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# YOLK UTILIZATION IN THE EGG OF THE OLIVE RIDLEY *LEPIDOCHELYS OLIVACEA*

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ABSTRACT

Yolk utilization has been studied in the eggs of olive ridley *Lepidochelys olivacea* from laying to pipping (hatching), pipping to emergence and in hatchlings. Chemical and calorimetric changes in the whole egg, in yolk, in the embryo and in the post emergence hatchling have been discussed. Cumulative conversion efficiencies of the whole egg and of yolk and net utilization effidiency of yolk have been determined using chemical and caloric values. Cumulative conversion efficiency of whole egg and of yolk at pipping in terms of energy are 71.97% and 30.42% respectively. At the time of emergence these values further reduced to 66.97% and 26.17% respectively. Based on the utilization of yolk energy, embryonic development in *L. olivacea* has been divided into three phases.

## INTRODUCTION

Yolk is the primary nutritional source of the developing embryos of oviparous and ovoviviparous animals. Much attention has been given recently to study the utilization of yolk by the embryo. Chemical and energy conversion of yolk to hatchling have been studied in terrestrial and aquatic eggs and based on this and the capacity of the egg to absorb water from the environment the eggs are classified as cleidoic and non-cleidoic (Needham, 1931).

There has been some vagueness about the categorisation of the eggs of turtles, laid in nests not far from the high water mark, either as cleidoic or non-cleidoic. Silas and Vijayakumaran (1984) have discussed this aspect based on earlier available literature on the subject as well as from observations on the development of the egg of olive ridley. They conclude that turtles lay non-cleidoic eggs since the egg is not a 'closed box' (as defined for cleidoic egg by Needham 1931) and based on the utilization of protein and lipid during development and the excretion of nitrogenous wastes.

There is no study on yolk utilization, chemical or calorimetric, in marine turtle eggs except on the metabolism of developing eggs of *Thalassochelys corticata* from Japan. The only report on yolk utilization in marine turtles by Kraemer and Bennet (1981) is on post-hatching yolk utilization in the loggerhead turtle *Caretta caretta*. The present study reports the chemical and calorimetric changes in the egg of olive ridley *Lepidochelys olivacea* from laying to pipping (hatching), pipping to emergence and from emergence to the 16th day of post-hatching yolk.

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## MATERIAL AND METHODS

The eggs of *L. olivacea* used in the present study were collected immediately after oviposition on 5-3-81 at 0200 hours from Kovalam beach, Madras, along the South East Coast of India, a natural nesting site. A total of 104 eggs were present in the clutch. The eggs were brought to the laboratory where they were numbered with marking pen from 1-102 (two eggs were damaged while removing), and weighed to 1 mg accuracy in an analytical balance. The eggs were then transferred to the hatchery in the same beach and burried in two pits. Eight eggs were removed for initial analysis ; of these 4 were dried intact while the rest were

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dissected and yolk, albumen (egg white) and shell were separated for analysis. Four eggs, two from each pit, were removed at 5 day intervals up to the 40th day and then four hatchlings on 42nd, 44th and 45th days and treated similarly. Pipping (breaking of the shell) is considered as hatching in marine turtles and the emergence is the time when the young turtle comes out of the nest (Ewert, 1979). The same definitions are used in the present study also. Pipping (hatching) in this study took place on the 42nd day. Of the 4 hatchlings removed from 42nd to 45th day, two were kept intact and the other two were dissected and the yolk and the hatchling minus yolk were taken separately for analysis.

On 45th day the rest of the hatchlings numbering 16, emerged. These were weighed and reared individually in 10 litre aquaria and were starved for post emergence studies. One or two hatchlings were sacrificed on 2, 3, 4, 6, 8, 10, 12, 14 and 16th day for analysis.

In addition to this, eggs and hatchlings from different clutches were collected at random at 5 day intervals upto 40th day and then on 42nd, 44th and 45th days, from the turtle hatchery of the Kovalam Field Laboratory of the Central Marine Fisheries Research Institute, where about 30,000 eggs were incubated. These samples were also treated similarly for analysis. Initial sample and initial weight of the eggs, however, could not be obtained from the general hatchery. Pipping took place on 42nd or 43rd day and emergence on 45th or 46th day in the hatchery.

Whole egg/hatchling and the tissues were weighed and dried to constant weight at 60°C in an electric oven to determine the water content. The dried tissues were then minced, homogenised, made into a fine powder and stored in airtight bottles in desiccators. Chemical analysis were performed on such homogenised samples. Protein was estimated by modified biuret method (Sumitra Vijayaraghavan and Vijayakumaran, 1976), lipid by choloroform, methanol (2 : 1) method (Raymont *et al.*, 1964) and caloric content by Gallencamp ballistic bomb calorimeter (Model No. CB 370). The temperature in the nest varied from 24.8 to 34.2°C during the whole period.

Since values for eggs from a single clutch and those obtained from the general hatchery were similar in yolk utilization, both the values were pooled for calculations. Values given are mean of 3 or more samples upto emergence and of two or single samples after emergence.

## Results

The proportionate weights of shell, albumen (egg white) and yolk in the freshly laid egg of olive ridley is given in Table 1. The clutch size varied from 79 to 160 (Av. 126) and the average weight of the egg in a clutch was 29.0 g (Range 22.9-36.5 g) (Silas and Ragagopalan, 1984).

