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## Abstract

Theory predicts that in long-lived organisms females should invest less energy in reproduction and more in growth and self-maintenance early in life, with this balance shifting as females age and the relative value of each reproductive event increases. We investigated this potential trade-off by characterizing within-population variation in resource allocation to eggs by female painted turtles (*Chrysemys picta*) and relating this variation to their nesting ecology and life history. We examined lipid and protein allocation to yolks, accounting for both relative female age and seasonal effects (first vs. second clutches within a female). Older females appear to increase their investment in reproduction by producing larger eggs, but these eggs are not disproportionately more lipid or protein rich than the smaller eggs from younger females. Within the nesting season, first clutches have more lipid and protein than second clutches. We also found that younger females nest closer to the water than older females. Our results indicate that trade-offs involving resource allocation and nesting behavior do occur both seasonally and with age, suggesting ontogenetic variation in life-history strategies in this long-lived organism.

## Disciplines

Behavior and Ethology | Ecology and Evolutionary Biology | Population Biology | Terrestrial and Aquatic Ecology

## Comments

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# Age and Season Impact Resource Allocation to Eggs and Nesting Behavior in the Painted Turtle

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## ABSTRACT

Theory predicts that in long-lived organisms females should invest less energy in reproduction and more in growth and self-maintenance early in life, with this balance shifting as females age and the relative value of each reproductive event increases. We investigated this potential trade-off by characterizing within-population variation in resource allocation to eggs by female painted turtles (*Chrysemys picta*) and relating this variation to their nesting ecology and life history. We examined lipid and protein allocation to yolks, accounting for both relative female age and seasonal effects (first vs. second clutches within a female). Older females appear to increase their investment in reproduction by producing larger eggs, but these eggs are not disproportionately more lipid or protein rich than the smaller eggs from younger females. Within the nesting season, first clutches have more lipid and protein than second clutches. We also found that younger females nest closer to the water than older females. Our results indicate that trade-offs involving resource allocation and nesting behavior do occur both seasonally and with age, suggesting ontogenetic variation in life-history strategies in this long-lived organism.

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## Introduction

According to the relative reproductive rate hypothesis, allocation to reproduction is expected to increase over time in long-lived organisms (Williams 1957; Kozlowski 1992; Ricklefs and Wikelski 2002; Congdon et al. 2003). As a female ages and growth slows, and the relative value of each reproductive event increases, she should shift resources from growth to reproduction. Moreover, theory predicts that a female should allocate these reproductive resources to propagules such that an optimum combination of quality and quantity is reached, thereby maximizing female fitness (Smith and Fretwell 1974). At the same time, offspring fitness in oviparous organisms often depends on propagule size (reviewed in Azevedo et al. 1997) and the location of the oviposition site, which affects the probability of nest predation (e.g., Paton 1994), the environmental conditions experienced during and after embryonic development (e.g., Weisrock and Janzen 1999), and the posthatching environment for dispersal (e.g., Kolbe and Janzen 2001). It is the complex interplay of all such parental and offspring fitness factors that yields the life-history pattern of a population.

Turtles comprise a model taxon with which to explore these issues. They are long lived and exhibit indeterminate growth, growing throughout their lifetimes, although growth slows considerably after reproduction begins (Wilbur 1975; Zweifel 1989). For example, female painted turtles (*Chrysemys picta*, Schneider 1783) grow three times faster at the onset of reproduction than females that have been reproducing for several years or more (Congdon et al. 2003; Bowden et al. 2004). This age-linked disparity in growth rates sets up the potential for a trade-off in how a female allocates energy and resources between growth and reproduction.

Folliculogenesis may span as many as 10 mo in turtles (Ernst et al. 1994). Given this lengthy period of follicle maturation, the opportunity exists for variation in maternal allocation of resources to yolk. Since yolk is the primary source of nutrition utilized during embryogenesis in oviparous organisms, it must contain all the necessary materials and energy to complete embryonic development (Ewert 1979). Because of the lack of post-ovulatory parental care in many reptiles, the yolk must also contain sufficient resources to sustain the hatchling after emergence from the egg, until it is able to find an external source of nutrition. Thus variation in resource allocation, which could differ both within and among populations depending on their life history, could impact offspring fitness.

