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Embryonic energetics in the egg of the green turtle *Chelonia mydas*

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Abstract

Water, nutrient and energy contents in the developing eggs and hatchlings of the green turtle *Chelonia mydas* were monitored during 66 day of incubation period in a beach hatchery at Saurashtra coast (northwest coast of India). The egg imported water from the surrounding medium in addition to utilization of 24.1 g water from the yolk and albumen. The developing embryo utilized 27 mg protein/day and 11.6 mg lipid/day. On an average, each developing egg spent 86.7 kJ for embryonic development. Protein, lipid and carbohydrate contributed 48.5%, 35.2% and 16%, respectively to the energy expenditure. The energy conversion efficiency of the egg was 75%. Nearly 68% of growth was in the last 21 days, when 58% of yolk energy was utilized. At emergence, the mean dry weight of hatchlings and residual yolk were 5.8 g and 0.9 g, respectively. The hatchling reserved 11.3% of the initial dry weight as well as energy content of the yolk as residual yolk.

Key words: Egg development strategies, protein, lipid and energy utilization, green turtle

Introduction

The allocation and pattern of utilization of nutrients and energy within the eggs of marine turtles play a critical role in determining hatching success and survival (Ewert, 1979).The lecithal eggs of these turtles contain all the nutrients required for the development of embryo (Thompson and Russell, 1999). Monitoring the physiological changes in the developing eggs provides information on the energetic strategies adopted by the sea turtles for embryonic development. Information is available on various aspects such as the water intake by the egg from the surrounding medium and its influence on the growth of embryo (Ackerman, 1997; Filoramo and Janzen, 1999); the utilization pattern of protein and lipid and its effects on the body size of hatchlings and the size of residual yolk (Thompson and Russell, 1999); and the parental investment in embryogenesis that determines the growth rate of embryo (Nagle *et al.*, 2003). The growth rate of embryos is not uniform through the incubation period, and is influenced by the nest environment, organogenesis and the consequent expenditure for development (Booth and Astill, 2001). The eggs of sea turtles contain a large quantity of yolk, which is more than the requirement of the developing embryo. The unutilized yolk is attached to the hatchling at the time of emergence. The residual yolk provides energy to the hatchlings for dispersal from the nests, subsequent swimming frenzies in aquatic habitats, and to tide over the initial non-feeding period (Kraemer and Bennett, 1981; Marlen and Fisher, 1999).

Information on the embryonic energetics in the eggs of the green turtle, *Chelonia mydas*, from India is meager. This is a species that nests sporadically during December – February along the Saurashtra coast (northwest coast) of India (Venkatesan, 2003). Hence a study on this aspect was undertaken by collecting developing eggs from a single clutch at periodic intervals from the above nesting area. The water, energy, lipid, protein and carbohydrate contents were estimated in the yolk, albumen and shell. The analysis was used to (i) quantify water intake by the non-cleidoic eggs; (ii) determine the proportion at which lipid and protein provide energy for embryonic development; (iii) estimate the amount of energy utilized for development., and (iv) monitor embryonic growth.

Materials and methods

Eggs (n=84) of *C. mydas* were collected from a clutch immediately after oviposition on 29.12.2001 at 23:30 hrs from Mangrol Beach (Lat. 21° 6' N; Long. 70° 6' E) in Saurashtra coast, a natural nesting site in the northwest coast of India. The eggs were transferred and buried in pits for incubation in a beach hatchery, 18 km away from the nesting site. The nest temperature was measured by using a temperature data logger. The temperature ranged from 24°C to 30°C during incubation. Four eggs were sampled from Day 0 (D 0 = freshly laid) to D 25 of incubation at 5 days interval, and thereafter every 10th day up to D 55. The final collection was four

hatchlings at the time of emergence on D 66. On each day of sampling, the yolk, albumen and shell were separated, dried in a hot air oven (at 70°C), powdered by using mortar and pestle, and stored in desiccators. The newly emerged fresh hatchlings were cut open on the ventral side; the yolk was separated, dried and stored. Aliquots from the dried samples were taken for analysis of biochemical components and energy content.

Total protein was estimated by modified biuret method (Gornall *et al.*, 1949), total carbohydrate by phenol-sulphuric acid method (Linford, 1965), and the total lipid content by the method suggested by Dubois *et al.* (1956). Caloric values of dry samples were estimated by using an Advance isothermal bomb calorimeter using standard specifications as given in the instruction manual. Cumulative efficiency of an egg was calculated by considering the wet and dry weights, carbohydrate, protein, lipid and energy contents of the freshly laid egg and the contents in the hatchlings at the time of emergence.

