60 days in Painted Turtles, and hatchlings overwinter in the nest cavity in many populations (Ernst et al., 1994). The apparent lack of predictability between the environmental conditions that are experienced by the mother and those that her offspring will experience suggests that females do not manipulate egg size to suit future environmental conditions.

Perhaps the most important consideration when framing patterns of maternal investment in *C. picta* into a life-history context is that clutch size, mean egg mass, and clutch mass increase with female body size (Iverson and Smith, 1993). Indeed, female body size (MPL) was the most important predictor (i.e., it had the largest standardized coefficient) of each reproductive parameter in our study (Table 1). It is intuitive that larger females have a larger abdominal cavity than their smaller counterparts, and accordingly, that more eggs can be packed into such an area. Moreover, it is understood that larger *C. picta* tend to have greater lipid reserves, both in relative and absolute terms, than their smaller counterparts (Congdon and Tinkle, 1982; Rollinson and Brooks, 2007), so clutch size likely increases with body size because packing constraints become more relaxed with increasing body size, and because larger females may have more energy to devote to reproduction. However, the fact that egg mass increased with body size is more difficult to explain, especially because optimality models generally predict that egg mass should be conserved among females in a given environment (Smith and Fretwell, 1974).

It has been commonly assumed that egg size is "constrained" in many populations of Painted Turtles (Clark et al., 2001). This notion stems from a seminal paper by Congdon and Gibbons (1987), who showed that the width of the pelvic aperture (through which eggs must pass during oviposition) increases with female body size, and that there is a strong concordance between aperture width and egg width in small-bodied turtles, like *C. picta*. They argued that the architecture of the pelvic girdle can limit egg width (and accordingly, egg mass), and that egg size increases with body size because of selection towards the optimal level of investment (Smith and Fretwell, 1974). This argument necessarily implies that smaller females suffer fitness consequences from producing relatively small eggs, and accordingly, that smaller females would have a greater fitness if they produced larger eggs.

However, numerous subsequent studies on small-bodied turtles show that even when the width of the pelvic aperture is not limiting, egg width (and egg mass) almost invariably increase with female body size (Iverson and Smith, 1993; Clark et al., 2001; Wilkinson and Gibbons, 2005). For example, data synthesized by Clark et al. (2001) show that in only three out of seven populations of *C. picta* studied to date is egg width likely limited by the width of the pelvic aperture. Similarly, egg width was likely not limited by aperture width in all three populations of *S. odoratus* studied by Clark et al. (2001), nor in the populations of *S. odoratus* and *Pseudemys floridana* studied by Wilkinson and Gibbons (2005). It is possible that other "constraints" on egg width exist, such as the size of the oviducts (Iverson and Smith, 1993), or possibly the width of the caudal gap (Clark et al., 2001), but such claims are largely unsubstantiated.

Little attention has been given to the possibility that the selective environment of a turtle's eggs and offspring are influenced by the maternal phenotype (Parker and Begon, 1986; Einum and Fleming, 2002; Hendry and Day, 2003; but see Rollinson and Brooks, 2008). That is, selection on egg/offspring size and quality may vary on spatial and temporal scales (Einum and Fleming, 1999), but maternal 'decisions' that are made by virtue of her phenotype may predict the selective environment of her offspring, resulting in variation in optimal egg size among maternal phenotypes (Hendry et al., 2001). For example, in the present study, body size (a maternal phenotype) was the most important predictor of mean egg mass, and mean egg mass was relatively conserved across a range of female body sizes. This may be indicative of different egg size optima that exist among females of different body sizes, and there are a number of scenarios in which body size-specific egg size optimization could result in a positive relationship between egg mass and body size (Rollinson and Brooks, 2008). Larger, older females, for instance, are generally in better condition than their younger, smaller counterparts (N. Rollinson and R. J. Brooks, unpubl.), and in proximate terms, this may explain why larger, older females are usually among the first to nest in a given season (Gibbons and Greene, 1990; Bowden et al., 2004; present study). Ultimately, laying early may be advantageous because these nests may stand a lesser chance of being depredated (Marchand and Litvaitis, 2004; but see Kolbe and Janzen, 2002), and because eggs in earlier nests may be more likely to complete development, especially in populations where the growing season is short (St. Clair and Gregory, 1990; Bobyn and Brooks, 1994). But because hatchlings emerge in the fall (when environmental conditions may not be conducive to feeding) or overwinter in the nest cavity (Ernst et al., 1994), it is possible that larger females must invest more energy in their offspring as their offspring will hatch earlier and spend more time in the nest cavity (or pond) using valuable energy reserves prior to overwintering. Smaller females, however, may not be able to lay their eggs as early as their larger counterparts, and because their eggs are more likely to hatch relatively late, a large investment per offspring may not be necessary to ensure that their hatchlings overwinter successfully. Though this hypothesis remains to be tested, it shows how phenotypic variation among females could result in a positive correlation between egg mass and body size (Iverson and Smith, 1993), independent of pelvic aperture constraints (Congdon and Gibbons, 1987; Rollinson and Brooks, 2008).

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