

# Does optimal egg size vary with demographic stage because of a physiological constraint?

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## Summary

1. Optimal egg size theory predicts that females should produce propagules of a size and number that maximize maternal fitness. However, studies of the allocation of resources to eggs have rarely provided evidence of such optimization. This is presumably because of constraints that limit reproductive allocation. One such example is that of pelvic aperture morphology constraining egg size in turtles.

2. Growing evidence suggests that even this classic example is incomplete. Hormones that regulate both the reproductive cycle and vitellogenic activity in turtles may provide a novel physiological mechanism for control of egg size.

3. This physiological constraint hypothesis was explored by examining the relationship between maternally derived yolk hormones and egg mass in the Painted Turtle (*Chrysemys picta*, Schneider).

4. Eggs from younger females contained significantly more testosterone in the yolk than did eggs from older females. Younger females laid eggs nearly 20% smaller than those of older females even where body sizes overlapped.

5. Elevated testosterone levels in younger females may thus constrain egg size physiologically, beyond the well-known physical constraints imposed by pelvic aperture morphology. The endocrine system may play an important but previously unrecognized role in the evolution of egg size in this model system.

*Key-words:* Maternal hormones, Painted Turtle, yolk testosterone

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## Introduction

The concepts of optimal propagule size and number are fundamental to organismal life history. Given that a female has limited resources, a trade-off must exist between investment in propagule size and number. Theoretically, resources should be allocated to optimize propagule size and number so as to maximize maternal fitness (Smith & Fretwell 1974). However, despite much progress, few examples clearly demonstrate such optimization (summarized in Roff 2002; but see Sinervo 1990). Further, evidence from a variety of systems indicates that larger propagule size positively influences offspring fitness, but many organisms still exhibit considerable amounts of intraspecific variation in propagule size. Biologists have thus proposed that, owing to constraints that inhibit the achievement of increases in size towards optimality, propagule size is not always maximized.

Constraints (genetic, phylogenetic, etc.) are frequently invoked to explain life-history traits that deviate from those predicted by theory (Roff 1992; Stearns 1992). In the context of optimal egg size theory, the width of the pelvic aperture in reptiles, and caudal gap height (the space between the carapace and plastron through which eggs must pass) in chelonians specifically, are classic examples of apparent physical constraints (Congdon & Gibbons 1987; Sinervo & Licht 1991a; Clark, Ewert & Nelson 2001). However, female turtles can minimize morphological constraints on egg size by continuing to grow as adults, and in some species of turtles, larger females have both larger pelvic apertures and caudal gaps (Congdon & Gibbons 1987; Iverson & Smith 1993; Rowe 1994; Clark *et al.* 2001). These larger females are capable of producing both larger and more eggs than smaller females of the species (Rowe 1994; Valenzuela 2001). Nevertheless, even the largest females in some of these species lay eggs smaller than both the physical constraint limit (Clark *et al.* 2001) and the size expected based on selection to maximize both hatching success (Gutzke & Packard 1985) and neonatal survival (e.g. Janzen, Tucker & Paukstis 2000a,b; Tucker 2000). Together

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these findings suggest that a physical constraint alone is insufficient to explain the lack of egg size maximization in at least some turtle species.