In most oviparous organisms, a decrease in egg size results

in a smaller hatchling, and these smaller hatchlings often exhibit decreased fitness when compared to larger conspecifics (Ewert 1979; Congdon and Gibbons 1987; Janzen 1993; Valenzuela 2001). Given that a female has a finite amount of resources to allocate toward reproduction within a reproductive season, a trade-off should exist between egg size and clutch size, where an increase in average egg size results in a decrease in the number of eggs a female can produce in a given clutch (Smith and Fretwell 1974). Complicating this potential trade-off, egg composition may also play an important role in determining offspring fitness (Rowe et al. 1995; Nagle et al. 2003); larger egg size does not guarantee increased hatchling success or survival. Instead, offspring fitness could depend on the nutritional composition of the egg (e.g., a hatchling from a larger egg containing a higher proportion of water might have lower fitness than one emerging from a somewhat smaller egg that is more nutrient rich).

Although lipid (Congdon and Gibbons 1985; Janzen et al. 1990; Rowe et al. 1995; Thompson et al. 1999; Nagle et al. 2003) and, to a lesser extent, protein (Wilhoft 1986; Janzen et al. 1990; Thompson et al. 1999) contents of turtle eggs have been characterized, and allocation of these resources has been compared across species, few studies have examined within-population variation. In one such study, Booth (2003) investigated yolk water, lipid, and protein variation over a large range of egg sizes in two species of Australian freshwater turtles and found that although lipid and protein allocation remained constant, water content was inversely correlated with egg size in one species.

We have previously shown that younger painted turtle females lay smaller eggs than relatively older females and that there is no difference in clutch size between these two groups (Bowden et al. 2004). This observation suggests that, all else being equal, the smaller hatchlings produced by these younger females are likely to experience a reduction in fitness compared to their larger counterparts from older females. Younger females should benefit if they are able to compensate for the fitness costs stemming from laying relatively smaller eggs. One method, other than creating eggs that are more nutrient rich, may be to adopt a nesting behavior that places their smaller hatchlings closer to the water after emerging from the nest. However, this nesting behavior could have its own cost: nests laid closer to the water often experience a higher rate of predation than nests laid farther inland (Christens and Bider 1987; Kolbe and Janzen 2002; Spencer 2002). As a result, each female should strike a balance between the costs and benefits of resource-allocation patterns and nest-site choice that confers the greatest fitness to her through maximal offspring survivorship.

Our objectives were to characterize within-population variation in resource allocation to eggs by female painted turtles and to relate this variation to their nesting ecology and life history. We examined lipid and protein contents of yolks, ac-

counting for both relative female age (based on years of previous nesting experience) and seasonal effects (first vs. second clutch per female within a season). We made three predictions concerning the patterns of resource allocation and nest-site choice within our painted turtle population. First, younger females (those with fewer years of nesting experience) would lay eggs with yolks that were less lipid and protein rich than those of eggs laid by relatively older females (those with more years of nesting experience) because younger females would be using a greater proportion of their resources for growth than older females and would thus have less available for investment in reproduction. Second, yolks of eggs from the first clutch of the season would have more lipid and protein relative to yolks from second-clutch eggs. Females allocate the majority of yolk to first-clutch eggs in the fall preceding laying (Congdon and Tinkle 1982; Bowden et al. 2002). Thus much of the resource allocation to the first clutch is taking place during the late summer to early fall when resources are relatively abundant. In contrast, allocation to the second clutch is primarily taking place in the spring when body reserves are low and the foraging substrate is less available. Finally, we predicted that younger females would nest closer to the water than older females, at a distance that could result in decreased predation on the hatchlings by decreasing the time between their emergence from the nest and when they reach the water. After testing these three predictions, we then integrate the results to provide a more complete picture of the reproductive aspect of the life history characterizing these long-lived organisms.

## Material and Methods

### *Study Site and Field Methods*

The population of painted turtles that nests at the Thomson Causeway Recreation Area in the Mississippi River, Carroll County, Illinois (41°57'N, 90°7'W), has been studied since 1988 (Janzen 1994; Janzen and Morjan 2001; Bowden et al. 2004). Consequently, nearly all females older than 7 yr of age (those with prior nesting experience) have been marked by filing a unique combination of notches into the marginal scutes of the carapace. For this study we monitored nesting females at Thomson Causeway from May 26 to July 2, 2002. Approximate age for each unmarked female observed nesting during this period was determined by noting the presence of fresh growth in plastral sutures, counting growth annuli on the pectoral scutes of the plastron, and assessing the overall shell condition. Since female painted turtles at this latitude typically become reproductively mature at 5–7 yr of age (Moll 1973) and are philopatric to nesting areas (Janzen and Morjan 2001; Valenzuela and Janzen 2001), we assumed that 2002 was the first reproductive season for any unmarked female that retained five to seven growth annuli and was found actively nesting. Any unmarked female thought to be more than 8 yr of age (no discernible growth annuli or fresh growth, and the shell was more

scarred and discolored than the former group) was marked but excluded from this study.