Results

The mean wet and dry weights and energy content of freshly laid eggs of the green turtle were 45 g and 10.4 g, and 244.7 kJ, respectively (Table 1). Lipid and protein contributed 28% and 51%, respectively, to the dry weight of egg. The energy content was 23.5 kJ/g dry weight. Yolk contributed 59% to the wet weight, 77% to the dry weight and 91% to the energy content of the egg. The dry weight of yolk to the fresh weight of whole egg ratio was 0.178. This compares favourably with the observations on 13 species of turtles (0.139–0.230., mean: 0.180) by Ewert (1979).

Albumen is the storehouse of water (95.5%) for the developing embryo. The shell contributed 16.4% to the dry weight, but only 6.2% and 2.6% to the wet weight and energy content of the egg, respectively.

Changes in the egg during development

The wet weight of the egg, which was almost constant up to D 45, decreased from 44.7 g on D 45 to 28.6 g on D 66. The quantity of water in the egg increased from 34.5 g on D 0 to 35.6 g on D 20, but gradually decreased to 33.7 g on D 55. In the last 12 days of incubation, water utilization was very high and only 21.8 g was present on D 66. However, the water content remained almost constant (76.3–80.5%) throughout the incubation period. It appeared that the egg actively imports water from the surrounding medium in the first 20 days and builds-up reserve in the yolk. During D 46–D 66, a total of 24g water was utilized from the yolk (7.5 g) and albumen (16.6 g). In the absence of data on the quantity of water import from the surrounding medium, it could be concluded that the egg utilizes 36.8% of the initial store of water for development.

The protein and lipid contents of the egg gradually decreased in the first 45 days, and the rates of utilization were 7.2 mg/day and 6.2 mg/ day, respectively. The rate of utilization accelerated to 69.4 mg protein/day and 23.3 mg lipid/day during D 46–D 66. Thus the rate of protein utilization accelerated by nearly ten times, but the lipid by only four times. In the 66 days incubation, the developing embryo utilized 1.4 mg carbohydrate/day (or 47% of initial store), 27mg protein/day (or 33.5% of initial store) and 11.6 mg lipid/day (or 26% of initial store).

The lipid content (28.3%) was considerably low in comparison to the protein content (51.1%) in the fresh egg. The lipid: protein ratio was 1.81:1. Since protein utilization was more than that of lipid, the protein/lipid ratio reduced to 1.62 at emergence. In the yolk, however, the protein/lipid ratio, which was 1.64 in the fresh egg, increased to 1.80 at emergence. Being the storehouse of energy, the yolk provided more lipid than protein for the developing embryo.

The energy content of the egg gradually got reduced at the rate of 0.47 kJ/day during D 0 to D 45, but the rate accelerated by about four times (1.9 kJ/day) during D 46 to D 66. The energy contributed by protein to egg development was marginally higher than that of lipid. This is evident from the reduction in the protein energy content at emergence. Protein (124.7 kJ) and lipid energy (116 kJ) contributed 51.1% and 47.5% to the total energy of

Table 1. *Characteristics of fresh egg of* C. mydas *(n=3); ± indicates standard deviation*

Tissue	Wet weight (g)	Dry weight (g)	Water content (%)	Energy (kJ)	Lipid	Protein	Carbohydrate
					(mg dry weight)		
Yolk	26.5 ± 1.04	8.0 ± 0.58	69.8 ± 2.3	223.1 ± 7.6	2810	4620	160
Albumen	15.7 ± 1.40	0.7 ± 0.14	95.5 ± 3.1	15.3 ± 1.05	45	576	45
Shell	2.8 ± 0.40	1.7 ± 0.06	39.3 ± 0.9	6.3 ± 0.21	82	111	82
Total	45.0 ± 0.18	10.4 ± 0.41	76.9 ± 0.9	244.7 ± 2.21	2940	5310	194

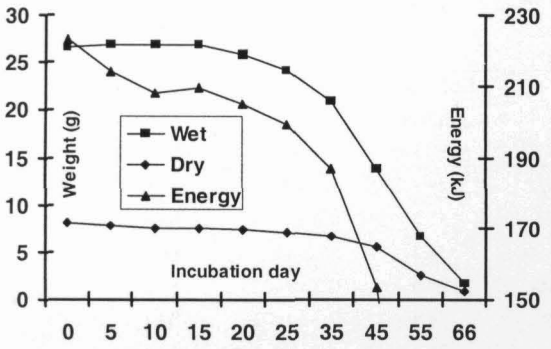

Fig. 1. Changes in mean wet and dry weights and energy content in the yolk of *C. mydas* egg during incubation

the fresh egg, respectively; at emergence, the corresponding contributions were 45.3% and 46.8%.