Egg size may be constrained physiologically. Indeed, the potential for testosterone to inhibit vitellogenesis has been previously established in turtles (Ho, Danko & Callard 1981; Ho *et al.* 1982; Callard *et al.* 1991). Ho *et al.* (1981) induced vitellogenin production in male Painted Turtles (*Chrysemys picta*, Schneider) via oestradiol administration and then reduced plasma vitellogenin levels through the subsequent administration of either testosterone or progesterone. The inhibitory effects of testosterone on vitellogenesis were longer lasting than those observed for progesterone, and were inversely related to the amount of testosterone administered, with smaller doses of testosterone resulting in a more rapid reduction in vitellogenin production (Ho *et al.* 1981). Although this study was conducted in male turtles, a similar mode of action could occur in female turtles. As in males, experimental administration of oestradiol results in the up-regulation of vitellogenin synthesis (Ho *et al.* 1981), and the administration of progesterone results in the down-regulation of vitellogenin synthesis in females (Custodia-Lora & Callard 2002). Testosterone and progesterone concentrations are elevated in plasma at the end of vitellogenesis in many female reptiles (Callard *et al.* 1978; Guillette *et al.* 1997; Edwards & Jones 2001). Together these studies indicate multihormonal control of vitellogenesis in reptiles, with oestradiol being primarily responsible for inducing vitellogenin synthesis and with testosterone and progesterone being responsible for suppressing vitellogenin synthesis. Despite a number of studies suggesting a role for testosterone in vitellogenesis, this relationship nonetheless remains correlative.

In general, the importance of androgens (including testosterone, dihydrotestosterone and androstenedione) in female vertebrates is poorly understood, in part due to the paucity of studies considering this topic. A recent review concluded that androgens are necessary for normal female development, and not just through the aromatization of androgens to oestrogens (Staub & De Beer 1997). For example, androgens are produced by ovarian follicles, they inhibit follicular growth, and the presence of androgen receptors in a variety of tissues underscores the significance of androgens in female reproductive physiology (Staub & De Beer 1997). These observations highlight the need for additional studies.

Maternally derived steroid hormones (progesterone, androstenedione, testosterone, oestradiol) have been detected in the yolk of freshly laid eggs of several reptile species (Conley *et al.* 1997; Janzen *et al.* 1998; Bowden, Ewert & Nelson 2000, 2002; Bowden *et al.* 2001; Lovern & Wade 2001; Elf, Lang & Fivizzani 2002). Experimental elevation of circulating plasma levels of testosterone, dihydrotestosterone and oestradiol in female Red-Eared Slider Turtles (*Trachemys scripta*) during the period of follicular development resulted in elevated concentrations in the yolk, indicating that yolk

steroid concentrations reflect circulating plasma steroid concentrations (Janzen *et al.* 2002). An analysis of hormone deposition at laying within yolk of both *C. picta* and *T. scripta* revealed that hormones are not evenly distributed throughout the yolk, resulting in 'layers' of hormones within the yolk that are consistent with reported patterns of plasma hormone cycling in females (Bowden *et al.* 2001). Testosterone and progesterone are most abundant in the outermost portion of the yolk as would be expected since both hormones are elevated in the plasma at the end of vitellogenesis, just prior to ovulation (Bowden *et al.* 2001).

The processes governing the determination of egg size and clutch size differ, at least in oviparous reptiles. Clutch size is largely controlled by gonadotropins, primarily follicle stimulating hormone, whereas egg size is determined by the number of follicles to which yolk will be added and vitellogenic capacity (Sinervo & Licht 1991a,b). Yolk is added to all follicles simultaneously in turtles (Callard *et al.* 1978), so once clutch size is determined (as number of recruited follicles), yolk is allocated to those follicles roughly equivalently, resulting in similarly sized eggs within a clutch.

We evaluate a potential physiological constraint on egg size, focusing on a well-studied natural population of the Painted Turtle, *C. picta*, a species that has often served as a model for life-history research. In particular, we investigated variation in egg size, clutch size and maternally derived yolk testosterone levels between clutches of eggs from the same female and among females with differing amounts of nesting experience.

Previous research with *C. picta* shows that female body size increases with age (Wilbur 1975; Zweifel 1989), and that pelvic aperture width and egg size increase as female body size increases (Rowe 1994). Follicular development can last as long as 10 months in *C. picta* (Congdon & Tinkle 1982), thus using a single plasma sample to assess a female's physiological state might not be biologically meaningful. Instead, we used total yolk steroid content as a cumulative index of female physiology throughout this period of follicular development, since yolk steroids reflect variation in plasma steroid hormones (Janzen *et al.* 2002). We demonstrate that age-related female physiology varies in a manner consistent with our physiological constraint hypothesis, and suggest that egg size may be constrained physiologically, at least under some conditions, thereby highlighting a previously undetected link between physiology and life-history evolution.

