

# Effect of shell type on the composition of chelonian eggs

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This study examined the hypothesis that composition of chelonian eggs, i.e. masses of shell, yolk and albumen, would be influenced by the structure of the eggshell. In particular, because albumen is a store of water in an egg, it was predicted that rigid-shelled eggs would have more albumen than pliable-shelled eggs because they have less scope for absorbing water from the incubation environment. Data were collected from the published literature for 23 chelonian species that exhibited either pliable or rigid-shelled eggs (11 and 12 species in each category, respectively). Linear regression analysis was used to describe relationships between mass of the egg and the three different components. For any given egg mass a rigid shell was heavier than a pliable shell and the exponent for rigid-shelled eggs was significantly higher than that for pliable-shelled eggs. By contrast, there were no significant effects of shell type on the masses of yolk or albumen. The ability of turtle eggs to absorb water during incubation was not, therefore, reflected in the mass of the albumen. Differences in shell structure in chelonians must have evolved for another reason that has yet to be investigated.

*Key words:* albumen, eggshell type, yolk

## INTRODUCTION

Bird eggs are characterized by a rigid eggshell surrounding yolk and albumen, the amounts of which vary between species on the basis of phylogeny and hatchling maturity (Sotherland & Rahn, 1987; Deeming, 2007). This means that no single allometric equation can be confidently applied to relationships between initial egg mass and any of the particular components. Bird eggs evolved from some kind of reptilian egg, although it is not possible to say for certain whether this was comparable to any egg type produced by an extant species (see Packard & Packard, 1980, and Deeming & Unwin, 2004, for discussions of the evolution of vertebrate eggs). Although egg composition has been described in reptilian eggs the emphasis has been more on chemical composition and its role in embryogenesis (see Thompson & Speake, 2004), or on energy and water contents (see Belinsky et al., 2004). There has not been any analysis of the relationships between egg mass and its constituent parts, which is important because it is how nutrients are provided by the female to the developing embryo.

A better understanding of gross composition would help develop our understanding of the formation, ecology and evolution of reptile eggs. This is important because although all reptiles produce hatchlings of comparable maturity (i.e. “super-precocial” – being able to survive independently of their parents), they emerge from eggs that have very different eggshell structures (Packard & DeMarco, 1991). Crocodilians all produce rigid-shelled eggs composed of calcite crystals. Chelonians calcify their eggs with aragonite but the structure ranges from rigid through to pliable shells. Almost all lizards and snakes, and tuatara, produce eggs with poorly calcified parchment-like shells. As an exception, some gekkonid

lizards produce substantial calcitic shells that are rigid (see review by Packard & DeMarco, 1991). These differences correlate with values for water vapour conductance (Deeming & Thompson, 1991) and affect the degree to which an egg can exchange water vapour with the incubation substrate (Ackerman et al., 1985b; Packard, 1991; Ackerman & Lott, 2004; Belinsky et al., 2004). However, the exact mechanism by which water is exchanged in reptile eggs is a matter of debate, with some evidence suggesting that liquid water can be absorbed across the eggshell (Thompson, 1987), whereas other data suggest that water is exchanged purely as vapour (Ackerman et al., 1985a). As this issue has not yet been resolved we hereafter simply refer to water exchange by eggs. Rigid-shelled eggs are physically constrained by the eggshell, have relatively low values for water vapour conductance and exhibit relatively little net gain of water from the incubation substrate (e.g. Booth, 2002; Booth & Yu, 2009). As the shell becomes less rigid conductance values progressively increase and the egg is able to absorb more water and so increase in size above that at oviposition (Deeming & Birchard, 2007).