Table 1. *Proportion of yolk, albumen (egg white) and shell in the freshly laid egg of the olive ridley* Lepidochelys olivacea

| Material | Wet weight (g) | % in wet weight | Dry weight (g) | % in dry weight |
|---|---|---|---|---|
| Sell .. | 1.50 | 3.24 | 0.73 | 10.38 |
| Albumen (egg white) | 12.18 | 39.42 | 0.20 | 2.84 |
| Yolk .. | 17.22 | 57.34 | 6.10 | 86.78 |
| | 30.90 | 100.00 | 7.03 | 100.00 |

Table 2. *Changes in the weight of a single egg of the olive ridley* L. olivacea *from the day of laying to emergence*

| Days after laying | Wet weight of the egg/ hatchling (g) | % increase/ decrease in wet weight (from initial) | Dry weight of the egg/ hatchling (g) | % dry matter in egg/ hatchling | % of water in egg/ hatchling |
|---|---|---|---|---|---|
| 0 | 30.90 | — | 7.03 | 22.74 | 71.26 |
| 6 | 30.22 | — 2.20 | 7.04 | 23.30 | 76.70 |
| 10 | 30.63 | — 0.87 | 6.95 | 22.69 | 77.31 |
| 15 | 31.21 | 1.00 | 7.01 | 22.47 | 77.53 |
| 20 | 31.07 | 0.55 | 6.91 | 22.24 | 77.76 |
| 25 | 30.97 | 0.21 | 7.04 | 22.75 | 77.25 |
| 30 | 31.33 | 1.36 | 7.04 | 22.48 | 77.52 |
| 35 | 31.32 | 1.35 | 6.54 | 20.88 | 79.12 |
| 40 | 29.27 | — 5.28 | 6.45 | 22.05 | 77.95 |
| 42 (Pipping embryo) | 16.74 | — 45.83 | 5.02* | 29.96 | 70.04 |
| 44 | 16.82 | — 45.56 | 4.99* | 29.66 | 70.34 |
| 45 (Newly emerged hatchling) | 15.96 | — 48.35 | 4.63 | 29.00 | 71.00 |

* Shell weight excluded

*Changes in the whole Egg*

The change in wet weight, dry weight and percentage dry matter and water in the egg over 45 days of development are presented in Table 2. The egg lost weight initially (upto a maximum of 2.2% of the initial) and from about the 10th day started gaining in weight upto 35th day (maximum gain 1.36%). From 35th day onwards the weight reduced rapidly. The percentage of water in the egg did not change considerably until the 35th day. On the 35th day the percentge of water increased from 77.52% recorded on the 30th day to 79.12% and thence onward decreased to 77.95% on the 40th day. On the 42nd day the egg hatched (pipping) and the water content dropped to 70.04%.

Chemical changes in the egg from laying to emergence of hatchlings are given in Table 3. The initial water content of the egg was 77.26%, lipid and protein contents were 30.0% and 52.94% respectively in dry weight.

TABLE 3. *Changes in chemical composition of the egg of olive ridley* L. olivacea *from laying to emergence*

| Days after laying | % water | % lipid in dry weight | % protein in dry weight | K. cal/g dry weight |
|---|---|---|---|---|
| 0 | 77.26 | 30.00 | 52.94 | 6.03 |
| 10 | 77.31 | 29.00 | 51.70 | 5.91 |
| 20 | 77.76 | 29.50 | 53.15 | 5.86 |
| 30 | 77.52 | 30.00 | 50.90 | 5.95 |
| 40 | 77.95 | 27.40 | 50.38 | 5.63 |
| 42* | 70.04 | 26.82 | 52.21 | 6.09 |
| 44 | 70.34 | 26.02 | 49.67 | 5.84 |
| 45@ | 71.00 | 25.80 | 49.38 | 5.93 |

* (Pipping embryo).

@ Newly emerged hatchling.

TABLE 4. *Changes in chemical composition of post emergence* L. olivacea *hatchling observed under starvation*

| Days after emergence | Live weight (initial) (g) | Live wt. on sampling day (g) | % water | % lipid (in dry weight) | % protein (in dryweight) | K.Cal/g dry weight |
|---|---|---|---|---|---|---|
| 1 | 15.96 | — | 71.00 (74.77) | 25.80 (21.64) | 49.38 (47.22) | 5.93 (5.69) |
| 2 | 16.98 | 16.16 | 67.85 (73.41) | 23.76 (21.16) | 65.08 (61.49) | 5.88 (5.69) |
| 3 | 17.64 | 16.42 | 68.74 | — | — | — |
| 4 | 16.69 | 16.13 | 71.95 (74.74) | 23.71 (20.31) | 58.90 (59.80) | 5.68 (5.61) |
| 6 | 16.61 | 16.62 | 71.15 (76.50) | 23.50 | 58.09 | 5.78 |
| 8 | 16.98 | 18.89 | 71.75 | — | — | — |
| 10 | 16.66 | 18.71 | 76.77 | — | — | 5.72 |
| 12 | 16.07 | 18.84 | 75.96 (77.20) | — (17.62) | — (64.27) | 5.27 (5.09) |
| 14 | 17.02 | 19.55 | 76.10 (77.41) | 20.27 (18.23) | 56.39 (57.48) | 5.79 (5.66) |
| 16 | 18.27 | 20.19 | — | — (16.28) | — (60.09) | — |

From 3th day onwards values of chemical composition given for whole hatchling are calculated from separate estimations of yolk and hatchling without yolk.

Values in parentheses are for hatchling minus yolk.

The caloric content was 6.03 K. cal/g dry weight. During development there was no appreciable change in the lipid content upto the 30th day, but by the 40th day the percentage of lipid dropped from 30.0 to 27.4 and further reduced to 26.82 in the pipping embryo on the 42nd day. By the 45th day when the hatchling emerged out of the nest, the lipid further reduced to 25.8. The protein content of the whole ‹ gg was seen to decrease gradually upto the 40th day from 52.94% to 50.38% (except on the 20th day with a value of 53.15%). The percent protein then increased to 52.21 in the pipping embryo but again reduced to 49.38% in the newly emerged hatchling. The caloric content of the whole egg reduced from 6.03 K. cal/g dry weight at the time of laying to 5.63 K.cal/g dry weight on the 40th day; the caloric value of the newly emerged hatchling was 5.93 K.cal/g dry weight.