## Materials and methods

### STUDY SITE AND FIELD METHODS

Nesting activity of Painted Turtles was monitored from 26 May to 2 July 2002 on the southern portion of an island in the Mississippi River, Carroll County, Illinois (41°57' N, 90°7' W). Painted Turtles nesting in this area have been studied for the past 15 years (Janzen 1994;

Janzen & Morjan 2001; Valenzuela & Janzen 2001), with essentially all females greater than 7 years of age (those females captured during prior nesting events) having been previously marked with a unique combination of notches in the marginal scutes of the carapace. For all unmarked females nesting in 2002, approximate age was determined by counting growth annuli on the pectoral scutes of the plastron, noting the presence of fresh growth, and by assessing the overall shell condition. The majority of unmarked females retained five to seven growth annuli. Given that female Painted Turtles at this latitude typically do not become reproductively mature until roughly 5–7 years of age (Moll 1973) and that they are philopatric to nesting areas (e.g. Scribner *et al.* 1993), we deduced that 2002 was the first nesting year for these females. Any unmarked females that were thought to be greater than 8 years of age were marked but not included in this study. Females in this latter group lacked discernable growth annuli and fresh growth, and their shells were generally more scarred and discoloured than females in the former group. For this study we used a combination of previously marked females and newly marked females (see below).

Because we have limited information on female age, we grouped our females into two categories based upon prior nesting experience. Although nesting experience *per se* is unlikely directly to affect variables such as egg size, we used nesting experience as a proxy for female age, allowing for the assignment of females to groups by leveraging our long-term data set. High nesting experience (HNE) females had at least 6 years of prior nesting experience (based upon previous capture data), were at least 12 years of age (based upon timing of onset of reproduction in *C. picta*), and had nested and produced multiple clutches within a nesting season in at least 3 years. Low nesting experience (LNE) females had 0–2 years of prior nesting experience, placing them at approximately 5–9 years of age. Because of limited or no previous data on these females, no requirements were placed on number of prior years producing multiple clutches.

All females that came ashore were monitored, but were allowed to nest undisturbed. Immediately after nest completion, females were captured by hand for identification and to take a standard linear measurement of body size (plastron length to the nearest mm). Within 4 h of nest completion, target nests were excavated to count and weigh eggs (to the nearest 0.01 g), and two eggs/clutch were collected for subsequent hormone analyses. In turtles, yolk is added simultaneously to all eggs in a clutch, and yolk steroid concentrations within clutches are repeatable (Janzen *et al.* 1998; Bowden *et al.* 2000), so two eggs are sufficient to estimate steroid concentrations for the entire clutch. These latter eggs were immediately frozen (–20 °C) and later transported to the laboratory where they were stored frozen until hormone analysis. The remaining eggs were returned to the nest to complete incubation.

All females that laid at least two clutches of eggs in 2002 were included in the clutch size analyses, including the females chosen for hormone analysis, resulting in 170 total clutches (85 first clutches and 85 second clutches) from 57 females with HNE and 28 females with LNE. All females that nested in 2002 and could be accurately assigned to either the HNE ( $n = 100$ ) or LNE ( $n = 71$ ) categories were used in the analysis of egg size. We evaluated all HNE ( $n = 22$ ) and LNE ( $n = 16$ ) females that had been collected and measured in 2001 in an analysis of relative female growth rates (as measured by the change in plastron length between nesting seasons 2001 and 2002).