We used a combination of previously marked females, for whom a minimum age could be calculated based on prior known nesting experience and average age at first reproduction, and newly marked females in our study. In other words, we included in our analysis only females we could classify as having either high nesting experience or low nesting experience. Females with high nesting experience (HNE;  $n = 10$ ) had at least 6 yr prior nesting experience, were at least 12 yr of age (based on previous capture data and timing of onset of reproduction in *Chrysemys picta*), and had laid multiple clutches within a nesting season in at least 3 yr before 2002. Females with low nesting experience (LNE;  $n = 10$ ) had 0–2 yr prior nesting experience and had visible recent growth with discernible annuli on plastral scutes, placing them between 5 and 9 yr of age. Several comparisons have been made among females grouped by these criteria to confirm that our classifications based on nesting experience represented functional groups: plastron length, total clutch mass, egg mass, and wet yolk mass all differed significantly between the LNE and HNE females, each being greater in HNE than LNE females, while clutch size and oviposition date did not (see Bowden et al. 2004).

All females were allowed to nest undisturbed but were closely monitored and were hand-captured immediately after nest completion for identification and to measure plastron length to the nearest millimeter. Within 4 h of completion, nests were excavated to count and weigh eggs (to the nearest 0.01 g). Two eggs were collected from both the first and second clutches of the nesting season for each of the 20 females included in this study ( $n = 80$  eggs), with an average internest interval of 16 d. All remaining eggs were returned to their nests to complete incubation, and each nest was measured (to the nearest 0.01 m) to at least three permanent landmarks to facilitate relocation. Because yolk is added simultaneously to all eggs in a clutch in turtles (Congdon and Tinkle 1982; Congdon and Gibbons 1990), all eggs within a clutch should have similar compositions. Consequently, we sampled two eggs per clutch to estimate egg composition for each clutch; these eggs were immediately frozen at  $-20^{\circ}\text{C}$  and later transported to the laboratory. Eggs were collected under permit NH02.0073 from the Illinois Department of Natural Resources and Special Use permit 32576-02024 from the U.S. Fish and Wildlife Service. This research was conducted in accordance with Iowa State University Care and Use of Animals protocol 1-2-5036-J.

#### Lipid and Protein Quantification

In the laboratory, egg yolk was separated from the shell and albumen while still frozen, and the yolk was then homogenized. For each egg, total wet yolk mass was measured before a small sample ( $\sim 0.10$  g) of yolk was removed for steroid radioimmunoassay (RIA; see Bowden et al. 2004), after which the re-

maining yolk was placed into an oven at  $40^{\circ}\text{C}$  and dried to a constant mass for a minimum of 48 h. Because we removed a small, consistent portion from each yolk for the RIA ( $\sim 1.7\%$  of total yolk mass, range 0.097–0.103 g), and the sample removed was representative of the whole yolk because of homogenization, we discounted this portion of yolk and based our calculations for total water, lipid, and protein contents on the mass of the remaining yolk (hereafter referred to as whole wet or dry yolk mass, depending on the analysis). Total water content of the yolk was determined gravimetrically as the change in yolk mass after drying, and this value was divided by the whole wet mass to calculate percent water in the yolk, which we include for comparison to other studies.

From each dried yolk, a sample of  $\sim 0.6$  g was used for lipid extraction. Each sample was placed into a cone of Whatman #3 11-cm filter paper that had been dried to constant mass before use. Petroleum ether was refluxed over the samples for 4 h in a Goldfish lipid-extraction apparatus to remove all nonpolar storage lipids (Janzen et al. 1990; Nagle et al. 2003). The remaining sample and filter paper was then dried to a constant mass for a minimum of 8 h at  $40^{\circ}\text{C}$ , and sample lipid content was determined gravimetrically as the mass change pre- to postextraction. By dividing this value by the preextraction mass, we were able to calculate lipid percentage in each dry yolk sample. Since the samples were homogenized, the lipid percentage in the sample was equal to the lipid percentage in the whole yolk, so total lipid content of each yolk was calculated by multiplying the lipid percentage of each sample by its whole dry yolk mass.

