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## Impact of Egg Content on Post-Hatching Size, Body Composition, and Performance in the Common Snapping Turtle (*Chelydra serpentina*). Linnaeus Fund Research Report

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Correlations between initial egg mass and hatchling mass have been noted in chelonians (e.g., Morris et al., 1983; Brooks et al., 1991), and increased hatchling size, in turn, has been associated with increased survivorship (Janzen, 1993), presumably via increased competitiveness in intraspecific encounters (Froese and Burghardt, 1974) and perhaps with some degree of protection against predators (Christian and Tracy, 1981). This relationship between egg size and hatchling size is likely due to variation in the amounts of different materials (solids and water in the yolk and albumen) present in the eggs. While several researchers have examined solid nutrient content in relation to egg mass (e.g., Congdon and Gibbons, 1985), changes in the relative amounts of water and solids present within the eggs has been largely ignored. Ricklefs and Burger (1977) noted that larger eggs generally have higher water contents with relatively little difference in total solid content within and among clutches. This suggests that initial water content may be an important factor in determining hatchling mass and subsequent performance, given the influence of water availability on embryonic development and subsequent hatchling size and locomotor performance (Packard, 1991, and references therein).

The present study examined variation in initial egg composition with varying initial egg mass, and its subsequent effects on hatchling mass, hatchling water and dry content, locomotor performance, predatory efficiency, and growth in hatchling snapping turtles. I hypothesized that increased egg mass is mainly due to increased initial water content, and that this increased water content leads to increased hatchling mass with a corresponding increase in the juvenile's ability to capture prey items and avoid predators. Moreover, juvenile hatchlings from relatively large eggs with higher water contents should demonstrate an increased growth rate as a result of increased predatory efficiency.

**Materials and Methods.** — Four freshly-laid *Chelydra serpentina* clutches were collected from Cherry County, Nebraska. Eggs in each clutch were weighed, then each clutch divided by mass into quartiles, from each of which two eggs were randomly selected, opened, and the shell and egg contents dried separately to determine dry shell and solid content masses. The amount of water in each egg was determined by subtraction. Each of these measurements was used to form

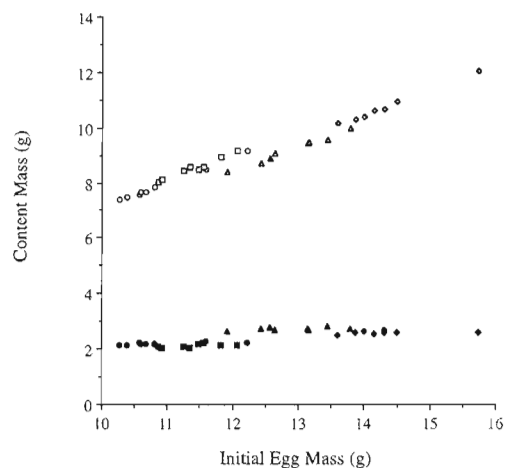
linear regressions for egg composition as a function of egg mass and to predict the initial composition of each egg. Remaining eggs were kept cool in moistened vermiculite until returned to the laboratory.

In the laboratory, eggs were weighed, half buried in fresh vermiculite with a water potential (sustained through the incubation period) of approximately -325 kPa (based on the curve generated from values in Morris et al., 1983), and incubated at  $29 \pm 0.5^\circ\text{C}$ . Eggs were weighed and candled weekly from the third to the eighth week of incubation.

Upon hatching, each hatchling was washed of clinging materials and weighed. The initial egg masses of all hatchlings in a clutch were divided into quartiles and from each quartile two randomly selected hatchlings were sacrificed. The entire yolk sac of each turtle was removed and weighed, and both it and the carcass were placed into a  $60^\circ\text{C}$  drying oven and dried to a constant mass.