The differing patterns of water exchange by these different shell types in reptiles should be reflected in their composition. Rigid-shelled bird eggs only lose water vapour during incubation and are incapable of absorbing water during incubation (Ar, 1991). Hence, all the water required for development is within the egg at oviposition and mainly within the albumen (Romanoff & Romanoff, 1949). It is predicted that as reptile eggshells become more rigid and less permeable to water the embryo should be more reliant on egg-based water stores for development. In rigid-shelled crocodilian and chelonian eggs albumen should be a significant part of the contents because the shell prevents absorption of large amounts



**Table 1.** Summary statistics (mean ± SD) for initial egg mass, shell mass, egg contents, yolk and albumen mass. Shell mass and egg contents mass are also described as a percentage of IEM. Yolk mass and albumen mass are expressed as a percentage of IEM and the egg contents (%CON). Results of comparisons of means for pliable- and rigid-shells eggs by independent t-tests are indicated at the end of each row. Significant differences are indicated by *P*-values in italics.

Shell type	All	Rigid	Pliable	Unequal variances	<i>t</i>	df	<i>P</i>
<i>n</i>	23	12	11				
Clutch size	33.3±41.3	11.6±9.4	57.0±47.7	Yes	−2.98	10.4	<i>0.013</i>
Initial egg mass (g)	22.7±19.9	15.0±10.4	31.1±24.6	Yes	−2.01	13.3	0.066
Shell mass (g)	2.1±1.6	2.5±1.9	1.7±1.1	No	1.18	21	0.250
Shell mass (%IEM)	11.6±6.1	16.3±4.3	6.4±2.0	No	6.98	21	<i>&lt;0.001</i>
Contents (g)	20.6±19.4	12.5±8.6	29.4±23.6	Yes	−2.23	12.4	<i>0.045</i>
Contents (%IEM)	88.4±6.1	83.7±4.3	93.6±2.0	No	−6.98	21	<i>&lt;0.001</i>
Yolk mass (g)	10.6±10.0	6.3±3.9	15.2±12.6	Yes	−2.23	11.8	<i>0.046</i>
Yolk mass (%IEM)	44.9±8.1	43.5±5.6	46.4±10.2	No	−0.87	21	0.392
Yolk mass (%CON)	50.8±8.2	51.9±5.9	49.5±10.3	No	0.71	21	0.489
Albumen mass (g)	10.1±9.7	6.3±4.8	14.2±12.0	No	−2.10	21	<i>0.048</i>
Albumen mass (%IEM)	43.8±8.1	40.8±5.4	47.2±9.4	No	−2.03	21	0.055
Albumen mass (%CON)	49.6±8.2	48.7±5.9	50.5±10.3	No	−0.51	21	0.622

of water from the environment. In pliable-shelled chelonian eggs albumen should be a smaller component of the contents because relatively more water can be absorbed from the nest environment. Evidence suggests that the absorbed water is not essential for development in chelonian and crocodilian eggs (Packard, 1991), but it is of interest to know whether eggs are provisioned with differing amounts of water at oviposition depending on the degree of calcification of the shell.

This report describes a study of the relationships between shell structure, mass and composition in chelonian eggs. Other reptile groups were considered for study but preliminary investigation showed that there were insufficient data for any meaningful analysis. Data are for gross egg composition, i.e. masses of shell, yolk and albumen, because it is in these chemical forms that water, nutrients and energy are incorporated into the egg. We hypothesized that eggshell type should be related to the proportions of yolk and albumen in the chelonian egg. Hatchling maturity is the same in all reptiles, which contrasts with analysis for bird eggs where all eggshells are rigid, but hatchling maturity is highly variable, ranging from super-precocial to altricial. The analysis allows a comparison to be made with the egg composition of bird eggs (Sotherland & Rahn, 1987; Deeming, 2007).