Protein contents of the dry yolk fractions remaining after lipid extraction were determined using the Dumas method (Jung et al. 2003). Samples were analyzed using a RapidN III nitrogen analyzer (Elementar Americas, Mt. Laurel, NJ; see Jung et al. 2003). Dry yolk samples were pelleted in aluminum foil for analysis; three samples of  $\sim 100$  mg were run for each yolk to control for possible interassay variation. Samples were run over four consecutive days, and at the beginning of each day three blanks, two run-in samples, and three aspartic acid standards were run to calibrate the system. Blanks consisted of an empty foil pellet and the run-ins and aspartic acid standards each contained 200 mg aspartic acid. Run-in samples determined whether the measured nitrogen levels were acceptable by comparing them to the known amount of nitrogen in aspartic acid, and the standards were used to calculate a daily conversion factor. Samples were combusted at  $800^{\circ}$ – $1,000^{\circ}\text{C}$  to reduce the yolk to ash and nitrogen gas, which could then be measured by a thermal conductivity detector. Protein percentage in each sample was determined by multiplying the nitrogen percentage by 6.25 (nitrogen-to-protein conversion factor; Card and Nesheim 1966; Thompson 1981), and values obtained were converted to proportions before calculating total yolk protein content. To account for running our protein analyses on lipid-free yolk, we multiplied the average proportion of protein for

Table 1: Egg composition of first and second clutches laid in the 2002 nesting season by HNE and LNE female *Chrysemys picta*

	HNE		LNE	
	First Clutch	Second Clutch	First Clutch	Second Clutch
Clutch size	10.5 ± .373	11.0 ± .683	11.3 ± .700	10.8 ± .573
Total egg mass (g)	7.57 ± .180	7.09 ± .180	5.70 ± .220	5.35 ± .177
Yolk mass (g):				
Wet	2.98 ± .080	2.76 ± .095	2.03 ± .084	1.96 ± .102
Dry	1.34 ± .041	1.23 ± .051	.90 ± .034	.81 ± .050
Water:				
Total (g)	1.52 ± .043	1.41 ± .049	1.01 ± .056	1.02 ± .060
% WYM	53.0 ± .005	53.6 ± .006	52.6 ± .009	55.7 ± .011
Lipid:				
Total (g)	.311 ± .012	.260 ± .013	.210 ± .007	.179 ± .014
% DYM	23.2 ± .006	21.1 ± .006	23.3 ± .007	21.7 ± .005
Protein:				
Total (g)	.775 ± .025	.730 ± .030	.528 ± .022	.482 ± .028
% DYM	57.8 ± .004	59.6 ± .005	57.9 ± .005	59.2 ± .005

Note. LNE = low nesting experience; HNE = high nesting experience. Values are presented as mean ± 1 SE; clutch size = number of eggs per clutch; WYM = wet yolk mass; DYM = dry yolk mass. Sample sizes: HNE,  $n = 10$ ; LNE,  $n = 10$ .

the sample from each egg by the proportion of lipid-free yolk, and total yolk protein content was then calculated by multiplying this value by whole dry yolk mass. Proportions of lipid and protein in the yolk are presented as dry yolk mass percentage (as opposed to wet yolk mass percentage) to facilitate comparison with other studies, the majority of which report similar measures.

#### Distance to Water

Upon completion of the nesting season, all nests were relocated and nest location coordinates were generated using INTERPNT (Harvard University, Petersham, MA). These coordinates were plotted onto a map of the nesting area with ArcView 3.2 (ESRI, Redlands, CA), and distance to water for each nest was determined by the shortest straight-line measurement to the waterline using the ArcView measuring tool.

#### Statistical Analyses

Because our study involved two response variables and we wanted to assess not only how relative nesting experience affected each variable individually but also how these variables were interrelated, we ran two multivariate analyses of variance (MANOVA) in SAS (SAS Institute, Cary, NC). All total lipid and protein content data were log transformed before statistical analyses, which allowed the data to meet the assumptions of normality and homogeneous variance. In addition, since we expected the groups to vary in relative composition, the MANOVA model will show this more clearly if the data are on

a log scale. The first MANOVA evaluated variation between LNE and HNE females in total yolk lipid and protein contents using values that were averaged across the two clutches from each female (two dependent variables, hereafter called the overall MANOVA). The second MANOVA evaluated how variation in the allocation of the two resources between the first and second clutches of each female contributes to any difference in resource allocation between HNE and LNE females (four dependent variables, hereafter called the clutch MANOVA). Standardized canonical coefficients were used to determine which response variables contributed most heavily to any significant differences (see Scheiner 1993). We report percentage data for egg components in addition to total content data to facilitate comparison with other studies.

