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ARE TURTLE EGGS CLEIDOIC OR NON-CLEIDOIC?

E. G. SILAS AND M. VIJAYAKUMARAN*

ABSTRACT

Experimental data to determine the true status of the turtle eggs as to whether they are cleidoic or non-cleidoic has been very meagre. Needham (1931) deduced that turtle eggs are non-cleidoic or 'imperfectly cleidoic'. Further reviewing the problem, Needham (1942) termed turtle egg as 'intermediate'. Recently Pritchard (1979) has indicated that turtles lay cleidoic eggs. In the case of the spiny softshell turtle *Trionix spiniferus* which lays rigid shelled eggs, Packard and Packard (1983) opined that the egg is cleidoic. Our observation on the flexible shelled egg of the olive ridley *Lepidochelys olivacea* has shown that the egg of this sea turtle is non-cleidoic. Based on the available data on capacity for water absorption, protein and lipid utilization and nitrogen excretion during incubation in turtle eggs we feel that the sea and freshwater turtles lay non-cleidoic eggs.

INTRODUCTION

In a recent review on the taxonomy, evolution and zoo-geography of turtles published in '*Turtles, Perspectives and Research*' Pritchard (1979) mentions that 'Turtles, being poikilothermous, laying cleidoic eggs, and having a typically scaled integument, are unquestionably reptiles.' During 1981-82 and 1982-83 nesting seasons of the olive ridley *Lepidochelys olivacea*, along the Madras coast, we have made a study of the yolk utilization during development and in the post hatching stages (Silas *et al.* 1984). Our findings indicate that the olive ridley egg evinces considerable differences from the typical cleidoic egg. A perusal of the literature indicates that a major effort to study chelonian egg was made in the late twenties (1929) by a team of Japanese workers (Tomita; Karashima; Nakamura and Sendju) who had studied the egg of the Japanese sea turtle, *Thalassochelys corticata*. Apparently, their findings led Needham (1931) to indicate in his review at two different places that the turtle eggs are 'non-cleidoic' (p. 899) and are 'imperfectly cleidoic' (p. 1142). Reviewing the evolution of cleidoic egg again, Needham (1942) opined that the eggs of modern chelonians may be 'intermediate'. It is obvious that with available data Needham could not clearly define the status of turtle egg, which is evident from the following statement '.... but for proper interpretation of the situation in the chelonia, we need to know a good deal more about the nitrogen metabolism of their embryos. As we have seen (p. 35), their eggs are non-cleidoic as regards water but they may be and, probably are, cledoic as regards the exit of nitrogenous waste poducts'. Recently Packard and Packard (1983) reported that the rigid shelled egg of the spiny softshell turtle, *Trionix spiniferus*, is 'fully cleidoic'.

At this stage it may be better to define what cleidoic and non-cleidoic eggs are. Needham (1931) used the term cleidoic to mean a 'closed box' which can be penetrated by matter only in a *gaseous* state. He has described the evolution of the cleidoic egg, the characteristics of which briefly stated would be as follows: (1) The cleidoic egg is not dependent on the environment for water or ash, and hence is a 'closed box'; (2) Oxidation of protein is supressed to a considerable extent and the end products of protein metabolism are accumulated in the form of non-soluble uric acid to save water for the developing embryo and to reduce the exchange of matter through the egg shell; and (3) consequently, oxidation of lipids is enhanced to meet the energy requirements of the developing embryo. By these characteristics, the hen's egg, the eggs of certain terrestrial reptiles and insects are all cleidoic. Eggs that absorb water and minerals from the environment, and utilize both protein and lipid as energy source for development, as seen in aquatic eggs, are non-cleidoic. The chelonia occupies a unique position

* Present address: Madras Research Centre of Central Marine Fisheries Research Institute, 29, Commander-in-Chief Road, Madras-600 105.

in that it has terrestrial as well as aquatic forms. Needham (1931, 1942), has used the expression 'imperfectly cleidoic' and 'intermediate' to denote the eggs of the trutles. The term 'imperfectly cleidoic' by itself does not definitely define the true characteristics but at that point of time and state of knowledge, perhaps, was of an indicative nature. Needham has heavily drawn on the results of the investigation of the team of Japanese scientists on the egg characteristics of *T. corticata* to come to such a conclusion. It is surprising that the ensuing years has not seen much work on this subject matter except for the related aspect dealt with by Cunningham and Hurtwitz (1936), Cunningham and Huene (1938), Cunningham *et. al.* (1939), Plummer (1976) and Packard *et al.* (1981).