#### RADIOIMMUNOASSAYS

We used a competitive-binding steroid radioimmunoassay (RIA) to measure levels of testosterone in *C. picta* yolk samples from 60 clutches (30 first clutches, 30 second clutches) from 30 females (15 HNE, 15 LNE). To prepare yolk samples for the assay, frozen yolks were separated from the other egg components and weighed, and each yolk was homogenized. A sample of approximately 50 mg of homogenized yolk was collected from each egg and suspended in 500 µl of dH<sub>2</sub>O. We followed the RIA procedure of Wingfield & Farner (1975), see also Bowden *et al.* (2000, 2001): 2000 cpm of tritiated testosterone (New England Nuclear, Boston, MA) was added to each sample to serve as a tracer. Samples were then vortexed and allowed to equilibrate overnight at 4 °C. Testosterone was extracted from the samples using petroleum and diethyl ethers and reconstituted in 90% ethanol (Schwabl 1993). The reconstituted samples were stored at –20 °C overnight to allow for sedimentation of neutral lipids and then were centrifuged and decanted. The supernatant was evaporated under nitrogen gas and resuspended in 10% ethyl acetate in isooctane in preparation for column chromatography. The columns consisted of a celite : ethylene glycol : propylene glycol upper phase and celite : water lower phase. Samples were directly applied to the columns and hormone separation was completed by eluting the testosterone fraction with 20% ethyl acetate : isooctane. Fractionated samples were dried under nitrogen gas and resuspended in phosphate buffer. Hormone concentrations were measured by competitive-binding radioimmunoassay using an antibody specific for testosterone (Wien Laboratories, Succasunna, NJ).

Yolk samples were run in duplicate and values for duplicates were averaged to obtain a single testosterone concentration for each sample. Testosterone concentrations were compared with a standard curve that ranged from 1.95 to 500 pg. Recovery values averaged 74.8% for testosterone, and the samples were run in three assays. The intra-assay variation, calculated as the coefficient of variation for the standards, was 7.89%, 6.83% and 5.05%, and the interassay variation was 7.04%. Testosterone values obtained from the RIA were also multiplied by total wet yolk mass to determine

the total testosterone content of each yolk. Total testosterone content for a clutch was determined by averaging the values obtained for the two eggs per clutch used for hormone analysis.

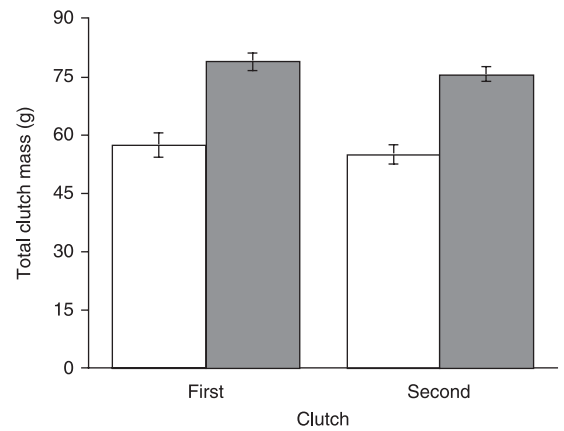
#### STATISTICAL ANALYSES

We ran regressions and repeated-measures ANOVAs to compare female age and size variables to egg variables and to examine variation in yolk hormone levels across nesting events within females using SuperANOVA (Abacus Concepts, Berkeley, CA). We used a nested ANOVA to partition variance in testosterone due to clutch and female, and an ANCOVA to assess the effects of plastron length and female nesting experience (high vs low) on egg mass with JMP (SAS Institute, Cary, NC). Data were log-transformed prior to statistical analysis as appropriate to meet assumptions of normality. Values presented in both the Results section and in figures reflect untransformed data for clarity.