Four animals were selected from each quartile of egg mass ( $n = 16$ ) in clutches B, C, and D to participate in growth and performance measures. As only eight animals from clutch A were available for growth and performance testing, all eight were included. Animals were housed individually in  $10 \times 10 \times 8$  cm containers containing water 3 cm deep, and maintained on a 12 hrs light:12 hrs dark photoperiod with a 25:15 °C temperature cycle. In addition to feedings provided during the weekly prey acquisition tests (see below), each animal was fed one piece of commercial cat food and one cricket following weekly locomotor performance tests (see below). Mass (g) was measured weekly prior to locomotor performance testing, and a growth rate (g/wk) for each hatchling was estimated based on the slope of a linear regression equation for that animal's mass measurements.

Locomotor performance (maximum aquatic velocity) was tested weekly for eight weeks beginning seven days post-hatching. A  $120 \times 5 \times 10$  cm linear water trough was lined with



**Figure 1.** Variation in egg content mass (solids and water) with initial egg mass. Filled symbols represent content solids; open symbols represent content water. Different symbol shapes represent different clutches as follows: circles = clutch A, squares = clutch B, triangles = clutch C, diamonds = clutch D. Linear regressions for egg contents among clutches are as follows: solid content:  $y = 0.675 + 0.141x$ ,  $r^2 = 0.612$ ,  $p < 0.0001$ ; water content:  $y = -0.662 + 0.789x$ ,  $r^2 = 0.969$ ,  $p < 0.0001$ .

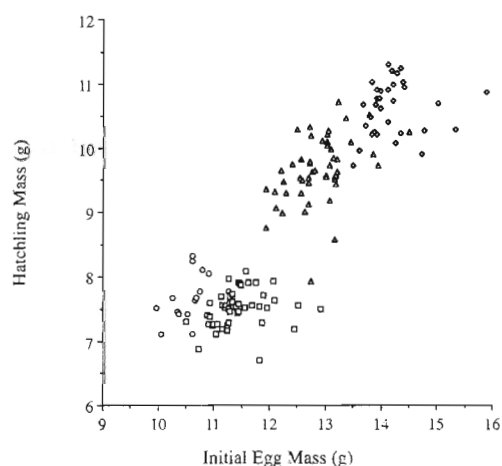
**Table 1.** Linear regressions of egg content mass (water and solids) and initial egg mass by clutch. \* =  $p < 0.05$ .

Clutch	Water			Solids		
	y-intercept	Slope	r <sup>2</sup>	y-intercept	Slope	r <sup>2</sup>
A	-1.917	0.902	0.994*	1.702	0.046	0.576*
B	-1.618	0.891	0.963*	1.350	0.068	0.203
C	-1.821	0.856	0.984*	2.088	0.049	0.230
D	-2.174	0.902	0.991*	2.239	0.026	0.097

sand and filled with 25 ± 1°C tap water to a depth of 6 cm. Aquatic velocity was assessed over the middle 1 m of the trough, which was partitioned into 25 cm intervals. The animal was coaxed to move as rapidly as possible down the length of the track with light taps to the tail. A second trial immediately followed the first. Time taken to cross each of the eight 25 cm intervals was determined, and the fastest crossing was used to calculate maximum absolute velocity (m/s). Animals failing to exhibit continuous locomotion were excluded.

Predatory efficiency was evaluated weekly beginning on day 17 post-hatching (week 2) until day 59 (week 8). Prey acquisition tests were conducted in the containers in which the turtles were housed. A live cricket 12.9 to 16.5 mm in length with its hind limbs broken was placed into the holding container of each animal. The predatory activity of the animals was recorded on video for 1 hr following the introduction of the cricket. The amount of time from when an animal first made a direct move toward capturing the cricket to when that turtle effectively subdued the cricket was recorded for each animal. Animals which did not attempt to capture their prey were excluded.

**Results.** — Within clutches, water content was significantly correlated with initial egg mass in all clutches (Fig. 1), whereas solid content was only significantly correlated with egg mass in clutch A (Table 1). Within clutches, differences in the amount of water present in the eggs accounted for approximately 88.8% of the difference in initial mass among eggs,



**Figure 2.** Variation in hatchling mass with initial egg mass. Different symbol shapes represent different clutches as follows: circles = clutch A, squares = clutch B, triangles = clutch C, diamonds = clutch D. The linear regression for hatchling mass as a function of egg mass among clutches is  $y = -2.50 + 0.920x$ ,  $r^2 = 0.789$ ,  $p < 0.0001$ .

whereas differences in the amount of solid contents accounted for only about 4.7% of the difference in initial mass. Among clutches, solid content made a more substantial contribution to differences in egg mass owing to a significant difference in the amount of solids present among the clutches (ANCOVA:  $F_{3,27} = 103.2$ ,  $p < 0.0001$ ).