*Post Emergence changes in the Hatchlings*

Post emergence changes in the hatchlings kept under starvation are presented in Table 4. The percentage of water reduced from 71.00 on the first day to 68.74 on the 3rd day and thence increased to 71.95 in the 4th day, which is also reflected in the live weight of the hatchlings, which decreased by 3.38% on the 2nd day and by 7.36% on the 3rd day. The reduction was only 3.18% on the 4th day and by the 5th day the live weight increased by 6.18% of the initial, the increase continued and reached 10.0% on the 14th day. The initial decrease was due to loss of water and the subsequent increase was due to absorption of water. Lipid in the dry matter decreased from 25.80% on the 1st day to 23.50% on the 6th day and further reduced to 20.27% on the 14th day. The percentage of protein increased from 49.38 to 65.08 on the 2nd day and thereafter declined to 56.59 on 14th day. Caloric value reduced from 5.93 K.cal/g dry weight on the 1st day to 5.79 K.cal/g dry weight on the 14th day.

*Changes in Yolk*

Yolk constitutes 55.73%, 86.77% and 96.70% of wet weight, dry weight and caloric content of the fresh egg of *L. olivacea* (Fig. 1). The combined values for yolk and albumen were 95.16%, 89.61% and 97.92% respectively for wet weight, dry weight and calories This indicates that even though albumen constitute about 40% of the wet weight of the egg, its contribution in dry weight and calories (2.84% and 1.22% respectively) are negligible. In other amniotes such as chick, however, albumen is reported to provide substantial amount of energy to the embryo (Needham, 1931; Romanoff, 1967). The dry weight of yolk to fresh weight of whole egg ratio for *L. olivacea* is 0.192+0.07.,

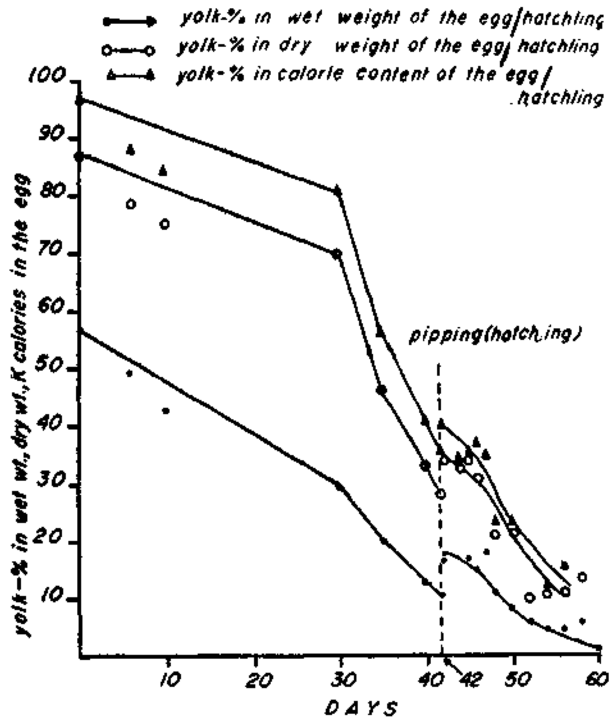


Fig. 1. Changes in the proportion of weight and caloric content of yolk in the whole egg upto pipping (hatching) and in the hatchlings upto the 16th day after emergence in the olive ridley *L. olivacea*.

This compares favourably with 12 species of turtles 0.139 to 0.230 (mean 0.180) and is close to the ratio of 0.165 for the chick (Ewert, 1979). Changes in per cent weight of yolk and yolk + albumen in the developing egg and subsequently upto 16th day after emergence (yolk alone) are represented in figures 1 and 2 respectively. The data on yolk + albumen is presented since yolk could not be separated from the 10th to tha 30th day in the egg.

The percentage of yolk in wet weight of egg reduced from 55.73% on the day of laying to 42.59% on the 10th day. Further data are available only on the 30th day, by which time the contribution of yolk in wet weight reduced to 29.50%. By 40th day the wet weight steeply reduced to 12.79% of the initial. The percentage of yolk in wet weight of pipping embryo (42nd day) and at the time of emergence (45th day) were 17.20 and 17.23% respectively.

The corresponding values for percentage dry weight of yolk in egg were 86.77 on the first day, 75.40 on the 10th day, 69.74 on the 30th day and 33.02 on the 40th day. Here also the sharp decline is noticed between the 30th and 40th days. On 42nd day, 34.26 of the dry weight of the pipping embryo was contributed by yolk. On emergence, this value was further reduced to 33.91%.

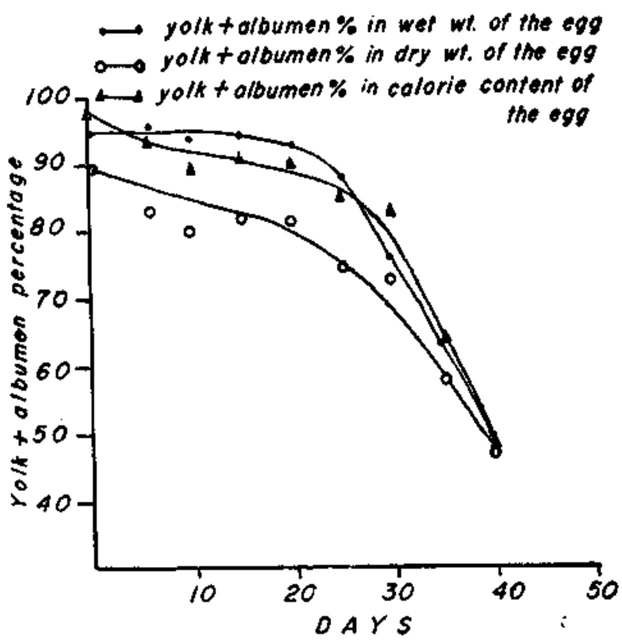


Fig. 2. Changes in the proportion of weight and caloric content of yolk + albumen in the whole egg upto pipping (hatching) in the olive ridley *L. olivacea*.