In the course of our work on the embryonic development of the olive ridley *L. olivacea*, we have found certain very interesting characteristics which clearly indicate that the egg of olive ridley is non-cleidoic. Perhaps if a critical reappraisal of the work of the Japanese workers (Tomita, Karashima, Nakamura and Sendju) is made in the light of the accepted definition of cleidoic egg, it will again turn out that the egg of *T. corticata* is also non-cleidoic. In order to clarify this position we shall discuss below the aspects connected with water absorption, protein and lipid metabolism and nitrogen excretion in turtle eggs during development.

Water Uptake

The investigations on *T. corticata* by the Japanese workers indicate that the egg of *T. corticata* absorbs 42% of the initial store of water during development. Needham (1931) refers to the work of Cunningham (1923) on the turtle *Chrysemys cinerea* where the turtle moistens the dry ground by water (urine) from a supernumerary bladder before laying the eggs. The eggs in turn, swells during development. According to Needham 'This is a notable link in the evolutionary chain, for here the turtle goes out of its way to provide a store of water for its terrestrial eggs, *yet* outside not inside them. This must be the furthest point to which a non-cleidoic egg could go in a terrestrial environment.' (Needham 1931, p. 899). Hildebrand and Hatsel (1927) and Deraniyagala (1930) have indicated that the eggs of the loggerhead turtle, *Caretta caretta*, absorb water during incubation. Cunningham *et al.* (1939) reported that the eggs of *Caretta caretta* increases its weight by 50% during incubation by uptake of water. Our observation on the eggs of olive ridley *L. olivacea*, clearly indicates that from the initial, there is a weight decrease in the egg upto the 5th day of incubation, from whence weight increment is seen and by the 15th day the egg swells up and the flexible shell becomes turgid; in other words, it does not indent on slight pressure. Expressed in terms of initial loss of water and later gain, the values for water absorption

TABLE I. *Evidence of absorption of water by flexible or rigid shelled egg of chelonians*

Species	Aquatic or terrestrial egg*	Water absorption	Reference
Thalassochelys corticata	A ?	42% of the initial quantity of water absorbed by the egg.	Karashima, 1929.
Chrysemys cinerea	A ?	Flexible shelled eggs swell by absorbing water.	Cunningham, 1923.
Caretta caretta	A ?	Eggs take up good quantity of water during incubation,	Hildebrand and Hatsel, 1927 ; Deraniyagala, 1930 ;
		50% increase in weight of egg by uptake of water during incubation.	Cunningham & Hurwitz 1936 ; Cunningham & Huene, 1938, Cunningham *et al.*, 1939.
Dermochelys coriacea	A ?	Eggs swell by absorbing water.	Deraniyagala, 1930.
Lepidochelys olivacea	A ?	Flexible shelled eggs swell by absorbing water—6.31% of the initial quantity of water absorbed.	Silas *et al.*, 1984.
Malachemys concentrata	A ?	Egg gains 28% weight by uptake of water during incubation.	Cunningham *et al*, 1939.
Trionix muticus	A ?	Some of the naturally incubated calcarious shelled eggs crack, probably due to absorption of water.	Plummer, 1976.
Trionix spiniferus	A ?	Calcarious shelled eggs that are in contact with the substratum of the nest are capable of absorbing water from the surroundings to compensate for the transpiration of water through the exposed region of the egg.	Packard *et al.*, 1981.

* Eggs are laid in nests above high water mark where the moisture content will be very high or in damp ground after heavy rain. Hence these cases represent a transitional phase from truely aquatic to terrestrial eggs.

in *L. olivacea* were —2.50% + 3.81% (Total 6.31% absorption) of the initial store of water. All the examples cited above are on the flexible shelled eggs. Packard *et al.* (1981) discussed water exchanges in artificially incubated eggs of the spiny softshell turtle, *Trionix spiniferus.* They have demonstrated that the rigid shelled eggs of this species, which are in contact with the simulated substratum of the nest, do absorb some amount of water to compensate for the water lost through transpiration of water vapour from the exposed part of the egg. Plummer (1976) attributed the cracking of some of the naturally incubating rigid shelled eggs of the softshell turtle, *Trionix muticus*, to the absorption of water. However, Packard *et al.* (1981) consider such occurrence as very unusual since 'uptake of sufficient liquid to cause cracking of the calcarious shell is not of frequent occurrence in nature.'