#### Results

Body size and reproductive effort generally differed with age (Table 1). Females in the LNE group had significantly shorter plastrons than females in the HNE group ( $F_{1,83} = 39.48$ ,  $P < 0.0001$ ). LNE females also grew significantly faster than HNE females, as evidenced by an approximately three-fold increase in the rate of plastron growth over 1 year (LNE =  $3.06 \pm 0.31$  mm; HNE =  $1.05 \pm 0.18$  mm;  $F_{1,36} = 35.81$ ,  $P < 0.0001$ ). LNE females had lower total clutch mass than HNE females ( $F_{1,83} = 42.92$ ,  $P < 0.0001$ ). Regardless of female age, first clutches did not differ significantly in mass from second clutches ( $F_{1,83} = 3.30$ ,  $P = 0.073$ , Fig. 1). The mean number of eggs per clutch did not vary either with female age ( $F_{1,83} = 2.34$ ,  $P = 0.13$ ) or between first and second clutches (first =  $10.6 \pm 0.2$ , second =  $10.8 \pm 0.2$ ;  $F_{1,83} = 0.46$ ,  $P = 0.50$ ). Oviposition date did not differ between LNE and HNE females for either first clutches ( $F_{1,18} = 3.74$ ,  $P = 0.07$ ), or second clutches ( $F_{1,18} = 3.10$ ,  $P = 0.10$ ).

We found a strong, positive relationship between female size and egg mass ( $r = 0.69$ ). However, because



**Fig. 1.** Total clutch mass for first and second clutches within the 2002 nesting season from low nesting experience (LNE) and high nesting experience (HNE) females. Open bars represent LNE females ( $n = 28$ ), filled bars represent HNE females ( $n = 57$ ). Values are reported as mean clutch mass (g)  $\pm$  1 SE.

we have two groups in our data set (LNE and HNE females), we were interested in examining whether or not this relationship was constant between these two groups. We performed an analysis of covariance on all females whose plastron length ranged between 145 and 170 mm (the primary zone of body size overlap in the data set). The ANCOVA revealed a significant interaction between female age and plastron length on average egg mass (Table 2), LNE females laid less massive eggs for a given body size (Fig. 2). Across the entire data set, LNE females produced strikingly smaller eggs than similarly sized HNE females (Table 1). Constraining the analysis to the zone of overlap, LNE females produced eggs that were ~20% smaller than those from HNE females; LNE females averaged  $5.56 \pm 0.12$  g egg<sup>-1</sup> ( $n = 41$ ) whereas HNE females averaged  $6.84 \pm 0.07$  g egg<sup>-1</sup> ( $n = 84$ ) across both clutches within the same nesting season (Fig. 2). Number of nesting events was also included in the ANCOVA, but egg mass was not affected by the number of nests laid by a female, so nest number was removed from the analysis. Because of the significant interaction term in the ANCOVA, we were unable to compare egg mass variation between LNE and HNE females directly.

**Table 1.** Measurements of female size, clutch size, egg size and oviposition date for females with low and high nesting experience. Clutch and egg measurements represent the mean of first and second clutch means

| Attribute                      |               | Low nesting experience ( $\pm$ SE) | High nesting experience ( $\pm$ SE) |
|--------------------------------|---------------|------------------------------------|-------------------------------------|
| Plastron length (mm)*          |               | 147 $\pm$ 1.41                     | 158 $\pm$ 1.14                      |
| Total clutch mass (g)*         |               | 56.37 $\pm$ 1.96                   | 77.35 $\pm$ 1.47                    |
| Egg mass (g)*                  |               | 5.30 $\pm$ 0.11                    | 6.87 $\pm$ 0.07                     |
| Wet yolk mass (g)*†            |               | 2.00 $\pm$ 0.65                    | 2.87 $\pm$ 0.66                     |
| Clutch size (number)           |               | 10.3 $\pm$ 0.3                     | 10.9 $\pm$ 0.2                      |
| Oviposition date (Julian date) | First clutch  | 159 $\pm$ 0.98                     | 155 $\pm$ 1.99                      |
|                                | Second clutch | 174 $\pm$ 0.52                     | 172 $\pm$ 1.38                      |