Wet and dry yolk masses were compared both between female groups and across clutches by performing a two-way factorial ANOVA in JMP v5.0 (SAS Institute). Using SuperANOVA (Abacus Concepts, Berkeley, CA) we ran repeated-measures analyses of variance (RMANOVA) with clutch (within female) as the repeated measure to determine whether there were differences in nest-site location relative to the waterline between LNE and HNE females.

#### Results

Whole wet yolk mass and dry yolk mass varied with both female nesting experience and clutch (Table 1), and there were no nesting experience × clutch interactions (wet:  $F_{1,18} = 1.83$ ,  $P = 0.193$ ; dry:  $F_{1,18} = 0.150$ ,  $P = 0.703$ ). Wet and dry yolk mass were greater for HNE females than LNE females (wet:

Table 2: MANOVA results showing relative contributions of yolk lipid and protein contents to the difference in resource allocation between HNE and LNE female *Chrysemys picta*

MANOVA and Source of Variation	Standardized Canonical Coefficient
Overall:	
Lipid	.802
Protein	1.287
Clutch:	
Lipid first	1.114
Lipid second	.147
Protein first	.816
Protein second	.247

Note. LNE = low nesting experience; HNE = high nesting experience. For the overall MANOVA, contents were averaged across the two clutches laid by each female, while in the clutch MANOVA, each clutch was included as a separate variable (first = first clutch of the season, second = second clutch) for each resource type. Sample sizes: HNE,  $n = 10$ ; LNE,  $n = 10$ .

HNE =  $2.75 \pm 0.07$  g, LNE =  $1.88 \pm 0.07$  g,  $F_{1,18} = 57.57$ ,  $P < 0.0001$ ; dry: HNE =  $1.28 \pm 0.03$  g, LNE =  $0.86 \pm 0.03$  g,  $F_{1,18} = 58.75$ ,  $P < 0.0001$ ). In addition, both wet and dry yolks from first-clutch eggs were heavier than those from second-clutch eggs when mass was averaged across both female classes for each clutch (wet: first =  $2.39 \pm 0.12$  g, second =  $2.24 \pm 0.12$  g,  $F_{1,18} = 6.85$ ,  $P = 0.0174$ ; dry: first =  $1.12 \pm 0.06$  g, second =  $1.02 \pm 0.06$  g,  $F_{1,18} = 11.23$ ,  $P = 0.0036$ ). Across all females, water averaged 53.7% of the wet yolk mass. Lipid accounted for 22.4% and protein for 58.7% of the dry yolk mass (Table 1).

#### Age-Related Variation in Resource Allocation

The overall MANOVA returned a highly significant effect of nesting experience on resource allocation (Pillai's Trace = 0.77,  $F_{2,17} = 28.67$ ,  $P < 0.0001$ ). The canonical coefficients for lipid and protein were similar in magnitude, so variation in allocation of each of these resources contributed equally to the observed statistical difference. The canonical coefficients for both response variables were positive (Table 2), indicating that each variable was positively correlated across the two groups of females and the allocation pattern was the same for each variable: higher contributions of total lipid and protein from HNE females than LNE females (Table 1).

#### Clutch-Related Variation in Resource Allocation

The clutch MANOVA detected variation in resource allocation between first and second clutches for LNE and HNE females, and it also returned a significant effect of nesting experience (Pillai's Trace = 0.804,  $F_{4,15} = 15.40$ ,  $P < 0.0001$ ). As in the overall MANOVA, the canonical coefficients for the clutch MANOVA were all positive (Table 2), so once again all four variables are positively correlated, and an increase in one variable is associated with an increase in the others. We interpret this outcome to indicate that HNE females are allocating higher total amounts of both resources to each of their clutches than are LNE females (Fig. 1). Further, the clutch MANOVA divides this variation into allocation to first clutches and allocation to second clutches. Lipid and protein from first clutches had the largest canonical coefficients, so HNE and LNE females differ most in how they allocate these resources to their first-clutch eggs. Both LNE and HNE females allocate more total lipid and protein to their first clutches than to their second clutches (as indicated by the points being above the dotted line in Fig. 1).