MATERIALS AND METHODS

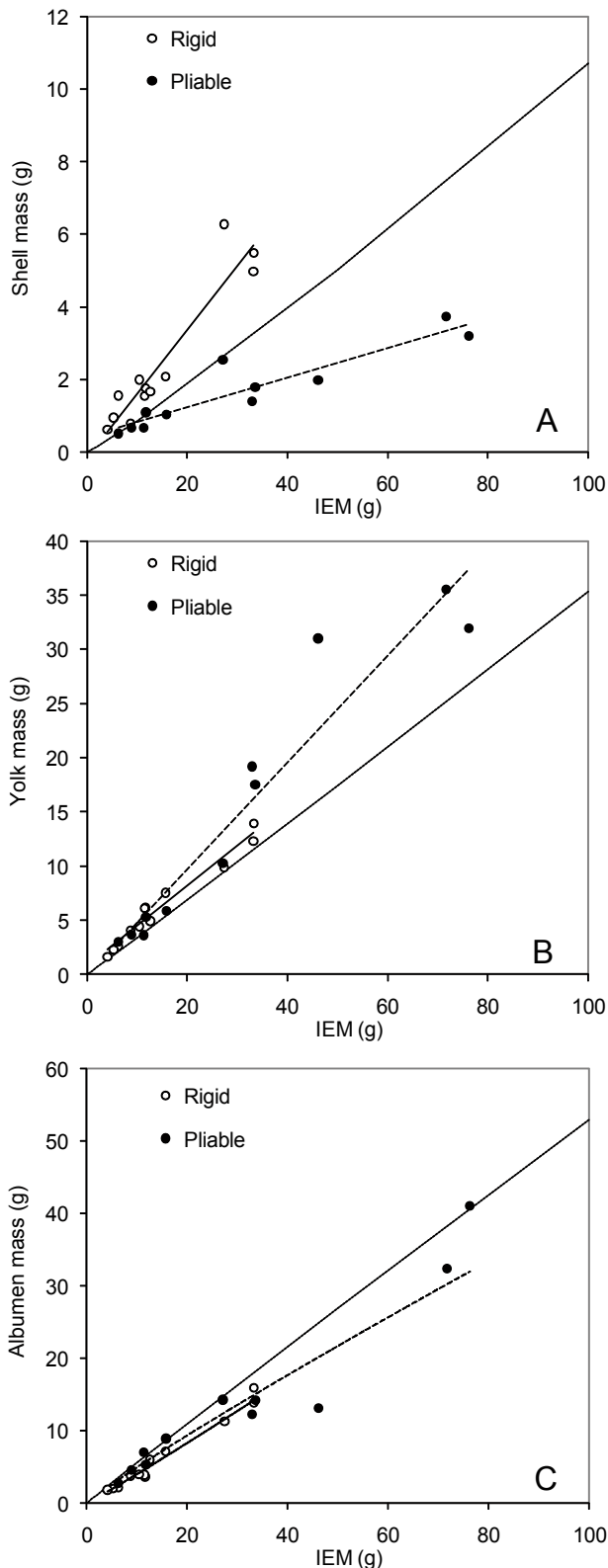
Records for egg composition were collected from previously published literature sources for as many species of chelonian as possible. In addition to initial egg mass (IEM, in g), the criterion for inclusion in the data set was at least values for the mass of yolk (YM, in g) and albumen (AM, in g). Shell mass (SM, in g) and mass of

the contents (EC, in g) were calculated if not provided in the report. Additional information included the family for each species, together with the average clutch size (Elgar & Heaphy, 1989) and the type of eggshell defined as pliable or rigid (see Deeming & Thompson, 1991). Many studies documented chemical composition, water or energy contents of chelonian eggs but, unless all of the component masses were described, these reports were ignored for this analysis.

Data for yolk and albumen masses were available for 27 reports from 23 species of chelonian in 11 different families (Ewert, 1979; Webb et al., 1986; Etchberger & Ehrhart, 1987; Lesham et al., 1991; Finkler & Claussen, 1997; Sahoo et al., 1998; Hewavisenthi & Parmenter, 2002; Booth, 2003; Wallace et al., 2006). Twelve chelonian species (from six families) had rigid-shelled eggs, and eleven species (from five families) laid pliable-shelled eggs (see Appendix 1).