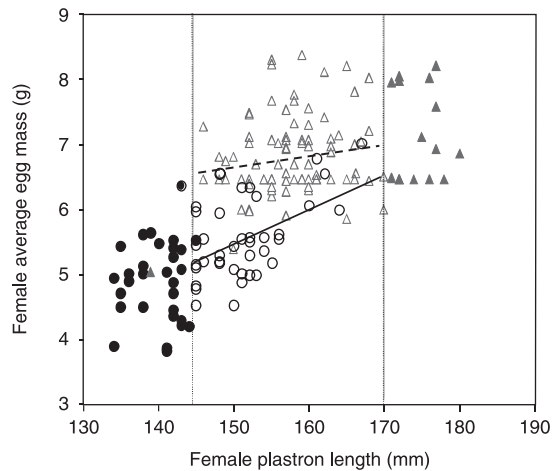
\*Denotes significant ( $P < 0.05$ ) difference between LNE and HNE means.

†Wet yolk mass was determined for 80 eggs representing 40 females (20 LNE, 20 HNE), a subset of these eggs are represented in the yolk steroid analyses.



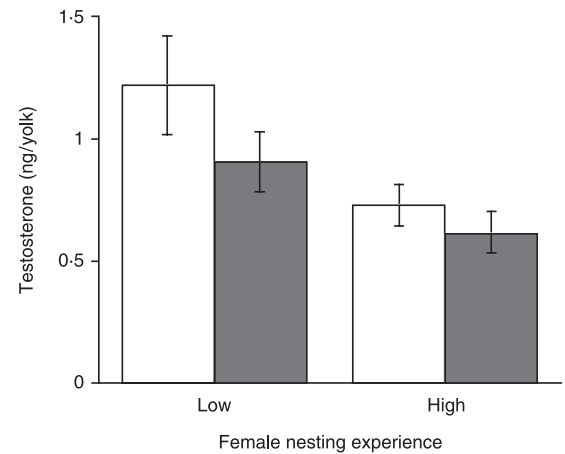
**Table 2.** Results of an analysis of covariance based upon log-transformed data for the effect of nesting experience and female size (plastron length) on egg mass in *Chrysemys picta*. The analysis represents 125 females with plastron lengths between 145 and 170 mm

| Source                               | df  | SS    | F-value | P-value |
|--------------------------------------|-----|-------|---------|---------|
| Plastron length                      | 1   | 0.122 | 15.44   | 0.0001  |
| Nesting experience                   | 1   | 0.472 | 59.72   | <0.0001 |
| Plastron length * nesting experience | 1   | 0.039 | 4.96    | 0.028   |
| Error                                | 121 | 0.956 |         |         |



**Fig. 2.** Average egg mass as a function of plastron length and female age. Circles represent LNE females ( $n = 71$ ), triangles represent HNE females ( $n = 100$ ). The vertical dotted lines delineate the primary zone of overlap in body size for LNE and HNE females, and all individuals contained within this zone (represented by open symbols) were included in the ANCOVA. Regression lines for the ANCOVA are as follows: LNE ( $y = e^{(1.444x - 5.536)}$ ;  $r = 0.50$ ), HNE ( $y = e^{(0.399x - 0.103)}$ ;  $r = 0.16$ ). Lines represent back-transformed regressions of log-log data.

The endocrine assays revealed clutch, age and seasonal effects. Testosterone concentrations were significantly correlated for the two eggs assayed from each clutch ( $F_{1,58} = 49.66$ ,  $P < 0.0001$ ). Sixty-three per cent of the variance in testosterone values was due to variation among females ( $F_{1,29} = 6.22$ ,  $P < 0.0001$ ), with variation among clutches accounting for 11% of the variance ( $F_{1,30} = 1.81$ ,  $P = 0.025$ ). Accounting for female age, testosterone concentrations varied significantly between first and second clutches ( $F_{1,28} = 4.33$ ,  $P = 0.047$ , Fig. 3), with testosterone concentrations being higher in first clutches from both LNE and HNE females. Mean testosterone concentrations were  $1.22 \pm 0.20$  and  $0.91 \pm 0.12$  ng g<sup>-1</sup> of yolk from first and second clutches of LNE females, respectively. Mean testosterone concentrations from HNE females were  $0.73 \pm 0.08$  and  $0.62 \pm 0.09$  ng g<sup>-1</sup> of yolk from first and second clutches, respectively. Despite being smaller, yolks from LNE females contained absolutely more testosterone than did yolks from HNE females ( $F_{1,28} = 4.93$ ,  $P = 0.035$ , Fig. 3). For all eggs used in the hormone analysis, a comparison of egg mass to wet yolk mass revealed a significant association between these two