The above observations clearly indicate that there is absorption in both the flexible shelled and rigid shelled eggs of chelonians (Table 1) studied so far. Hence we would consider that these eggs are not the 'closed box' type as envisaged by Needham, Needham (1942) himself has opined that as regards water absorption Chelonian eggs are non-cleidoic. This property of the egg is quite distinct from the eggs of the chick or even the water fowl (Grebe) (eggs of which are laid in water) which do not absorb water and are truely 'cleidoic' (Table 2).

Utilization of Protein

The few references on utilization of protein in turtle eggs we could find in literature is that of Tomita (1929); Karashima (1929); Nakamura (1929) and Sendju (1929), referred to also by Needham (1931) in the case of *T. corticata.* Here, 16.5% of the total protein are combusted for energy by the developing egg of this species (refer Needham, 1931, p. 1134). The total depletion of protein in yolk, calculated from nitrogen values given by Nakamura (1929), until the 45th day of incubation in the *T. corticata* egg (Total incubation period = 47 days) is 78.3%. By the 45th day 25.34% of the total nitrogen available in the egg has been utilized (Initial nitrogen in the egg = 592 mg; amount of nitrogen in yolk + embryo on the 45h day = 119 + 323 = 442 mg) in the *T. corticata* egg (calculated from Nakamura, 1929). This clearly indicates that a considerable amount of protein is metabolised for energy in *T. corticata* egg (Table 3).

Table 2. *Categorisation of absorption of water by developing eggs*

Type of egg	Aquatic or terrestrial egg	Water absorption	Reference
CLEIDOIC EGGS			
Chick (*Gallus domesticus*)	T	No absorption of water from the environment	Needham, 1931.
Water Fowl	A		
NON-CLEIDOIC EGGS			
(*a*) Marine demersal eggs which release planktonic larvae	A	Average percentage of water rises from 60.8 in egg to 84.0 in larva	
(*b*) Marine planktonic eggs which release planktonic larvae	A	Average percentage of water rises from 90.9 in the egg to 91.8 in the larva	Needham, 1931 and Pandian, 1970.

Table 3. *Utilization of protein and lipid as energy source in the developing eggs of turtles*

Species	Incubation time (days)	Aquatic or terrestrial egg	Depletion of protein in % of the respective initial value	Depletion of lipid in % of the respective initial value	Reference
Thalassochelys corticata	47	A ?	16.5	34.0	Karashima, 1929; Tomita, 1929; Nakamura, 1929.
			Upto 45th day calculated on the basis of total nitrogen content. 25.34		Calculated from the data of Nakamura, 1929.
Lepidochelys olivacea	45	A ?	Upto pipping (hatching) 42nd day. 29.57	26.49	Silas *et al.*, 1984.
			Upto emergence 45th day. 33.87	28.86	

Our work on the yolk utilization in *L. olivacea* (Silas *et al.* 1984) also indicates the utilization of a considerable amount of protein by the developing egg of *L. olivacea.* 29.57% and 33.87% of the initial store of protein in the egg is utilized at the time of pipping (hatching) and emergence respectively in the egg of *L. olivacea.* The reduction in the protein content of yolk at pipping and emergence are 73.31% and 76.03% respectively. The total incubation time of *L. olivacea* (45 days for emergence; pipping or hatching on the 42nd day) is not much different from that of *T. corticata* (47 days).

The utilization of protein seen in these two cases are typical of the non-cleidoic type of eggs (Table 4). This is quite distinct from the minimal utilization of protein—about 4-5%—in the truely cledoic eggs of chick and silkworm moth (Table 4).

Utilization of Lipid

Since protein metabolism is greatly suppressed in the cledoic egg, the energy for the developing embryo has to come mainly from the lipid store of the yolk. As indicated by Needham (1931) the total protein content in the cledoic egg is almost equal to the total lipid content of the egg, with the exception of silkworm moth (*Bombyx mori*). In the non-cleidoic eggs, the percentage of total lipid is considerably low in comparison to the total irotein content in the egg; the ratio of protein/fat varying between 1.5 and even 12.5 in fishes and marine invertebrates (Needham, 1931, Table 31). In tortoise he has given the protein/fat ratio as 2.271. In the olive ridley we find the ratio to be 1.75; in other words 52.94% protein and 30.0% lipid. This condition approximates closely to the non-cleidoic type. Thus, it is seen that even in the availability of organic material required for development, the tendency is to minimize utilization of protein during development in the cleidoic eggs.