Multiple records for three individual species (*Caretta caretta*, *Chelydra serpentina* and *Dermochelys coriacea*) were averaged before any analysis. Masses for the shell, contents, yolk and albumen were expressed as a percentage of the initial egg mass. Masses for the yolk and albumen were also expressed as a percentage of the contents. Independent *t*-tests were used to compare mean values of pliable- and rigid-shelled eggs for various parameters, and if Levene’s test for equality of variances was significant then *t*-values and degrees of freedom for unequal variances were used. Regression estimates for the relationships between IEM and egg components used the least squares method on untransformed data (Fowler et al., 1998). Analysis of covariance (ANCOVA) was used to determine the effects of shell type as a fixed factor, with IEM as a covariate. Direct phylogenetic relatedness





**Fig. 1.** Relationship between initial egg mass (IEM) and A) shell mass, B) yolk mass and C) albumen mass in rigid-shelled and pliable-shelled chelonian eggs (solid and dashed lines respectively). Details of regression estimates are shown in Table 2. A regression estimate for precocial bird eggs (Deeming, 2007) is included for each component for comparison.

between species represented was not of interest because shell type and family were confounded – for the species in the data set only one shell type was found in each family. Differences in the exponents of regression relationships were examined using the method of Bailey (1981). Analyses were performed in Minitab version 15.0 (Minitab Inc., PA, USA).

## RESULTS

Summary statistics for the chelonian eggs are shown in Table 1. On average, chelonian species laying pliable-shelled eggs had significantly larger clutches than species laying rigid-shelled eggs (Table 1). Rigid-shelled eggs were half the IEM of pliable-shelled eggs although this difference only approached significance (Table 1). When expressed in g, SM was not significantly affected by shell type, but when expressed as a percentage of IEM there was a highly significant effect of shell type ( $P < 0.001$ ), with rigid shells being a much higher percentage (Table 1). The relationships between IEM and SM were clearly divergent (Fig. 1A) and exponents for the regression estimates for SM versus IEM for the two shell types (Table 2) were significantly different ( $P < 0.01$ ). The exponent for the pliable-shelled eggs was smaller than that for the rigid-shelled eggs but still significantly larger than zero ( $t = 7.33$ ,  $df = 21$ ,  $P < 0.001$ ). ANCOVA showed that shell type did not significantly affect SM or EC, but for both variables, IEM and the interaction terms were both highly significant (Table 3).

Mean values in g for both YM and AM were significantly affected by shell type, although this was probably related to the smaller IEM values for the rigid-shelled eggs (Table 1). When expressed as a percentage of the IEM both YM and AM had higher values for pliable-shelled eggs than for rigid-shelled eggs, but these differences were not significant (Table 1). Scatter plots for YM and IEM (Fig. 1B) and AM versus IEM (Fig. 1C) show that the relationships for the eggs of the two shell types were similar. For YM, the exponent for the regression estimates for the relationship with IEM according to shell type (Fig. 1B; Table 2) was significantly lower ( $t = 2.25$ ,  $df = 21$ ,  $P < 0.05$ ). For AM the exponents for relationships with IEM showed no significant difference ( $t$ -test,  $P > 0.05$ ). ANCOVA showed that shell type as a fixed factor had no significant effect on YM or AM but IEM was a significant covariate in both instances (Table 3). Neither of the interaction terms in these analyses was significant (Table 3).

In comparison to the allometric relationship for precocial bird eggs of equivalent IEM (Deeming, 2007), SM for pliable-shelled turtle eggs was generally similar to or lighter than in bird eggs of equivalent mass, whereas rigid-shelled eggs tended to be heavier (Fig. 1A). Irrespective of shell type, YM was higher, and AM was lower, in chelonian eggs than in bird eggs of equivalent IEM (Fig. 1B,C).

In summary, for any given IEM, compared with pliable-shelled eggs, rigid-shelled turtle eggs had heavier shells and lighter yolk mass, but albumen mass was unaffected. Compared to precocial bird eggs rigid-shelled turtle eggs had heavier shells and yolks and less albumen mass.