The degree of correlation between hatchling mass and initial egg mass varied greatly within clutches (Fig. 2). Hatchling mass was significantly correlated with initial egg mass in all clutches except clutch D (Table 2). This clutch, however, demonstrated a significant correlation between hatchling mass and week 8 egg mass ( $n = 32$ ,  $r^2 = 0.256$ ,  $p = 0.0027$ ). Hatchling mass demonstrated a much more pronounced increase with increasing initial egg mass among clutches than within clutches, in part due to a significant clutch effect (ANCOVA  $F_{3,143} = 56.1$ ,  $p < 0.0001$ ).

Both wet yolk-free hatchling mass and dry yolk-free hatchling mass were significantly associated with initial egg mass among clutches (Table 3), whereas wet and dry yolk sac mass were not. Both measures of yolk-free hatchling mass varied significantly among clutches (wet yolk-free hatchling mass:  $F_{3,27} = 4.51$ ,  $p = 0.011$ ; dry yolk-free hatchling mass:  $F_{3,27} = 11.01$ ,  $p = 0.0001$ ). There was little evidence of a relationship between initial egg mass and yolk-free hatchling mass or yolk sac mass (wet or dry) within any of the individual clutches.

Total body composition of the hatchlings again provided mixed results for correlations with initial egg mass within individual clutches vs. those among clutches. Within clutches, only the solid content in clutch A was significantly correlated with initial egg mass ( $n = 8$ ,  $r^2 = 0.523$ ,  $p = 0.04$ ). Yet among clutches, there was a significant increase in both the amount of water present in the bodies of the hatchlings ( $y = -1.715 + 0.695x$ ,  $r^2 = 0.828$ ,  $p < 0.0001$ ) and the amount of solids ( $y = 0.617 + 0.109x$ ,  $r^2 = 0.568$ ,  $p < 0.0001$ ) with increasing egg mass, due to significant clutch effects in each (total water content:  $F_{3,27} = 6.65$ ,  $p = 0.002$ ; total solid content:  $F_{3,27} = 50.15$ ,  $p < 0.0001$ ).

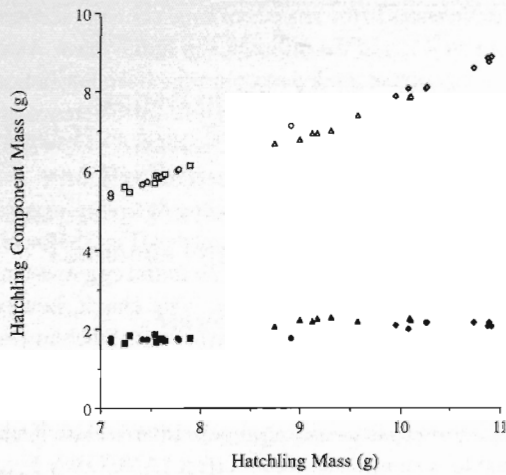
Analysis of the water/solid composition of the hatchlings as a function of hatchling mass (Fig. 3) provided results similar to those of the composition of the eggs with initial egg mass

**Table 2.** Linear regressions of hatchling mass (g) and initial egg mass (g) by clutch. \* =  $p < 0.05$ .

Clutch	n	y-intercept	Slope	r <sup>2</sup>
A	16	1.802	0.557	0.253*
B	48	5.492	0.178	0.101*
C	51	3.875	0.449	0.199*
D	32	8.999	0.114	0.019

**Table 3.** Linear regressions of hatchling wet and dry components (g) and initial egg mass among clutches. \* =  $p < 0.05$ .