Total energy of the yolk (Percentage in total calories of the egg) declined from 96.70 on the first day to 84.33 on the 10th day and 80.98 on the 30th day. As in the case of wet weight and dry weight the reduction in energy content of yolk from 30th to 40th day was steep, from 80.98% to 56.48% of the total energy of the egg. It further declined to 35.75% on the 42nd day and in the pipping embryo on the 42nd day yolk provided 40.03% of the total energy of the animal. On emergence this percentage dropped to 34.82. The mean caloric value of the yolk was 6.74 K.cal/g dry weight in the freshly laid egg and a maximum value of 7.26 K.cal/g dry weight was recorded in the yolk of the pipping embryo.

Similar trend was recorded in the yolk + albumen expended (on dry weight and energy basis). Except for the period from 5th to 30th day, the values for yolk + albumen were calculated from separate values obtained for yolk and albumen. Albumen or egg white represented here includes amniotic and allantoic liquids also.

Table 5 gives the data of expenditure of yolk and yolk + albumen in a single egg. Yolk wet weight dropped from 17.22 to 13.04 g on the 10th day. It further declined to 9.24 g on the 30th day. The reduction after 30th day was faster and the value dropped down to 3.67 g by the 40th day. 2.88 g of yolk was retained by the embryo at the time of pipping and this amount further declined to 2.75 g at the time of emergence. Corresponding values for dry matter were 6.10 g to 5.24 g on the 10th day, 4.91 g on the 30th day and 2.13 g on the 40th day. At the time of pipping, the hatchlings retained 1.72 g dry matter of yolk which again declined to 1.57 g on emergence.

Same trend was recorded for yolk + albumen also. An interesting observation was the reduction in percentage of water in yolk as the development progressed. The per cent dry matter of yolk increased gradually from 35.42 in the fresh egg to 57.3 in the newly emerged hatchling.

TABLE 5. *Changes in wet and dry weights of yolk and yolk + albumen in a single egg from the day of laying to emergence in the olive ridley* L. olivacea

| Days after laying (g) | Egg weight (wet) (g) | Yolk weight (wet) (g) | Yolk weight (dry) (g) | Yolk + albumen weight (wet) (g) | Yolk + albumen weight (dry) (g) |
|---|---|---|---|---|---|
| 0 | 30.90 | 17.22 | 6.10 | 20.94 | 6.30 |
| 6 | 30.22 | 14.75 | 5.53 | 28.96 | 5.85 |
| 10 | 30.63 | 13.04 | 5.24 | 28.88 | 5.56 |
| 15 | 31.21 | — | — | 29.54 | 5.76 |
| 20 | 31.07 | — | — | 28.74 | 5.66 |
| 25 | 30.97 | — | — | 27.56 | 5.28 |
| 30 | 31.33 | 9.24 | 4.91 | 23.74 | 5.13 |
| 35 | 31.32 | 5.86 | 3.01 | 18.51 | 3.81 |
| 40 | 29.27 | 3.67 | 2.13 | 14.34 | 2.75 |
| 42 (pipping embryo) | 16.74 (Pipping embryo) | 2.88 | 1.72 | — | — |
| 44 | 16.82 | 2.82 | 1.63 | — | — |
| 45 (Newly emerged hatchling) | 15.96 (Newly emerged hatchling) | 2.75 | 1.57 | — | — |

Changes in chemical composition of the yolk during the developmental period are recorded in Table 6. Since the contribution of albumen in chemical composition or in caloric value are negligible, the values for yolk + albumen are considered as good as those of yolk. Lipid formed 35.94% of dry matter in the yolk of fresh egg, the same value for the hatchling was 34.50%. No significant change in lipid content was apparent during development. Percentage of protein in yolk dropped from 58.02 to 55.01% in the pipping embryo and declined further to 53.88% at the time of emergence. The caloric value of yolk recorded in this study (6.61 to 7.26 K.cal/g dry weight) is much lower than the average (7.88 K.cal/g dry weight) recorded for yolk of hatchlings of *Caretta caretta* by Kraemer and Bennet (1981), but is quite comparable to the values obtained for *Chelyotra serpentina* (6.6 K.cal) and *Pseudemys scripta* (6.7 K.cal) by Slobodkin (1962) and the calculated value of 6.8 K.cal/g dry weight for *C. caretta* (from the data of Tomita, 1929).

TABLE 6. *Changes in chemical composition of yolk during development in the egg of olive ridley* L. olivacea

| Days after laying | % water | % lipid in dry weight | % protein in dry weight | K. cal/g dry weight |
|---|---|---|---|---|
| 0 | 64.58 | 35.94 | 58.02 | 6.72 |
| 10 | 59.82 | 37.37 | 55.98 | 6.61 |
| 20 | — | 35.82* | 58.50* | 6.85* |
| 30 | 46.81 | 35.60 | 55.23 | 6.91 |
| 40 | 42.03 | 36.00 | 53.67 | 6.74 |
| 42 (Pipping embryo) | 40.18 | 34.71 | 55.01 | 7.26 |
| 44 | 42.23 | 35.75 | 52.17 | 6.79 |
| 45 (newly emerged hatchling) | 42.70 | 34.50 | 53.88 | 6.81 |

* Values are for yolk + albumen.