**Table 2.** Least squares regression estimates on untransformed data for the relationship: component = a + b x IEM. Estimates are presented for turtle species split according to eggshell type. All regressions were significant ( $P\leq0.001$ ).

Component	Intercept (a) (SE)	Exponent (b) (SE)	R <sup>2</sup> (%)	n	Range for IEM	Range for component
<i>Pliable-shelled eggs</i>						
Shell	0.438 (0.215)	0.040 (0.006)	0.841	11	6.30–76.23	0.51–3.73
Yolk	–0.108 (1.888)	0.492 (0.049)	0.911	11	6.30–76.23	3.01–35.56
Albumen	–0.337 (1.834)	0.467 (0.047)	0.907	11	6.30–76.23	2.78–41.08
<i>Rigid-shelled eggs</i>						
Shell	–0.160 (0.349)	0.176 (0.019)	0.881	12	4.10–33.25	0.62–6.27
Yolk	0.773 (0.431)	0.369 (0.024)	0.956	12	4.10–33.25	1.64–13.93
Albumen	–0.534 (0.408)	0.455 (0.023)	0.973	12	4.10–33.25	1.83–15.94

DISCUSSION

The finding that shell type had no significant effect on the relative amounts of yolk or albumen in chelonian eggs was surprising because of the inability of rigid-shelled eggs to achieve a large net absorption of water vapour. This suggested that their albumen content should have been higher, but this was not the case and our working hypothesis was not supported.

The data set used in this analysis was very limited in its scope, which probably reflects the level of interest in basic egg composition in reptiles. This is in stark contrast to the situation in birds where detailed egg composition is known for 279 species from 21 orders (Deeming, 2007). Data for lizard and snake eggs were rare in the literature and those that were available (e.g. Mos et al., 2010) lacked any detail on the proportions of yolk and albumen in the egg. Although Tracy & Snell (1985) reported that the albumen content of eggs of various lizard species was very low (1–3%), they presented insufficient information on egg or shell mass to allow this information to be included in our analysis. It is interesting to see that Tracy & Snell (1985) reported that the two species of Galapagos iguanas had higher proportions of albumen (19 and 28%). It is unclear why this is so but it may reflect the relatively large egg size in these species. However, at oviposition lizard embryos are relatively well advanced compared to birds (Andrews, 2004) and so the “albumen” may be an extra-embryonic fluid. Indeed, Badham (1971) reported that the albumen content of *Amphibolorus* (*Pogona*) eggs

increased as incubation progressed. This was erroneous and the fluid was almost certainly within the allantois (Deeming, 1988). Lack of data is limiting our general understanding of development in these species and in particular why large lizard eggs do not absorb water during incubation while for small eggs (less than 1 g) it seems to be essential (see Deeming & Birchard, 2007). Careful analysis of egg composition at oviposition in squamates would help resolve these queries and provide invaluable data for comparative analysis.

For chelonians a relatively good set of data was available, although some studies (e.g. Nagle et al., 2003; Roosenburg & Dennis, 2005) failed to report values for gross composition even though they would have almost certainly been measured. Although the data were from a range of chelonian families, the effect of phylogeny on the masses of yolk and albumen could not be studied because of the confounding relationship between family and shell type. Comparable data for crocodilians were limited to the three most commonly studied species (*Alligator mississippiensis*, *Crocodylus porosus* and *Crocodylus johnstoni*; Manolis et al., 1987; Deeming & Ferguson, 1989). In all reptiles there is considerable scope to increase the data set for basic egg composition at oviposition and hopefully future research will aim to supplement the existing data set for this basic measure of reproductive biology.

Water vapour conductance values for different types of reptilian eggshell differ by orders of magnitude (Deeming & Thompson, 1991). Conductance values of pliable-shelled chelonian eggs are around ten times greater than

**Table 3.** Results of analysis of covariance of chelonian data with shell type as a fixed factor and initial egg mass (IEM) as a covariate for the three egg components.