The egg converted 64.8% of its dry mass and 74.9% of its energy into hatchling (Table 2). Compared to lipid (73.8%) the conversion efficiency of protein (66.5%) was low indicating enhanced oxidation of protein.

Subtraction of energy content of the hatchling (158 kJ) from that of fresh egg (244.7 kJ) provided an estimate of the energy expended (86.7 kJ) during development. By considering combustion values of 23.5 kJ/g dry weight for protein, 39.5 kJ/g for lipid and 17.3 kJ/g for carbohydrate (Pandian and Vivekanandan, 1990), it was estimated that protein contributed 48.5%, lipid 35.2% and carbohydrate 16.0% to the energy expenditure during development. The total energy expenditure for the experimental clutch (84 eggs) was 7,282 kJ or 110.3 kJ/day.

Changes in yolk and albumen

Based on changes in the wet and dry weights of the yolk and albumen, the incubation duration of 66 days could be categorized into three phases. Phase 1 is the first ten days when the wet weight of yolk increased from 26.5 g to 26.8 g, but the dry weight decreased from 8g to 7.5 g (Fig. 1), i.e., by 53 mg/day. During this phase, the wet weight of yolk gained 0.3 g, but that of albumen lost 0.7 g, indicating utilization of water from the albumen by the embryo. Phase 2 extended from D 11 to D 45, when the wet and dry weights of yolk decreased at the rate of 371 mg/day and 57 mg/day, respectively. On D 45, the yolk was only about 50% and 70% of what it was in the fresh egg in terms of wet and dry weights, respectively. Phase 3 is from D 46 to D 66, when the wet and dry weights of yolk decreased rapidly at the rate of 577.6 mg/day and 219 mg/day, respectively. The wet weight of albumen increased from 15.0 g to 17.2 g in Phase 2, but it was fully utilized at the rate of 819 mg/day in Phase 3.

The yolk (dry weight: 8g) in the freshly laid egg consisted of 2.8 g lipid and 4.6 g protein, contributing 35% and 58% respectively to the dry weight of the yolk The utilization of lipid and protein was almost at the respective same rate during the developmental period. The yolk energy decreased from 223 kJ on D 0 to 152 kJ on D 45 (Fig. 1) and further to 25 kJ on D 66; i.e., 198 kJ yolk energy was utilized by the developing embryo, in addition to, perhaps converting a portion of it into fat bodies. In Phases 1 and 2, only 31.2% of yolk energy was utilized (Table 3) compared to 83.6% in Phase 3. At emergence, the yolk was attached to the ventral side of the hatchlings, and it is used as reserve energy during the initial free-swimming, non-feeding stage by the hatchling. The dry weight of this residual yolk was 0.9 g consisting of 0.3 g lipid and 0.54 g protein on D 66 (Fig. 2); the lipid (33%) and protein (59%) contents in the yolk were almost in the same proportion as they were in the fresh egg. For development, the embryo utilized 2.5 g lipid (equivalent to 98.8 kJ) and 4g protein (equivalent to 96

Table 2. *Conversion efficiency (%) of the egg of* C. mydas; *D = refers to Day of Incubation; ± indicates standard deviation*

Parameter	D 0	D 66	Conversion efficiency(%) D 0–D 66
Wet weight (g)	45.0 ± 0.18	28.6 ± 1.37	63.6
Dry weight (g)	10.4 ± 0.41	6.8 ± 0.16	64.8
Energy (kJ)	244.7 ± 2.21	183.2 ± 1.34	74.9
Lipid (g dry weight)	2.9 ± 0.13	2.17 ± 0.023	73.8
Protein (g dry weight)	5.3 ± 0.07	3.53 ± 0.037	66.5
Lipid energy (kJ)	116.1	85.7	73.8
Protein energy (kJ)	124.8	83.0	66.5

Table 3. *Yolk utilisation efficiency (%) in the egg of* C. mydas; *D refers to Day of Incubation; ± indicates standard deviation*

Parameter	D 0	D 45	Utilization efficiency D 0–D 45	D 66	Utilization efficiency D 0–D 66
Wet weight (g)	26.6 ± 1.04	13.8 ± 1.22	48.1	1.6 ± 0.23	94.0
Dry weight (g)	8.0 ± 0.29	5.5 ± 0.24	31.2	0.9 ± 0.05	88.8
Energy (kJ)	223.1 ± 7.60	153.5 ± 5.90	31.2	25.2 ± 1.1	88.7
Lipid (g dry weight)	2.8 ± 0.12	2.0 ± 0.09	28.6	0.3 ± 0.02	89.3
Protein (g dry weight)	4.6 ± 0.19	3.3 ± 0.14	28.3	0.5 ± 0.03	89.1
Lipid energy (kJ)	111.0	79.4	28.6	11.85	89.3
Protein energy (kJ)	108.6	76.4	28.3	12.69	89.1

kJ) from the yolk. The hatchling reserves 11.3% of the initial dry weight equivalent to the same amount of the initial energy content of the yolk for the non-feeding stage.