#### Distance to Water

Analysis of a data set that included only the 20 females used in the egg-component study revealed no significant difference in distance to water between nests laid by LNE and HNE females ( $F_{1,18} = 3.71$ ,  $P = 0.070$ ; Fig. 2a). However, a more comprehensive RMANOVA including those 20 females plus 61 other females (22 LNE and 39 HNE, for a total of 81 females)

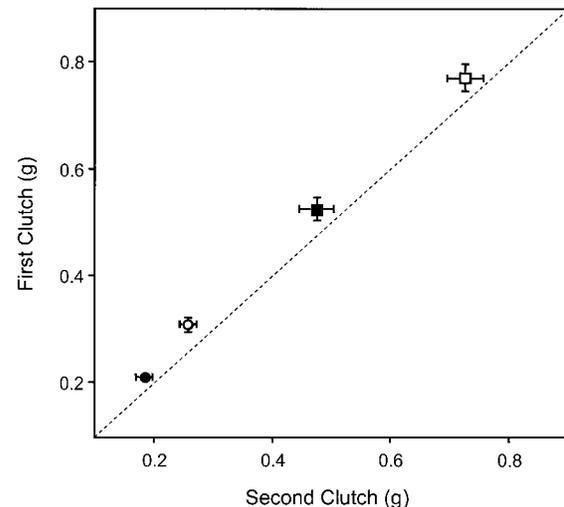


Figure 1. Lipid (circles) and protein (squares) allocation to first and second clutches of *Chrysemys picta* females with low nesting experience (LNE;  $n = 10$ ) and high nesting experience (HNE;  $n = 10$ ). Filled symbols represent LNE; open symbols represent HNE. Untransformed data are presented for clarity, and points represent means  $\pm$  SE. The dotted line has slope = 1 and indicates where resource allocation to each clutch is equal.

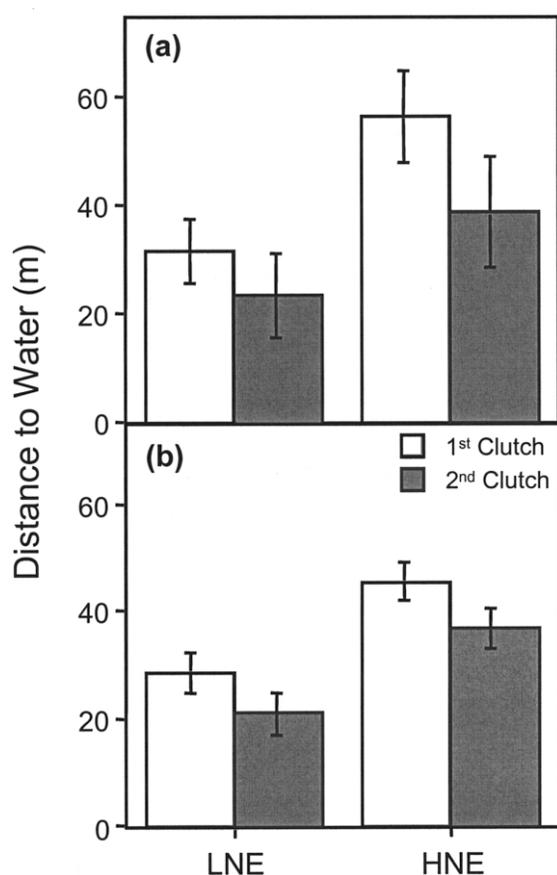


Figure 2. Distance to the water from nests laid by *Chrysemys picta* females with low nesting experience (LNE) and high nesting experience (HNE). *a*, Includes only those females used for the egg-component analyses (LNE,  $n = 10$ ; HNE,  $n = 10$ ). *b*, Includes all females that laid two clutches in the 2002 nesting season (LNE,  $n = 32$ ; HNE,  $n = 49$ ). Data are means  $\pm$  SE.

that also laid two clutches in the 2002 nesting season revealed that HNE females laid nests farther from the water than LNE females ( $F_{1,79} = 13.48$ ,  $P = 0.0004$ ; Fig. 2*b*). Although we did not predict that nesting behavior would vary seasonally with clutch, both the ANOVA using the 20 females ( $F_{1,18} = 7.58$ ,  $P = 0.0131$ ; Fig. 2*a*) and the subsequent comprehensive RMANOVA ( $F_{1,79} = 7.65$ ,  $P = 0.0071$ ; Fig. 2*b*) indicate that first clutches were laid farther from the water than second clutches.

## Discussion

This study is one of the first to examine the nature of the postulated trade-off between growth and reproduction in long-lived organisms and within-population variation in resource allocation. Using the painted turtle as our model species, we first characterize this allocation pattern and then show how it may both influence and be influenced by life-history strategy.