*Post Emergence changes in Yolk of Hatchlings*

Reduction in weight and changes in weight and chemical composition of post emergence yolk are recorded in Table 7. Weight of yolk dropped from 2.75 g (wet) and 1.57 g (dry) to 0.22 g (wet) and 0.12 g (dry) by the 16th day respectively. The reduction in weight of post emergence yolk was 92.2% and 92.5% (wet and dry) respectively by the 16th day. The reduction in quantity of yolk until 6th day was gradual but thenceon the decline was sharp. Percentage dry matter varied from 57.3-51.40. Percentage of lipid reduced from 34.50% in the first day to 32.81%, on the 16th day. Percentage of protein increased initially but declined to 51.49%, from the initial 53.88%, on the 13th day. Caloric value of the yolk varied from 6.18 to 6.89 K.cal/g dry weight. Post emergence study was possible only upto the 16th day on account of limitation of number of hatchlings for the experiment. By the 16th day 92.5% of the yolk had been utilized. Elsewhere, Vijayakumaran *et al.* (1984) have shown that on starvation *L. olivacea* hatchlings could survive upto 36 days. The utilization of the balance 7.5% of the yolk could thus take place during part of this period

TABLE 7. *Changes in weight and chemical composition of yolk in hatchlings (post emergence) of olive ridley* L. olivacea

| Days after emergence | Wet weight of animal (g) | Yolk wet weight (g) | Yolk dry weight (g) | % dry matter in yolk | % lipid in dry wt. (yolk) | % protein in dry wt. (yolk) | K. cal/g dry wt. (yolk) |
|---|---|---|---|---|---|---|---|
| 1 | 15.96 | 2.75 | 1.57 | 57.30 | 34.50 | 53.88 | 6.89 |
| 2 | 15.14 | 2.27 | — | — | 35.40 | 55.75 | 6.79 |
| 3 | 15.93 | 2.88 | 1.52 | 52.60 | 30.35 | 54.65 | 6.95 |
| 4 | 16.61 | 1.83 | 1.00 | 54.79 | 33.00 | 53.65 | 6.34 |
| 6 | 19.21 | 1.62 | — | — | — | — | 6.18 |
| 8 | 18.89 | 1.05 | 0.57 | 54.24 | — | — | — |
| 10 | 18.71 | 0.90 | 0.46 | 51.40 | — | — | 6.72 |
| 12 | 18.84 | 0.88 | 0.45 | 56.25 | — | — | — |
| 14 | 19.55 | 1.22* | 0.68 | 55.56 | 32.81 | 51.49 | 6.70 |
| 16 | 20.19 | 0.22 | 0.12 | 55.00 | — | — | — |

* The single hatchling analysed contained high yolk material

*Changes in the Embryo*

Davelopment of embryo is represented as proportion of embryo in the egg on wet weight, dry weight and calories in Fig. 3. The embryo can be separated on the 15th day onwards and on that day it constituted 0.96%

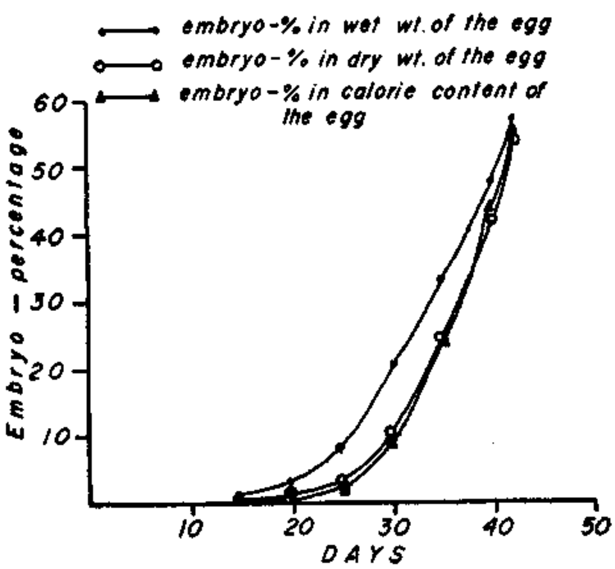


Fig. 3. Changes in the proportion of weight and caloric content of the embryo in the whole egg upto pipping (hatching) in the olive ridley *L. olivacea.*

and 0.44% of the wet and dry weights of the egg respectively. Increase in weight of the embryo was slow upto 30th day. Percentage of weight of embryo in egg weight increased sharply from 20.75% (wet) and 9.82% (dry) on the 30th day to 47.38% (wet) and 41.2% (dry) on the 40th day. At the time of pipping (42nd day) these values went upto 55.75% (wet) and 52.46% (dry). The sharp increase in the growth of embryo from the 30th day onwards was correlated with the sharp decline in weight and energy of the yolk in the egg.

Growth of embryo in a single egg is given in Table 8. From 0.31 g (wet) and 0.025 g (dry) weight on the 15th day, the embryo grew to 6.50 g (wet) and 0.68 g (dry) on the 30th day. From 30th to 40th day it increased its weight upto 13.86 g (wet) and 2.70 g (dry). The increase in growth rate was continued upto the 42nd day (pipping day) when the embryo reached 16.74 g (wet) and 5.02 g (dry). At the time of emergence, on the 45th day, weight of the hatchling declined to 15.96 g (wet) and 4.63 g (dry).

In the initial stages of development water content of the embryo was very high. It declined from 91.96% on the 15th day to 89.47% on the 30th day ; and then sharply to 80.55% on the 40th day. Water content of the pipping embryo (42nd day) was 70.04% and there was no appreciable change thereafter upto the emergence.

The percentage of lipid increased from 15.78% in the 20th day embryo to 25.80% at the time of emergence. Protein content of the embryo decreased from 64.66% on the 20th day to 51.52% on the 40th day and again increased to 52.21% at the time of pipping. From pipping to emergence, protein content further declined to 49.38%. Caloric value of the embryo increased from 4.55 K.cal/g dry weight on the 20th day to 6.09 K.cal/g dry weight on the 42nd day (pipping embryo). The value at the time of emergence was 5.93 K.cal/g dry weight.