Component	Wet Mass			Dry Mass		
	y-intercept	Slope	r <sup>2</sup>	y-intercept	Slope	r <sup>2</sup>
Yolk-free Hatchling	-2.156	0.783	0.804*	0.066	0.113	0.687*
Yolk Sac	-1.058	0.020	0.019	0.588	-0.008	0.010



**Figure 3.** Variation in yolk-intact hatchling content mass (solids and water) with hatchling mass. Filled symbols represent content solids; open symbols represent content water. Different symbol shapes represent different clutches as follows: circles = clutch A, squares = clutch B, triangles = clutch C, diamonds = clutch D. Linear regressions for egg contents among clutches are as follows: solid content:  $y = 0.768 + 0.135x$ ,  $r^2 = 0.675$ ,  $p < 0.0001$ ; water content:  $y = -0.768 + 0.865x$ ,  $r^2 = 0.988$ ,  $p < 0.0001$ .

(Fig. 1). Within clutches, almost all of the variation in hatchling mass (an average of 96.3%) was due to differences in the amount of water present in the clutchmates, with essentially no difference in dry mass (3.8%) among individuals within a clutch (Table 4). Among clutches, total dry mass increased significantly with increasing hatchling mass owing to significant differences in dry mass among clutches ( $F_{3,27} = 37.82$ ,  $p < 0.0001$ ), but its contribution to differences in total hatchling mass was still appreciably lower than that of water.

Growth rate (g/wk) was not significantly associated with initial egg mass, nor was there a significant clutch effect in association with initial egg mass. However, growth rate was significantly affected by both hatchling mass ( $F_{1,51} = 9.17$ ,  $p = 0.004$ ) and clutch with respect to hatchling mass ( $F_{3,51} = 3.33$ ,  $p = 0.03$ ). To ascertain the duration that each of these effects lasted, ANCOVAs were performed on the individual weekly mass measurements. While clutch continued to influence the mass of the neonates throughout the eight week testing period, hatchling mass was not a significant determinant of the mass of the juveniles after week 3 post-hatching (Table 5).

Absolute maximal velocity (m/s) was significantly influenced by mass measurements only during week 1 velocity measurements (egg mass:  $F_{1,38} = 4.97$ ,  $p = 0.03$ ; hatchling mass:  $F_{1,38} = 6.39$ ,  $p = 0.016$ ; week 1 mass:  $F_{1,38} = 4.96$ ,  $p =$

**Table 4.** Linear regressions of hatchling content mass (water and solids) and hatchling mass by clutch. \* =  $p < 0.05$ .

Clutch	Water			Solids		
	y-intercept	Slope	r	y-intercept	Slope	r <sup>2</sup>
A	-1.478	0.965	0.997*	1.478	0.035	0.308
B	-1.438	0.958	0.893*	1.438	0.042	0.015
C	-1.587	0.934	0.985*	1.587	0.066	0.247
D	-2.025	0.993	0.977*	2.025	0.007	0.002

0.03). In all cases, the significant mass influence was attributable to a significant correlation in a single clutch.

Tests of the influence of egg mass, mass at hatching, and weekly mass failed to detect any significant influence of these measures on how quickly an individual can capture a prey item ( $F \leq 3.05$  (variable d.f.),  $p > 0.05$ ). In part, this may be due to the severely reduced number of individuals who actually pursued and captured prey items during the weekly tests. Similarly, clutch had little influence on pursuit time.

**Discussion.** — Egg content composition at oviposition varied markedly with initial egg mass both within and among clutches, primarily due to increases in the amount of water present within the eggs. Within clutches, differences in the amount of solid materials present within the egg accounted for relatively little of the difference in mass among the eggs, suggesting little difference in nutrient investment among the eggs of a single female. Females which tend to lay larger eggs, however, also tend to include more nutrients in those eggs as well as more water.