The females in our study laid eggs with yolks that were

slightly more than half water (Table 1), which is substantially lower than the value previously reported for whole *Chrysemys picta* eggs (68.3%, Congdon and Gibbons 1985; 75.5%, Rowe et al. 1995). However, we did not include the shell or albumen in our samples, which likely accounts for most of the observed difference. Our overall nonpolar lipid percentage in the dry yolk (22.4%) is also lower than previously reported values for *C. picta* obtained using similar extraction methods (28.2% [Congdon and Tinkle 1982] to 37.1% [Gutzke et al. 1987]), possibly because of population differences such as the longitudinal variation of egg components found in *Chelydra serpentina* by Finkler et al. (2004). Alternatively, our exclusive use of double-clutching females or differences in egg-preparation and extraction techniques could have contributed to the observed difference. No other study has measured protein percentage in painted turtle yolks, but our overall protein percentage in dry yolk (58.7%) falls in the middle of the range of reported values for other species, although these studies include the dry albumen fraction (*C. serpentina*, 54.9% [Wilhoft 1986] and 60.9% [Janzen et al. 1990]; *Emydura macquarii*, 62.0% [Thompson et al. 1999]; *Emydura signata*, 60.4%; *Chelodina expansa*, 54.0% [Booth 2003]).

The overall MANOVA showed that lipid and protein allocation varied between females with different amounts of nesting experience. HNE females produced eggs with higher total lipid and protein contents than eggs from LNE females (overall MANOVA; Tables 1, 2), but these eggs were also significantly larger and contained larger yolks than those from LNE females, accounting for most of this variation. Once egg mass is taken into account, HNE females did not produce eggs that were more lipid and protein rich than eggs from LNE females (Table 1), suggesting that allocation of these resources may be constrained. In other words, females allocated a set percentage of lipid and protein to their egg yolks regardless of egg mass. Thus, in contrast to our expectation and the pattern observed in other organisms (e.g., Künkele 2000), LNE females produced eggs of similar composition to those of older females.

The clutch MANOVA revealed that total lipid and protein content of yolk declined from first to second clutches laid by each female in a nesting season (Fig. 1). This decline was associated with a seasonal decrease in both whole wet and dry yolk mass as well as in total egg mass (Table 1). Though not measured as part of this study, an increase in albumen water content in second-clutch eggs most likely explains why total egg mass decreases more from the first to second clutch than do wet and dry yolk mass. The seasonal decline in total lipid and protein content suggests that these resources may be limited, and diet most likely plays a role in this decline. Previous research has found that energy for first-clutch eggs derives primarily from harvested resources, as food is generally easily obtainable in late summer into early fall, when yolk is being added to first-clutch follicles. In contrast, most energy for second-clutch eggs derives from stored resources because of

limited food availability in early spring (Congdon and Gibbons 1990). Therefore, females must balance resource allocation between reproduction and self-maintenance/growth in the spring such that they are able to both produce viable offspring and have the highest possible chance of surviving until the next reproductive season. Although we did not examine energy reserves of females in this study, after relying on stored resources throughout the winter they may be energy limited in the spring, which could result in the seasonal decline in yolk resources detected in the current study.

Although we did not include yolk water content in our MANOVAs, as it should not be a limiting resource for these aquatic turtles, water allocation, although quite variable between females, did show an interesting pattern that differed from that of lipid and protein allocation. While LNE females allocated similar amounts of water to both first- and second-clutch eggs, for HNE females, total yolk water content decreased in second-clutch eggs (by ~0.1 g) along with the decreasing levels of lipid and protein (Table 1; Fig. 1). Water availability during development affects hatchling fitness, but not necessarily in a straightforward fashion. Previous research has found that eggs incubated on wet substrates yield larger hatchlings than eggs incubated on dry substrates (Packard and Packard 1986; Janzen et al. 1990; Miller and Packard 1992). In painted turtles, this size difference is caused by a lengthening of development for the eggs incubated on wet substrates (Packard et al. 1983; Cagle et al. 1993). Interestingly, the smallest eggs with the fewest resources (i.e., second-clutch eggs from LNE females) contained proportionally more water than other eggs (Fig. 1). However, water uptake from and loss to the surrounding soil most likely have a greater influence on hatchling fitness than water allocated by the female to the yolk.

