CHAPTER II

LITERATURE REVIEW

General History

Turtles are descended from ancient animals that evolved a shelled form over 200 million year ago. They are part of the Class Reptilia of Subphylum Vertebrata. Turtles found a successful life in the Triassic period. The key to their success is shell morphology. However, this shell probably limited morphological diversity. Thus, flying or gliding turtles have never existed, and even arboreality is not at all developed. Shell morphology can reflect the ecology of a turtle species. The most terrestrial forms have high domed shell but some species, like the gopher tortoises of North America have a secondarily reduced domed shell for burrowing. Living in rocky foothill regions, the shell of the pancake tortoise of Africa is flat and flexible. This adaptive shell provides them with the means to escape efficiently from a predator. Two different groups of box turtles (*Terrapene* and *Cuora*), some mud turtles (*Kinosternon*), and some african side-neck turtles (*Pelusios*) have all evolved flexible regions in the lower shells that allow the front and rear lobes to be pulled upward to close the openings of the shell. Some freshwater turtles, like the soft-shells, have much reduced bony shells so that they can move through water very rapidly.

Although all living shelled reptiles are turtles, the terms tortoise and terrapin have different meaning in various parts of the world. Goin and Goin (1971) commented that the name "tortoise" may be restricted to the members of the family Testudinidae and use "terrapin" for small to medium-sized, more or less aquatic, hard-shelled turtles that are used for food.

Systematic Background

Turtles are in the Order Chelonia (also known as Order Testudines). Chelonians are divided into 2 Suborders : Pleurodira (side-necked turtles) and Cryptodira (hidden-necked turtles). The Suborder Pleurodira is represented by 2 Families, Chelidae and Pelomedusidae, while the Suborder Cryptodira is represented by 10 Families; Carettochelyidea, Chelydridae, Dermatemyidae, Emydidae, Kinosternidae, Platysternidae, Testudinidae, Trionychidae, Cheloniidae, and Dermochelyidae.

At present, *Malayemys subtrijuga* (Schlegel & Müller,1844), or "Tao Na" in Thai, meaning field turtle, is placed in the Family Emydidae, Genus *Malayemys*, species *Malayemys subtrijuga*. This species was originally described as a member of the Genus *Emys* by Schlegel and Muller in 1844, because at that time that genus included all aquatic hardshelled Testudines. Subsequently the taxon was placed in the genera *Geoclemys* in 1859 and *Damonia* in 1889, until Lindholm (1931) commented that *Damonia* was preoccupied and proposed the new Genus *Malayemys* for this species. No other species besides *M. subtrijuga* are included in the Genus *Malayemys*. This was accepted by most subsequent workers, but because Smith (1931) reviewed the species as *Damonia*, this name is still sometimes used in S.E. Asia (Nutphand,1979). No subspecies are recognized.

Morphology

Turtles are among the most bizarre vertebrates: Covered in bone, with the limbs inside the rib cage, and with horny beaks instead of teeth. Thus, the morphology of turtles is very specialized.

Ashley (1962) who described turtle morphology pointed out that its longitudinal axis is relatively short while its transverse axis is broad. Therefore the body is morphologically shorter than in any other vertebrate except the frog. Young (1981) proposed that this shortening and broadening may be the result of some quite simple change in morphogenesis. Ashley divided the body of turtles into 5 parts as follows.

1. <u>Head</u>

The head is composed of the strong bony skull which protects the special sense organs. The head is covered by skin, except for several turtle species in the Families Platysternidae and Chelidae in which the head is covered by a large horny shield.

2. Neck

Turtles have relatively long necks, especially snapping turtles, soft-shelled turtles and some side-necked species of the Family Chelidae.

3. Trunk

The trunk contains the main visceral mass of the body. It is firm and rigid from the carapace above, and the plastron below. Both carapace and plastron are strongly united on each side by bony bridges varying in width with the species. Horny scutes cover the carapace, the plastron and the bridges. The scutes of the carapace are named nuchal, vertebrals, costals, marginals and supracaudals while on the plastron they are gular, humeral, pectoral, abdominal, femoral and anal. On the bridge are the small axillary and inguinal scutes (Figure 1, 2, and 7).