**Fig. 3.** Yolk testosterone content for first and second clutches within the 2002 nesting season from LNE and HNE females. Open bars represent first clutches ( $n = 15$ ), filled bars represent second clutches ( $n = 15$ ). Values are reported as mean total testosterone per yolk  $\pm 1$  SE. Data were log-transformed prior to statistical analyses.

variables ( $F_{1,58} = 60.01$ ,  $P < 0.0001$ ;  $r = 0.88$ ). Thus, increases in egg mass strongly correspond to increases in yolk mass (Table 1) rather than to increases in the aqueous albumen fraction of the eggs.

## Discussion

Although intuitively appealing, life-history theory surrounding the apportionment of resources between propagule size and number has been fraught with controversy and relatively few clear-cut cases of support. The potential physical constraint on egg size in turtles has long served as a classic cautionary example of the limitations of this fundamental theory. In this paper, we provide evidence for the existence of yet another type of constraint in turtles. Younger females, with body sizes similar to those of older females, laid significantly (nearly 20%) smaller eggs than older females (Fig. 2), and this pattern is not readily explained by morphological constraints within the zone of body size overlap.

A trade-off between growth and reproduction might explain this striking disparity in reproductive allocation. The majority of growth occurs prior to reproductive maturity in turtles, but growth continues throughout an individual's lifetime at a much-reduced rate (Wilbur

1975; Zweifel 1989). We found that younger (LNE) females grew more rapidly than older (HNE) females, whereas HNE females invested more in offspring as evidenced by an increase in total clutch mass (Fig. 1). This finding suggests that the LNE females were not resource limited compared with their HNE counterparts, but rather that the LNE females favoured growth over reproduction. Moreover, despite overlap in body size between LNE and HNE females, LNE females produced smaller clutch masses and less massive eggs (Fig. 2). These data are consistent with a trade-off in the younger females between allocation to growth and allocation to offspring as well as a trade-off between egg size and number. Surprisingly, the difference in resource allocation to clutch mass was not the result of LNE females producing fewer eggs of more similar size to those of HNE females, a strategy that would have mitigated any potential negative effects of reduced egg size. Instead, we found that younger females produced clutches of eggs similar in number to, but consisting of smaller eggs than, clutches from older females. This is consistent with younger females either overestimating their available resources at the time when clutch size is determined, or with clutch size not being directly under female control. Although clutch size may be constrained, this possibility seems unlikely, as clutch size has been shown to be more variable than egg size in many species of turtles (Roosenburg & Dunham 1997; Clark *et al.* 2001; Valenzuela 2001). Alternatively, the lack of variation in clutch size may be attributable to optimization of clutch size by selection in this population, but resolution of this issue will require further study.

In addition to laying smaller eggs than older females, younger females also produced eggs with higher concentrations of testosterone. Further, for females within the zone of body size overlap, those with less nesting experience produced disproportionately smaller eggs, suggesting that egg size was limited in these individuals but probably not by physical constraints (see also Clark *et al.* 2001). Studies across several populations of *C. picta* found that the width of the pelvic aperture increased with female body size (Congdon & Gibbons 1987; Iverson & Smith 1993; Rowe 1994), further suggesting a lack of physical constraint on egg size, at least in the larger LNE females.