*Cumulative Conversion Efficiency in the Egg*

Cumulative efficiency in a single egg in terms of wet weight, dry weight, protein, lipid and calories are presented in Table 9. Upto the time of pipping on the 42nd day, *L. olivacea* spent 11.88 K.cal. of energy which amounted to retention of 71.97% of energy in the egg. Cumulative efficiencies for lipid and protein at pipping were 63.51% and 70.43% respectively. It has been reported that turtle hatchlings spend considerable energy in their struggle for emergence from the nest (Evert, 1979 ; Kraemer and Bennet, 1981) which usually happens 3 to 4 days after pipping. In the present study, *L. olivacea* spent energy equivalent to 2.12 K. cal (0.16 g protein and 0.05 g lipid) for emergence from the nest. Cumulative efficiency for the whole egg at the time of emergence was 66.97%, 61.14% and 66.13% for calories, lipid and protein respectively.

*Cumulative Conversion Efficiency of Yolk*

Cumulative conversion efficiencies for yolk alone were 30.42%, 27.40% and 26.55% in terms of calories, lipid and protein respectively at the time of pipping (Table 9). At the time of emergence these values reduced to 26.17%, 24.66% and 24.01% for calories lipid and protein respectively.

Total combustion of lipid through ignition in a calorimeter provides 9400 calories/g dry weight where as protein gives 5600 cal/g dry weight (West *et al.*, 1966). Computing this value in the present study, out of the total 40.99 K.cal of yolk 20.61 K.cal was contributed by lipid and 19.82 K. cal by protein. Based on these values cumulative energy conversion of yolk lipid and yolk protein have been calculated (Table 9). Cumulative efficiencies at pipping are 32.02% for yolk lipid and 26.69% for yolk protein energy. At the time of emergence these values reduced to 24.79% and

TABLE 8. *Changes in weight and chemical composition of the developing embryo of the olive ridley* L. olivacea

| Days after laying | Wet weight of the egg (g) | Wet weight of the embryo (g) | Dry weight of the embryo (g) | % water in the embryo | % lipid in dry weight of the embryo | % protein in dry weight of the embryo | K. cal/g dry weight |
|---|---|---|---|---|---|---|---|
| 0 | 30.90 | — | — | — | — | — | — |
| 15 | 31.21 | 0.31 | 0.025 | 91.96 | — | — | — |
| 20 | 31.07 | 0.96 | 0.075 | 92.17 | 15.78 | 64.66 | 4.55 |
| 25 | 30.97 | 2.52 | 0.206 | 91.90 | — | — | 4.63 |
| 30 | 31.33 | 6.50 | 0.68 | 89.47 | 21.50 | 63.11 | 5.32 |
| 35 | 31.32 | 10.26 | 1.60 | 84.37 | — | — | 5.76 |
| 40 | 29.27 | 13.86 | 2.70 | 80.55 | 23.43 | 51.52 | 5.84 |
| 42 (Pipping embryo) | 16.74 (Pipping embryo) | 16.74 | 5.02 | 70.04 | 26.82 | 52.21 | 6.09 |
| 44 | 16.82 | 16.82 | 4.99 | 70.34 | 26.02 | 49.67 | 5.84 |
| 45 (Newly emerged hatchling) | 15.96 (Newly emerged hatchling) | 15.96 | 4.63 | 71.00 | 25.80 | 49.38 | 5.93 |

TABLE 9. *Cumulative conversion efficiency in a single egg of the olive ridlly* L. olivacea

| Parameter | | Initial | 42nd day (pipping) | Cumulative efficiency at pipping (%) | 45th day (emergence) | Cumulative efficiency at emergence (%) |
|---|---|---|---|---|---|---|
| *Whole Egg* | | | | | | |
| Wet weight of egg (g) | .. | 30.09 | 16.74 | 55.63 | 15.96 | 51.65 |
| Dry weight of egg (g) | .. | 7.03 | 5.02 | 71.40 | 4.63 | 65.86 |
| Energy (K. cal) | .. | 42.39 | 30.51 | 71.97 | 28.39 | 66,97 |
| Lipid (g) | .. | 2.11 | 1.34 | 63.51 | 1.29 | 61.14 |
| Protein (g) | .. | 3.72 | 2.62 | 70.43 | 2.46 | 66.13 |
| *Yolk* | | | | | | |
| Wet weight (g) | .. | 17.22 | 2.88 | 16.61 | 2.75 | 15.96 |
| Dry weight (g) | .. | 6.10 | 1.72 | 28.20 | 1.58 | 25.90 |
| Energy (K. cal) | .. | 40.99 | 12.47 | 30.42 | 10.73 | 26.17 |
| Lipid (g) | .. | 2.19 | 0.60 | 27.40 | 0.54 | 24.66 |
| Protein (g) | .. | 3.54 | 0.94 | 26.55 | 0.85 | 24.01 |
| Lipid energy (K. cal)* | .. | 20.61 | 6.60 | 32.02 | 5.11 | 24.79 |
| Protein energy (K. cal)* | .. | 19.82 | 5.29 | 26.69 | 4.75 | 23.97 |

* Calculated by using complete combustion values of 9400 cal/g for lipid and 5600 cal/g for protein (West *et al.*, 1966).

23.97% respectively for lipid and protein energy in the yolk.

*Net Utilization Efficiency of Yolk*

Yolk utilization efficiency at different stages of the embryo are given in Table 10. Upto 30th day 18.64% calories, 20.09% lipid and 23.45% protein have been utilized. The development was faster from the 30th day and the values went upwards to 69.58% for calories, 72.61% for lipid and 73.16% for protein at the time of pipping. At the time of emergence 73.82% calories, 75.34% lipid and 75.99% of protein were uitilzed by the embryo. Of the total energy expended until emergence 25.25% was utilized during the first 30 days, 69.03% between 30th day and pipping and 5.72% for emergence. The embryo conserved 26.18% of total yolk energy for post emergence requirements.