One might expect that species whose offspring overwinter in the nest after hatching would allocate more energy to their yolks than turtles whose offspring emerge from the nest soon after hatching in early fall, thus providing a ready source of energy for the hatchling; this is, in fact, often the case with lipids (see Congdon et al. 1983*b*; Congdon and Gibbon 1985). However, even though painted turtle hatchlings, including those at our study site (Weisrock and Janzen 1999), generally overwinter in the nest (Gibbons and Nelson 1978; Ernst et al. 1994), the lipid percentage in the yolks from our population is lower than values for species whose offspring do not overwinter in the nest, while protein percentage in our yolks was higher than that for some species that do not overwinter, but lower than others (see Wilhoft 1986; Janzen et al. 1990; Thompson et al. 1999; Booth 2003).

Protein contributes mostly to growth, while lipids constitute the majority of energy stores (Wilhoft 1986), so the lack of a definitive relationship between yolk protein content and hatchling overwintering is not surprising. In our case, the lower lipid values may reflect a nutritionally rich environment for neonates after emergence from the nests in the spring. Alternatively,

Rowe et al. (1995) found that *C. picta* hatchlings used a smaller percentage of their egg lipids during embryonic development than *C. serpentina* or *Emydoidea blandingii*, species that do not typically overwinter in the nest (Congdon et al. 1983*a*; Ernst et al. 1994; Rowe et al. 1995). This interspecific variation could mean that even though *C. picta* yolks have less stored lipid to begin with, the embryos might metabolize more efficiently such that the nest-bound hatchling has enough lipid reserves to survive the winter.

Instead of producing eggs that are less lipid and protein rich (having a lower percentage of dry yolk mass) than those of HNE females, LNE females appear to limit resource allocation to reproduction by producing smaller eggs, even after the effect of body size has been removed (i.e., LNE females of the same size as HNE females still lay smaller eggs; Bowden et al. 2004). In general, smaller eggs produce smaller hatchlings (Packard et al. 1987; Tucker et al. 1998; Valenzuela 2001), and smaller hatchlings often have lower fitness and survival rates than larger hatchlings (Ewert 1979; Congdon and Gibbons 1987; Brodie and Janzen 1996; Tucker 2000). In some cases, this lower survival may stem from predation during terrestrial migration from nests to the water, since larger hatchlings are able to move faster than smaller ones and thus may be exposed to predation risks for less time (Janzen et al. 2000). However, there are potential benefits to being a smaller hatchling, including that smaller hatchlings often have proportionally larger yolk reserves to sustain them after hatching or emergence (Packard 1999).

Nesting closer to the water, as observed for LNE females (Fig. 2), may help to mitigate the risk of predation while traveling to the water. On the other hand, this pattern may simply relate to a female's nesting experiences: females may become less skittish on land the more times they nest uneventfully. In the case of our nesting area, which often has a substantial presence of people and their pets, turtles may take years to acclimate to these disturbances before nesting farther from the water. If so, LNE and HNE females may differ both in how they assess predation risk and how they respond to this perceived risk. Nests laid closer to the water are more likely to be depredated (Kolbe and Janzen 2002; Spencer 2002), and in our population, natural predation of adults is low and the biggest influences are human related (e.g., road vehicles and hunting/fishing), so the optimal distance from water to lay a nest may vary with female age and/or level of nesting experience. That is, HNE females may nest farther inland, risking higher individual mortality to increase their chances of a successful nest (since their future fecundity is relatively lower than that of LNE females). In turn, the smaller hatchlings from the LNE females would have a shorter distance to traverse from the nests to the water (although the nests have a higher probability of predation). Therefore, the decrease in predation risk to the female as a result of reduced time spent on land may compensate for the decreased fitness resulting from producing smaller hatchlings.

The observed differences in resource allocation, egg size, and nesting behavior between LNE and HNE females in our study seem to indicate the presence of two reproductive strategies related to female life-history stage. When young, females produce smaller eggs that can effectively use the limited resources they are able to put toward reproduction while still growing, leaving a smaller hatchling with enough residual yolk to be able to sustain itself until it can reach the water and begin feeding. Once the females are older and growth has slowed considerably, they are able to invest more energy in reproduction, producing larger eggs, which in turn produce large hatchlings capable of traversing longer distances more quickly.

In sum, we have documented the impacts of maternal age and seasonal variation on yolk mass and on allocation of lipid and protein to egg yolks in a free-ranging population of turtles. These findings yield evidence of ontogenetic changes in the physiological ecology of reproductive females. In combination with the observed differences in spatial nesting behavior of younger and older turtles, our study revealed trade-offs in energy allocation and between fitness components that may influence the life-history strategy of these long-lived organisms. The stage is now set for manipulative laboratory and field experiments to test the mechanistic hypotheses identified herein.

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