	Shell type (df=1,19)		IEM (df=1,19)		Interaction (df=1,19)		R <sup>2</sup>
	F	P	F	P	F	P	
Shell	2.11	0.162	144.48	<0.001	56.58	<0.001	0.874
Contents	2.12	0.161	9811.78	<0.001	56.59	<0.001	0.999
Yolk	0.21	0.652	104.53	<0.001	2.13	0.161	0.929
Albumen	0.01	0.917	127.21	<0.001	0.02	0.884	0.928



for rigid-shelled eggs of chelonians and crocodilians. Such differences correlate with the degree to which reptile eggs can absorb a net gain of water during incubation. Parchment-shelled eggs can absorb considerable amounts of water, particularly when small (Deeming & Birchard, 2007). It is unknown whether this reflects the maternal provision of water, but in smaller species (IEM 0.1–36 g) there appears to be no significant relationship between IEM and proportion of water in the egg (data in Belinsky et al., 2004).

Pliable-shelled turtle eggs can absorb a lot of water without cracking (e.g. Packard et al., 1983), but the rigid shell of chelonian eggs limits net water uptake (e.g. Booth & Yu, 2009). It was anticipated that the constraint imposed by a rigid shell on water exchange would mean that the egg would need to be provisioned at oviposition with sufficient water for embryonic development. For pliable-shelled eggs the possibility of absorbing water vapour from the nest environment would allow the female to restrict the amount of liquid water required in the egg at oviposition. In birds, provision of water in the egg at oviposition correlates closely with the water content of the hatchling and is reflected in the proportion of albumen invested in the egg (Sotherland & Rahn, 1987). This does not appear to hold true for reptiles (Belinsky et al., 2004).

The results for chelonian eggs presented here indicate that, when controlled for IEM, there was no significant difference in the albumen and yolk contents of pliable- and rigid-shelled eggs. It is interesting that the albumen content of super-precocial chelonian eggs, 49–50% of IEM, is less than that for precocial birds but is comparable to the proportions of albumen in super-precocial megapode eggs (Sotherland & Rahn, 1987), which, like chelonian eggs, are buried (Booth & Jones, 2002). Water content of bird eggs correlates with hatchling maturity and the water content of hatchlings (Sotherland & Rahn, 1987); perhaps the relatively low albumen content of chelonian eggs also relates to their super-precocial level of hatchling maturity. The limited data for the water content of chelonian eggs also shows no clear effect of shell type, with pliable-shelled eggs ranging from 78 to 83% water, compared with 73–83% for rigid-shelled eggs (Belinsky et al., 2004). For pliable-shelled turtle eggs the degree of water uptake has effects on the lipid and nitrogen metabolism of embryos (Packard et al., 1984). For instance, urea concentrations in snapping turtle (*Chelydra serpentina*) eggs are inversely correlated with net change in egg mass, yet there is no direct effect of urea concentration on growth (Packard & Packard, 1989). Moreover, compared with rigid-shelled chelonian eggs, pliable-shelled eggs produce significantly heavier hatchlings relative to IEM (Deeming & Birchard, 2007), though this may simply reflect the difference in the proportion of IEM represented by SM. There is about a 5% difference in both SM (Table 1) and hatchling mass (Deeming & Birchard, 2007).

Therefore, the role of water uptake in chelonian eggs is still unclear, although Booth (2002) has suggested that absorbing water from the environment during the long incubation period may serve to insure against potential

periods when the nest dries out. If there is no difference in the albumen (and hence water) content at oviposition in chelonian eggs then why should there be such differing degrees of shell calcification in the Chelonia? This question has not been considered in depth, perhaps because of an assumption that egg composition reflected eggshell type, but if this is not important, what could be the reason for this difference?