The relationship between initial egg mass and hatchling mass within a clutch was verified, with significant correlations in three of the four clutches. Other studies have also noted significant correlations between initial egg mass and hatchling mass within clutches (Morris et al., 1983; Brooks et al., 1991), lending support to the notion that increased initial water content in the eggs leads to increases in hatchling mass. Moreover, the clutch which demonstrated no significant increase in hatchling mass with egg mass demonstrated significant correlations between hatchling mass and week 8 egg mass, suggesting that differences among eggs in the amount of water gained or lost over the incubation period may influence hatchling mass in a manner not related to initial egg mass.

Both wet and dry yolk-free hatchling mass increased with increasing egg mass among clutches, whereas wet and dry yolk sac mass did not. As there was little evidence that differences in initial water content within clutch affected any of these measures, differences are likely due to clutch-related differences in the ability of the embryos to utilize their egg contents during development. The influence of initial water content, however, cannot be ruled out, as there was relatively little overlap in the initial egg mass distributions of the samples taken from each clutch, increased water availability to the

**Table 5.** Summary statistical analyses for tests of differences in weekly mass (g) among clutches with hatchling mass (g) as the covariate.

Week	Hatchling Mass		Clutch Mass	
	F <sub>3,51</sub>	p	F <sub>3,51</sub>	p
1	28.61	0.0001	9.54	0.0001
2	10.07	0.0026	20.86	0.0001
3	5.90	0.0187	13.83	0.0001
4	1.00	0.3227	12.39	0.0001
5	1.30	0.2595	10.17	0.0001
6	0.25	0.6177	10.16	0.0001
7	0.73	0.3971	5.84	0.0016
8	0.18	0.6712	5.98	0.0014



embryos in larger eggs may have allowed for increased mobilization of yolk nutrients (Packard, 1991).

Differences in hatchling mass were accompanied by differences in the water/solid composition of the hatchlings. Within clutches, almost all of the variation in hatchling mass observed was due to differences in the amount of water present within the bodies and yolk sacs of the hatchlings, with little variation in dry mass. Similarly, the major factor accounting for differences in hatchling mass among clutches was variation in the amount of water present in the bodies of the animals, although dry mass increased in a clutch-based manner (see above).

While embryos appear to demonstrate differential growth as a result of differing amounts of water available during incubation, little evidence of differential growth post-hatching has been demonstrated (Bobyne and Brooks, 1994). Similarly, the present study provides little evidence of a relationship between the initial water content of the egg and post-hatching growth rate. Curiously, during the first three weeks post-hatching, growth rates were higher in smaller hatchlings. Miller (1993) noted that, after transfer to aquatic housing conditions and commencement of feeding, hatchlings from eggs incubated on dry substrates demonstrated a greater increase in mass than did hatchlings from eggs incubated on wet substrates, and suggested that this was due to the hydration of the hatchlings from the dry treatment upon introduction to water. The relatively lower growth rates of large hatchlings may also be due in part to increased metabolic needs, as large hatchlings may need to devote more of their total energetic uptake and reserves to maintenance of the body as opposed to growth.

In contrast to studies which have demonstrated that increased water availability in the nest environment may lead to differential performance in hatchling turtles (Miller et al., 1987; Miller, 1993), there was little evidence that variation in initial water content of the eggs influenced locomotor performance in the hatchlings, perhaps because differences in the amount of water present in the eggs at oviposition were not great enough to markedly impact locomotion. As clutch-related differences in performance did not appear to be related to differences in egg mass among different clutches, it appears that genetic differences may have a more pronounced influence on locomotor performance than does variation in egg mass or initial water content of the egg.

I detected no influence of egg or neonate size on the ability of the juveniles to quickly capture and subdue prey items. Nor was there much evidence suggesting that clutch-related factors influenced predatory efficiency. The sample sizes for this highly variable measure were likely too small to appreciably detect such differences, especially in light of the exclusion of animals that did not attempt to capture the prey presented to them.

In light of the present study, it appears that although hatchlings from larger eggs with higher water contents have larger masses at hatching, these differences have little demonstrable influence on post-hatching growth or per-

formance in aquatic conditions. My findings support other studies (e.g. Brooks et al., 1991; Bobyne and Brooks, 1994) that have indicated that egg size and hatchling size are relatively poor indicators of post-hatching quality and success.

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