That physical constraints do not appear to limit egg size in turtles, under some conditions, indicates that another mechanism probably functions to limit egg size in the larger LNE females. A late vitellogenic surge of plasma testosterone in females is common in reptiles (Guillette *et al.* 1997; Edwards & Jones 2001; Weiss, Jennings & Moore 2002). This surge has been associated with courtship and mating in alligators (Guillette *et al.* 1997) and with sexual receptivity in Leopard Geckos (Rhen *et al.* 2003). The higher testosterone contents of the egg yolks from LNE females probably indicates that these females had higher levels of circulating testosterone while they were undergoing vitello-

genesis than did HNE females, although plasma was not collected to confirm this conjecture. The present data are consistent with the hypothesis that elevated testosterone levels constrain egg size by limiting vitellogenic activity as previously demonstrated for *C. picta* (Ho *et al.* 1981, 1982; Callard *et al.* 1991). Alternatively, testosterone may impact egg size via an association with increased female growth rates rather than through reproduction *per se*.

Androgens are directly implicated in the sexually dimorphic growth of humans during adolescence; bone growth and vertebral size tend to be greater in males than females (Khosla, Spelsberg & Riggs 1999). Painted Turtles also express sexual dimorphism, but females are larger than males at maturity and there is some evidence that females grow faster than males (Zweifel 1989; Ernst, Lovich & Barbour 1994; Janzen & Morjan 2002). Growth is commonly measured as an increase in shell length of either the plastron or the carapace (Zweifel 1989; Rowe 1994; Clark *et al.* 2001). Turtle shell is made up of bone and a highly keratinized epithelial cover, and testosterone is capable of enhancing growth of both bone (Khosla *et al.* 1999) and keratinized structures such as rooster combs and deer antlers (Nelson 2000). Experimental evidence suggests that testosterone may indeed be capable of enhancing growth. Experimentally elevated testosterone results in accelerated posthatching growth rates in some birds (Schwabl 1996; Eising *et al.* 2001), but the longevity of this effect has not been explored. If testosterone actually functions to enhance growth in *C. picta*, we predict that testosterone levels should decline as growth slows in adults. Experimental studies are needed to clarify the effects of testosterone on growth and reproduction in reptiles.

What are the ecological and evolutionary implications of this potential constraint? Neonate size, which is strongly affected by propagule size, is a critical aspect of the life history of most organisms, typically impacting early growth and survival. For example, larger hatchling turtles tend to experience higher survival in the field and in the laboratory (Janzen *et al.* 2000a,b; Tucker 2000; Valenzuela 2001; but see Congdon *et al.* 1999). Larger hatchlings are capable of more rapid locomotion, and thus should decrease the amount of time needed to move from nest to water, thereby decreasing the amount of time they are exposed to terrestrial predators (primarily avian) during this highly vulnerable migration period (Janzen *et al.* 2000b). Despite the apparent importance of hatchling size to offspring success, LNE females appear to begin reproducing at a stage when their smaller offspring are less likely to thrive. This suggests that Painted Turtles follow a bet-hedging strategy for reproductive investment and that selection is acting on age at first reproduction as well as offspring size.

Elevated concentrations of testosterone may mediate a functional trade-off in younger females between investment in reproduction and investment in growth. Given the high mortality rates experienced both at the

egg and hatchling stages in turtles, younger females are likely to benefit more from producing additional offspring rather than larger offspring, provided that egg size exceeds the minimum survival threshold (e.g. an egg must contain enough energy to produce a viable offspring capable of overwintering in the nest for *C. picta*). Contrary to elementary optimality theory (Smith & Fretwell 1974), it appears that selection should act to accelerate female growth when young and to increase offspring size toward an optimum only after a female fully matures. As a consequence, optimal egg size should vary with female age, not simply with female size, as has been found in a population of *T. scripta* (Tucker 2001).

Our findings shed new light on an old standard in life-history evolution. Specifically, we identify a potential endocrinological constraint on the achievement of an optimal egg size in turtles, which has served as a classic example of morphological constraint on egg size. This study highlights the importance of examining functional links between physiological state, demographic stage and life-history evolution in nature. Empirical studies are now needed to confirm the mechanistic basis underlying the physiological constraint on egg size in this model system.

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