TABLE 10. *Net yolk utilization efficiency on different days of incubation in the egg of the olive ridley* Lepidochelys olivacea

(Values in parantheses are percentage reduction from initial value)

| Days after laying | Dry weight of yolk (g) | Total lipid in yolk (g) | Total protein in yolk (g) | Total energy in yolk (K. cal) |
|---|---|---|---|---|
| 0 | 6.10 | 2.19 | 3.54 | 40.99 |
| 10 | 5.24 (14.08) | 1.96 (10.50) | 2.93 (17.23) | 34.01 (17.03) |
| 30 | 4.91 (19.49) | 1.75 (20.09) | 2.71 (23.45) | 33.35 (18.64) |
| 40 | 2.13 (65.08) | 0.74 (66.21) | 1.19 (66.38) | 14.36 (64.97) |
| 42 (Pipping) | 1.72 (71.80) | 0.60 (72.61) | 0.95 (73.16) | 12.47 (69.58) |
| 45 (emergence) | 1.57 (74.26) | 0.54 (75.34) | 0.85 (75.99) | 10.73 (73.82) |

## DISCUSSION

While the terrestrial cleidoic eggs are closed to the envrionment, the non-cleidoic aquatic eggs depend mainly on the environment for water and minerals. Water uptake by perchment shelled or flexible shelled reptilian eggs (eggs that indent easily when laid and tend to expand through uptake of water during incubation) are well documented (Cunningham and Hurwitz 1936; Cunningham and Huene, 1938). The present study on *L. olivacea*, with perchment shelled egg, supports this observation as modest uptake of water is observed in this egg. Initially, upto 10 days, the egg shrinks losing water and dry matter, but from thence on it absorbs water, becomes turgid, chalkywhite and attains a perfect round shape.

There is no appreciable difference in water content of the egg until the 30th day, it increases from 77.52% on the 30th day to 79·12% on the 35th day and then reduces to 77.95% on the 40th day. This increase in water content after the 30th day corresponds to the intensive growth of the embryo. The uptake of water at this stage may facilitate the combustion of protein. The percentage of water reduces to 70.04% in the pipping embryo, the reason for the immediate drop in water content being the loss of albumen and other fluids which surround the embryo inside the egg and the inclusion of 'spare' yolk in the embryo.

Reviewing water content and growth rate in embryos in general, Needham (1931; p. 884) remarks that 'several authors have concluded that a decrease in water content, is a universal accompaniment of growth, and the more rapidly the latter takes place, the more rapidly does the drying up of the tissues go on'. This is true of the development of *L. olivacea* where we find maximum growth between the 30th day to pipping (hatching) on the 42nd day and the water content of the embryo shows a decreasing relationship.

The most important change in the chemical composition of the whole egg is reduction in percentage of lipid from 30.0% in freshly laid eggs to 26.82% at the time of pipping and to 25.8% at emergence. The reduction is mainly from the 30th day onwards, which is the period of maximum growth of the embryo. The percentage of protein reduces by 2% just before pipping, but the embryo retains the percentage of protein of the fresh egg. From pipping to emergence, however, the protein content reduces again by 3%. Reduction in lipid and increase in protein from eggs to newly hatched larvae have been reported in many marine and fresh water invertebrates (Pandian, 1970 b, 1972; Pandian and Schuman, 1967; Shakuntala, 1977). In *L. olivacea*, however, lipid reduces but protein content does not increase. No appreciable difference in the energy content (K. cal/g dry weight) has been noticed in the egg and the newly emerged hatchling, because of the yolk carried over to the hatchling which contributes about 33.91% in the dry weight at this stage.

Due to significant reduction in the percentage of water in the yolk, the latter gets concentrated as development progresses. Lipid content of the yolk does not reduce, but the protein of the yolk reduces to 55.01%

and 53.88% at the time of pipping and emergence respectively from the initial value of 58.02%. Since there was no appreciable change in chemical composition, the caloric value of the yolk also did not vary much during the course of development.

Romenoff (1967) referred to the early developmental period as that of differentiation and late development as that of growth. In *L. olivacea* only from the 15th day the embryo was observed to have the characteristic shape and profile and the development was slow until that 30th day.

On the basis of energy utilization it is possible to broadly define the embryonic development of olive ridley, in which the emergence was on the 45th day, into 3 phases as follows :

Phase—I : Period of slow growth

Duration : Upto 30th day of development.

Features : (*a*) Percentage of water in the embryo is very high (>89.5%) and

(*b*) Of the total energy utilized until emergence, 25.25% is used during this period.

Phase-II : Period of fast growth

Duration : From 30th day to pipping (42nd day)

Features : (*a*) Percentage of water reduces considerably (from 89.5% on the 30th day to 70.04% in the pipping embryo) and

(*b*) 69.03% of the total energy expended until emergence is utilized during this period.

Phase-III : Pipping to emergence—period of intense activity for emerging out of the nest.

Duration : 3 days, from 42nd to 45th.

Features : 5.72% of the total energy utilized is spent for the emergence.

It is interesting that a similar trend of growth in terms of utilization of organic materials (lipid and protein) and growth of embryo has been observed earlier by the Japanese workers (Tomita, Karashima and Nakamura, 1929) in the marine turtle *T. corticata*, although they had not tried to categorise growth phases during embryonic development (Tables 11 a, b and c).

TABLE 11a. *Movement of nitrogen within the egg during development in* Thalassochelys corticata (Nakamura 1929)

| Day | Milligram Nitrogen | | | | |
|---|---|---|---|---|---|
| | Whole egg | Egg white | Egg yolk | Amniotic & Allantoic liquids | Embryo |
| 0 | 592 | 41.2 | 548 | 3 | — |
| 16 | 586 | 17.8 | 544 | 24 | — |
| 30 | 549 | 12.2 | 501 | 25 | 10 |
| 45 | 506 | — | 119 | 64 | 323 |

Total $N_2$ lost by 45th day is 25.34% of the initial (Initial-592 mg; 45th day-119 (yolk) + 329 (embryo) = 442 mg).