Superimposing eggshell type on a phylogenetic tree for turtles (e.g. Shaffer et al., 1997) does not show any particular patterns of relatedness associated with the degree of eggshell calcification (Deeming, personal observation). This implies some other physiological or ecological factor is involved. Eggshell structure may reflect differences in the nesting environment and the potential for microbial spoilage of the egg contents during incubation, which has been suggested as a driving force for the evolution of a calcified egg (Packard & Packard, 1980). If this were true it would be expected that rigid-shelled eggs would be found in nest locations that pose a greater risk of microbial contamination.

Given that the structure of a pliable shell is comparable to that of the earliest stages of formation of a rigid shell, differences in eggshell structure in chelonians may reflect the duration of shell formation (Packard & DeMarco, 1991). Hence, species laying rigid-shelled eggs invest more in the mass of eggshell than species laying pliable-shelled eggs. The difference does not appear to reflect differences in deposition of calcium ions in the yolk (Packard, 1994), which suggests that rigid-shelled eggs are not providing a greater reserve of calcium ions (although the mass of the shell would be far in excess of the requirements of the embryo). It is interesting to note that clutch size in species laying pliable-shelled eggs is significantly larger than in those species laying rigid-shelled eggs (Table 1). This would impact upon the dietary calcium required to shell these eggs – pliable shells mean that individual eggs require less calcium than a rigid-shelled egg of the same IEM, but the demands for calcium ions for the whole clutch may be very much higher. For example, *Geochelone elegans* and *Macrolemmys temminckii* have eggs of comparable mass (27 g) but rigid and pliable shells with shell masses of 6.2 and 2.6 g respectively. In the herbivorous *G. elegans* clutch size is limited to three eggs. By contrast, the mostly carnivorous *M. temminckii* produces a clutch of 30 eggs, but to produce rigid-shelled eggs it would take 2.4 times more calcium than is used for the pliable-shelled eggs. Perhaps the diet in the latter species allows a larger clutch but provides insufficient calcium to produce rigid-shelled eggs. It is possible that these examples represent different reproductive strategies but it would be interesting to investigate whether differences in shell type and clutch size between these chelonians reflect differences in the availability of calcium ions in their diets.

To conclude, although shell mass differs between rigid- and pliable-shelled eggs, composition of the contents of chelonian eggs does not correlate with the degree of eggshell calcification and appears not to be directly related to water relations of the egg. The exact reason for



differences in shell type is not clear at this time but the relationship between nutrition and calcium availability would be worthy of further investigation.

## ACKNOWLEDGEMENTS

Many thanks go to Geoff Birchard, David Booth and four anonymous referees for their constructive comments on previous versions of this manuscript.

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Accepted: 14 April 2010

APPENDIX 1

Species included in the analysis

Taxon	Shell type
Carettochelyidae	
<i>Carettochelys insculpta</i>	Rigid
Chelidae	
<i>Chelodina expansa</i>	Rigid
<i>Emydura signata</i>	Rigid
Geoemydidae	
<i>Rhinoclemmys areolata</i>	Rigid
<i>Melanochelys trijuga</i>	Rigid
Kinosternidae	
<i>Kinosternon scorpioides</i>	Rigid
<i>Sternotherus minor</i>	Rigid
<i>Sternotherus odoratus</i>	Rigid
Testudinidae	
<i>Geochelone elegans</i>	Rigid
Trionychidae	
<i>Apalone ferox</i>	Rigid
<i>Apalone mutica</i>	Rigid
<i>Trionyx triunguis</i>	Rigid
Cheloniidae	
<i>Caretta caretta</i>	Pliable
<i>Chelonia mydas</i>	Pliable
<i>Lepidochelys olivacea</i>	Pliable
<i>Natator depressus</i>	Pliable
Chelydridae	
<i>Chelydra serpentina</i>	Pliable
<i>Macrolemys temmincki</i>	Pliable
Dermochelyidae	
<i>Dermochelys coriacea</i>	Pliable
Emydidae	
<i>Chrysemys picta</i>	Pliable
<i>Pseudemys concinna</i>	Pliable
<i>Terrapene carolina</i>	Pliable
Pelomedusidae	
<i>Pelusios sinuatus</i>	Pliable