Growth of embryo

The embryo could be dissected and separated from the yolk on D 25 only. On D 25, the weight of embryo (0.3 g) was 3% of the initial dry weight of the egg. The growth was not uniform. It accelerated after D 35, and attained 2.2 g on D 45, and 4.4 g on D 55. The growth rate was fastest (219 mg/g) between D 45 and D 55 and decreased (138 mg/g) in the last 11 days (D 56–D 66). Nevertheless, in terms of energy, 68% of growth was during Phase 3, i.e., in the last 21 days, when 58% of initial yolk energy was utilized.

On D 25, the protein (0.19 g) and lipid (0.06 g) contributed 61.3% and 19.4% to the dry weight of the embryo. During development, it accumulated more lipid than protein, and at emergence the respective quantity was 1.9 g and 3.0 g. During D 25–D 66, protein was added by 16 times and lipid by 34 times. Consequently the energy content of the embryo increased substantially from 14.5 kJ/g to 26.9 kJ/g dry weight.

Discussion

Several studies on turtle indicate that their eggs are non-cleidoic (Silas *et al.,* 1984). In this type, the most fundamental need is water and these eggs absorb water from the surrounding medium. The quantity of water in the egg of *C. mydas* increased from 34.5 g on D 0 to 35.6 g on D 20. In spite of utilization of water by the developing embryo for its metabolic processes, the water content in the egg remained almost constant (76.3–80.5%)

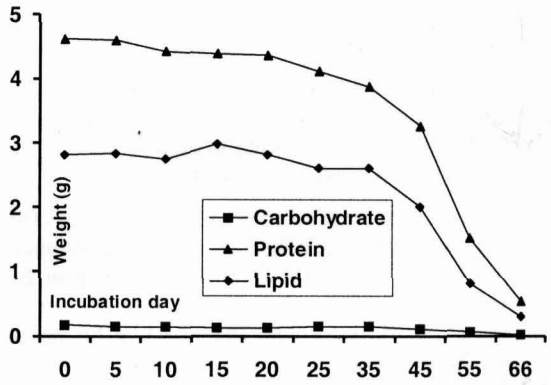

Fig. 2. Changes in mean carbohydrate, protein and lipid contents in the yolk of *C. mydas* egg during incubation

through the incubation, indicating continuous import of water from the surrounding medium. The nesting intensity of this species along the Saurashtra coast was maximum between 40 and 60 m from the high tide line (HTL) where the average moisture content of the soil was 2.4% (Venkatesan, 2003). Clearly, the eggs absorb water through the flexible, thin shell from the surrounding medium for development.

The yolk is comprised predominantly of protein (57.5% of dry weight) and lipid (35%). During development, the embryo utilized 64% more protein (4.1 mg dry weight) than lipid (2.5 mg) from the yolk. However, both contributed approximately equal quantity of energy for development. The embryo of the olive ridley *Lepidochelys olivacea* also utilized 64% more dry weight of protein than lipid, but they contributed approximately equal quantity of energy to the developing embryo (Silas *et al.,* 1984). Nevertheless, the developing embryos of turtles oxidize more protein than lipid for their energy source.

The temperature at which the eggs are incubated determines the sex of the developing embryos. For the green turtle the all-male-producing temperature is 26° C and for all females it is 30° C (Miller and Limpus, 1981). Along the Saurashtra coast of India, the green turtle nests during December – March when the beach soil temperature increases from 24° C to 30° C. All hatchlings from eggs deposited in December and January (temperature: 24–27° C) were found be males and those during February and March (temperature: 28–30° C), females.

After breaking (the stage is known as pipping) the eggshell, the hatchlings spend 3 or 4 days inside the nest before emerging out. Silas *et al.* (1984) found that at this stage, the hatchlings of *L. olivacea* utilized the residual yolk, but lost weight. For *C. mydas*, the changes in the hatchling between pipping and emergence were not assessed. After pipping the eggshell, the hatchlings climb the nest surface involving collective thrashing, and by negative geotaxis and allelomimetic behaviour exhibiting periodic bursts of activity (Carr and Ogren, 1960). These activities are likely to enhance the metabolic rate and decrease/arrest the growth rate in the last few days before emergence.

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