In Table 4, the values of total lipid utilized during development by cleidoic and non-cleidoic eggs in percentage of total lipid present are given. The data of lipid utilization in the turtles *T. corticata* (Karashima, 1929) and *L. olivacea* (Silas *et al.*, 1984) are recorded in Table 3. It is evident from the data presented that in the marine turtles the utilization of lipid is distinctly lesser than in the true cleidoic egg *e.g.*, chick, lackey moth, etc. Here again the evidence points to the more non-cleidoic condition of the egg of sea turtles.

TABLE 4 *Utilization of protein and lipid as energy source in developing eggs*

Type of egg and species	Aquatic of terrestrial egg	Depletion of protein in % of the respective initial value	Depletion of fat in % of the respective initial value	Reference
CLEIDOIC EGGS				
Chick (*Gallus domesticus*) ..	T	4.5	60.0	
Silkworm moth (*Bombyx mori*) ..	T	3.9	48.0	
Sheep blowfly (*Lucilia sericata*) ..	T	0.5	44.5	
Grasshopper (*Melanoplus* sp.) ..	T	—	54.0	
Lackey moth (*Malacosoma* sp.) ..	T	—	85.0	
Potato beetle (*Leptinotarsa* sp.) ..	T	—	36.5	
Average ..		3.0	54.7	Needham, 1942 and Pandian, 1970.
NON-CLEIDOIC EGGS				
Frog (*Rana temporaria*) ..	A	29.6	28.0	
Salamander (*Cryptobranchus* sp.) ..	A	27.8	30.8	
Salamander (*Hynobius* sp.) ..	A	—	25.5	
Carp (*Cyprinas carpio*) ..	A	41.0	—	
Trout (*Salvelinus fontinalis*) ..	A	19.5	—	
Trout (*Salmo iredius*) ..	A	32.5	51.0	
Salmon (*Salmo fario*) ..	A	36.3	—	
Average ..		31.1	33.8	

Nitrogen Excretion

In the cleidoic eggs any substantial combustion of protein would result in the accumulation of undesirable residues of ammonia, urea or uric acid which are toxic and will be detrimental to development. Hence total cessation of combustion of protein should be the ideal situation. However, in nature this does not happen as we find that even in the truely cleidoic eggs of chick or the silkworm moth a minimal combustion of 4 to 5% protein takes place (Table 4). The inability of the cleidoic egg to absorb water necessitates the disposal of the waste products of protein metabolism in the form of uric acid crystals which are deposited in the walls of the embryonic membranes thereby saving the water reserve for use of the developing embryo. Needham (1942) had suggested that disposal of nitrogenous waste products by chelonian eggs probably are uricotelic as seen in cleidoic eggs. But the data on nitrogen excretion in the developing eggs of turtles presented by Needham (1931) himself and the recent study by Packard and Packard in *T. spiniferus* egg (Table 5) cleiarly indicates that nitrogen excretion in turtle egg is predominantly ureotelic. This points to the non-cledoic nature of the turtle egg. However, Packard and Packard (1983) have indicated that the egg of the spiny softshell turtle *T. spiniferus* is 'fully cleidoic'. Since the nitrogen excretion in this egg is predominantly ureotelic, they have opined that 'our results raise the possibility that uricotely is not a necessary outcome of natural selection for mechaism to conserve water during embryonic development and indicate a need to reassess the adaptive significance of urecotely among embryos of other terrestrial vertebrates.'

The type of nitrogen excretion in the egg of turtles (ureotely) is the same as obtains in the adult. Similarly, the adults laying cleidoic eggs exhibit the uricotelic metabolism. Form of nitrogen excreted by an animal depended primarily on the conditions under which its embryo had to live (Needham 1942). Hence study of nitrogen excretion in the adults will give an indication of what happens to the nitrogenous wastes in the developing embryos.

According to Moyle (1949) the turtles which are almost wholly aquatic, semi aquatic or live in damp places frequently entering water are predominantly ureotelic, while the tortoises living in very dry, almost desert conditions are uricotelic (Table 6). This is reflected in the mechanism of excretion in turtle eggs also.

In the light of these facts we feel that the condition obtaining in the spiny softshell turtle *T. spiniferus*, discussed by Packard and Packard (1983) should be viewed taking into consideration the following points :

1. The *T. spiniferus* egg is not 'fully cleidoic' and allows absorption of water linked with transpiration of water vapour from the egg. The eggs do not swell due to the hard shell.