TABLE 11b. *Weights of egg white, egg yolk, amniotic and allantoic liquids and embryo, at different stages of incubation in* T. corticata (Nakamura, 1929)

| Day | Grams weight of the fractions | | | |
|---|---|---|---|---|
| | Egg white | Egg yolk | Amniotic & Allantoic liquids | Embryo |
| 0 | 18.7 | 11.4 | 3.3 | — |
| 16 | 10.5 | 10.8 | 12.5 | — |
| 30 | 5.8 | 10.4 | 15.1 | 1.1 |
| 45 | — | 2.6 | 12.7 | 17.5 |

TABLE 11c. *Movement of fatty acids in the egg of* T. corticata *during incubation* (Karashima 1929b)

| Days of development | Grams fatty acid per egg |
|---|---|
| 0 | 1.64 |
| 15 | 1.68 |
| 30 | 1.63 |
| 45 | 1.36 |
| Hatched | 1.12 |

The amount of energy contributed by the lipid and protein in the fresh yolk of *L. olivacea* was almost equal (50.3% was contributed by lipid and 48.4% by protein). The cumulative efficiency of lipid energy at pipping and emergence are 32.02% and 24.79% respectively and that of protein energy are 26.69% and 23.97% rspectively. This indicates that both lipid and protein of the yolk are equally utilized for development. But the cumulative efficiency of the total lipids and proteins in the whole egg are 63.51% and 70.43% and 61.14% and 66.13% respectively for pipping and emergence. It is possible that part of the protein energy has been utilized for structural build up of the hatchling.

Only 77% of the potential energy contained in protein, as determined by combustion in a calorimater, is utilizable by the embryos whereas more than 95% of the potential energy of lipid is (Ewert, 1979). If this correction in energy calculation is applied to the present study it will become evident that the contribution of lipid to metabolizable energy is about 20% more than that of protein.

A comparison of the cumulative conversion efficiency of energy, lipid and protein of *L. olivacea* and *T. corticata* and other cleidoic, non-cledoic and non-cledoic eggs with certain cleidoic properties (Table 12) reveals that the eggs of *L. olivacea* and *T. corticata* belong to the non-cleidoic group. Protein metabolism is not 'suppressed' in *L. olivacea* egg and the lipid metablism is high, if not 'geared up'. But unlike the other non-cleidoic eggs uptake of water is very much restricted in *L. olivacea* egg (Silas and Vijayakumaran, 1984).

Irrespective of the property of the egg, whether protein metabolism is suppressed or not or lipid metabolism is 'geared up' it is imperative that 28 to 40% of the available energy has to be used for development (Table 12). The cumulative conversion efficiency of the egg does not vary much (between 60.6% and 72.5%).

Until emergence 73.82% of the total energy of yolk is utilized by *L. olivacea* embryo and the rest 26.18%, which corresponds to the cumulative conversion efficiency of yolk, is available to the hatchling for the entry into its new habitat, which is a strenuous process involving crawling to the sea, new experience of swimming, feeding and escapement from predators.

*L. olivacea* spends 19% of yolk energy available at the time of pipping for emergence from the nest. This value is considerably lower than the energy spent (50% of post hatching yolk) by the loggerhead turtle *C. caretta* (Kraemer and Bennet, 1981). This point needs further consideration since in our studies with remova of eggs for analysis there was a decrease in clutch size to a minimal number at the time of pipping and emergence. Hence the intense metabolic activity involved in the action of emergence, as experienced in a normal clutch may be reduced. Our observations also indicate that utilization of yolk in post hatching period may be extended over several days. The utilization of 'spare' yolk from the time of emergence to the 16th day shows that 92.5% is utilized upto that period.

In conclusion we would like to emphasize that there is a paucity of information on energy utilization during development in turtles. Priority attention is needed

TABLE 12. *Cumulative energy conversion efficiency in developing eggs*

| Habitat/species | Property of the egg | Cumulative conversion efficiency (%) | | |
|---|---|---|---|---|
| | | Protein | Lipid | Energy |
| Terrestial eggs (1) | Cleidoic | 97.00 | 45.30 | 64.40 (2) |
| Marine demersal eggs (1) | Non-cleidoic with certain cleidoic properties | 85.30 | 46.10 | 60.60 (2) |
| Fresh water eggs (1) | Non-cleidoic | 68.90 | 66.20 | 67.20 (2) |
| Marine turtle eggs : | | | | |
| *Thalassochelys corticata* | Non-cleidoic | 83.50 (3)<br>74.66 (5) | 66.00 (3)<br>66.00 | 72.50 (4)<br>69.23 (5) |
| *Lepidochelys olivacea* (6) | Non-cleidoic | 70.43<br>upto pipping (hatching) | 63.51 | 71.97 |
| | | 66.13<br>(upto emergence) | 61.14 | 66.97 |

(1) from Pandian (1970).

(2) Recalculated values from Pandian (1970).

(3) From Needham (1931).

(4) Calculated from the values given by Needham (1931).

(5) Calculated from the nitrogen values upto 45th day of incubation given by Nakamura (1929).

(6) Present study.

in the following areas of basic research for a better understanding of the problem.

1. An indepth study of the metabolism of protein, lipid and inorganic matter during development of sea turtle eggs.

2. Energy expended from pipping (hatching) to emergence in relation to clutch size, depth of the nest and soil conditions.

3. The incubation period could extend or decrease due to changes in temperature. The pattern of energy utilization under such conditions needs study.

4. The complete utilization of 'spare' yolk in the post emergence phase has not been investigated. In the olive ridley, it is seen that feeding is resorted to only from the 6th day onwards. The onset of feeding after hatching needs study in the different species under different conditions.

We hope that this paper would stimulate more work in this less trodden ground.

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