2. The nesting takes place soon after rains (Plummer, 1976) indicating a behavioural preadaptation or 'aquatic affinity'. Does the timing of the nesting soon after rain indicate a behavioural instinct for ensuring availability of water for absorption for the developing eggs ?

TABLE 5. *Pattern of nitrogen excretion in turtle eggs*

Species	Aquatic or terrestrial egg	N_2 partition in % total N_2 excreted: Ammonia	Urea	Uric acid	Amino acids, creatine & creatinine	Purines other than uric acid	Reference
Turtle							
Chrysemes pinta ..	A ?	15.3	39.0	18.8	11.5 and 18.6 (undetermined)		Needham, 1931 (Table 163)
Chelonia mydas ..	A ?	16.1	45.1	19.1	—	—	
Trionix spiniferus ..	A ?	24.0	70.0	6.0	—	—	Packard & Packard, 1983
Tortoise							
Testudo gracea ..	A ?	—	90.0	trace	—	—	Needham, 1931 (Table 164)

TABLE 6. *Partition of nitrogen in the urine of turtles (in per cent of total nitrogen excretion). The most aquatic species excrete almost no uric acid, where as this compound dominates in the most terrestrial species (Moyle, 1949)*

Species	Habitat	Urine component *				
		Uric acid	Ammonia	Urea	Amino acids	Unaccounted for
Kinostemon subrubrum	Almost wholly aquatic	0.7	24.0	22.9	10.0	40.3
Pelusios derbianus	Almost wholly quaatic	4.5	18.5	24.4	20.6	27.2
Emys orbicularis	Semiaquatic ; feeds on land in marshes	2.5	14.4	47.1	19.7	14.8
Kinixys erosa	Damp places ; frequently enters water	4.2	6.1	61.0	13.7	15.2
K. youngii	Drier than above	5.5	6.0	44.0	15.2	26.4
Testudo denticulata	Damp, swampy ground	6.7	6.0	29.1	15.6	32.1
T. gracea	Very dry, almost desert conditions	51.9	4.1	22.3	6.6	4.0
T. elegans	Very dry, almost desert conditions	56.1	6.2	8.5	13.1	12.0

* There were small amounts of allantoin, guanine, xanthine and creatinine and variable amount not accounted for.

3. The habitat of the adult is aquatic and the method of excretion in the adult must be ureotelic which is also reflected in the developing eggs.
4. Data on utilization of protein during development is not available for this species ; but in the two marine turtles that have so far been analysed, namely *T. corticata* (Japanese workers, 1929) and *L. olivacea* (Silas *et al.*, 1984) a good amount of protein is utilized during growth of the embryo which is quite evidently not a cleidoic property.
5. Unless subjected to very extreme condition of desiccation the eggs will not resort to uricotely since the energy requirement for this is extremely high (Needham, 1931). We do not feel that with water available in the surroundings and absorption of water possible through the shell, the *T. spiniferus* egg would have been subjected to extreme water scarcity.

It is our feeling, that the egg of the spiny softshell turtle *T. spiniferus*, even though possessing a hard shell, should, in view of the points raised above, be classified as non-cleidoic and this will account for the ureotelic nitrogen excretion.

CONCLUSION

The most fundamental need of the non-cleidoic egg is for water (Needham, 1942). We have seen that all turtle eggs, flexible shelled or rigid shelled, studied so far do take in water during incubation, which clearly defines the turtle egg as non-cleidoic. The metabolic properties exhibited by eggs of turtles that have been studied so far—*T. corticata, L. olivacea* and *T. spiniferus* (nitrogen excretion only)—are truely non-cleidoic Since the nitrogen excretion in adult turtles that have some association with water are predominantly ureotelic, the eggs of these also may exhibit the pattern of ureotelic excretion, which is a non-cleidoic property. Based on all these informations we would classify the turtle eggs—marine or fresh water—as non-cleidoic. There is no data, except for nitrogen excretion in the adults which is predominantly urecotelic, on the uptake of water of metabolic properties of egg in the tortoise that live in very dry, almost desert conditions, to classify them as cleidoic or non-cleidoic. But if the urecotelic nitrogen excretion of the adult is an indication the tortoise that experience extreme water scarcity may be laying cleldoic eggs.

We would strongly urge that studies on the eggs of other species of turtles and tortoises be undertaken to elucidate water uptake, protein and lipid matabolism and nitrogen excretion. There is a need for understanding variabilities in these parameters for eggs of different species of turtles and tortoises to ascertain whether eggs of all species could be clearly denoted as cleidoic or non-cleidoic or as 'intergrades'.

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