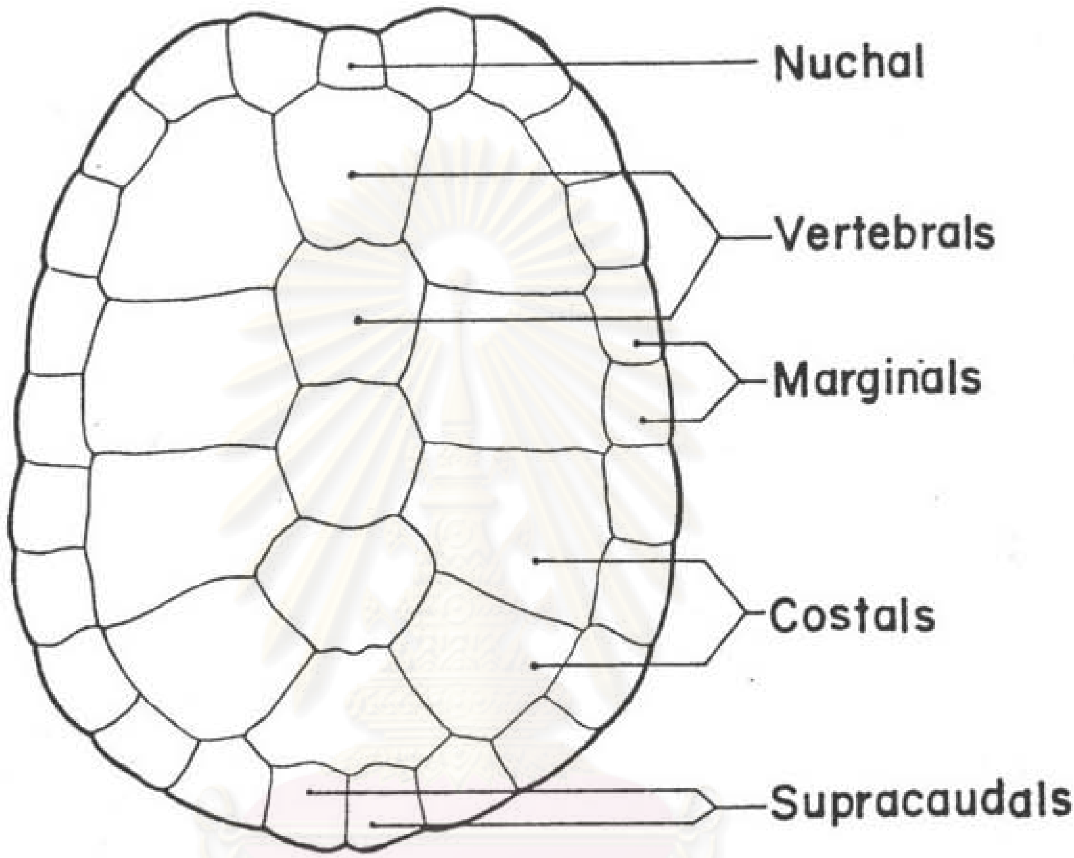

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Figure 1. Scutes of the carapace of *M. subtrijuga* (Drawing from specimen no. CUMZ (R) 1994-03-22,3)

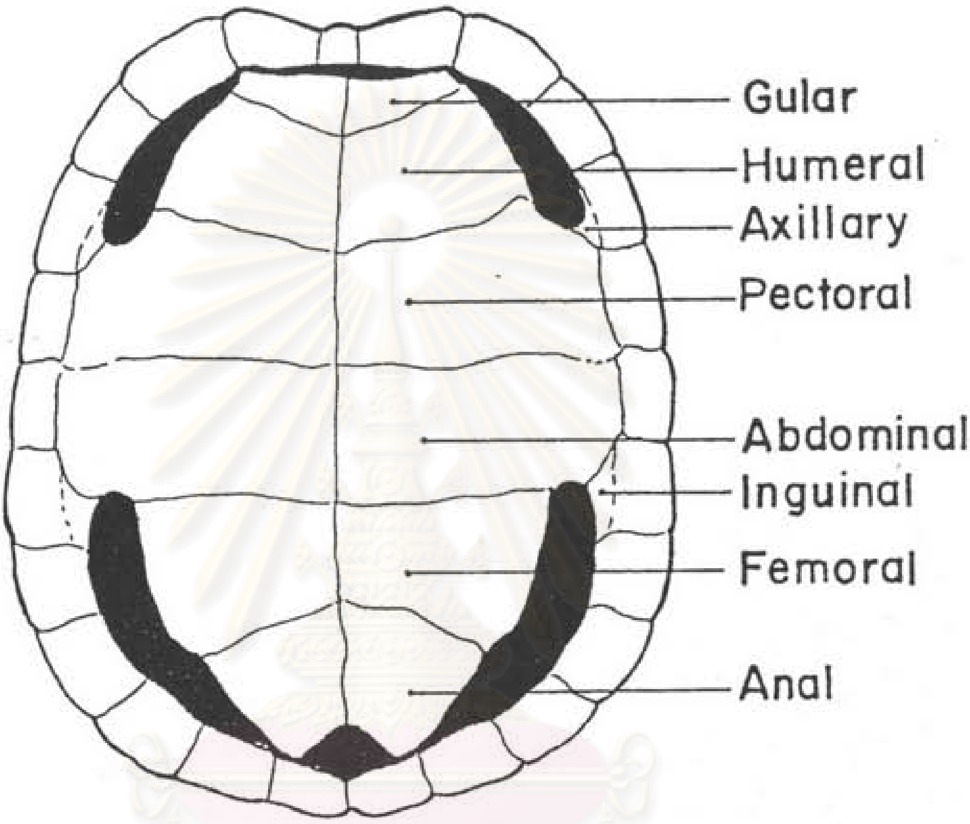

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Figure 2. Scutes of the plastron of *M. subtrijuga* (Drawing from specimen no. CUMZ (R) 1994-03-22,3)

4. Limbs

Forelimbs and hindlimbs each have five toes. Each toe exhibits a claw except the fifth toes on the hindlimbs. The legs are supplied with powerful muscles to provide for rapid swimming and walking on land.

The limb morphology is related to habitat. The tortoises have elephant-like hindlimbs. Some species have flattened the forelegs into scoops to burrow in the soil. The freshwater turtles, such as emydids, have webbing between their toes for swimming. The forelimbs of sea turtles have developed to form flippers to be able to swim efficiency.

5. Tail

This appendage is short in most Emydid and Testudinid turtles but is rather long in the Snapping turtles (Chelydridae) and the Big head turtle (Platysternidae).

The morphology of *M. subtrijuga*, reviewed by Boulenger (1889), de Rooij (1915), Smith (1931), Bourret (1941), Taylor(1970), Nutphand (1979), Ernst and Barbour (1989) and van Dijk and Thirakhupt (1994), was that the oval carapace is moderately arched, unserrated, notched posteriorly and bears three discontinuous keels. The nuchal scute is moderately large. The first vertebral is longer than wide but the rest are wider than long. The color of the carapace ranges from medium to darker brown, with a yellow or cream-colored border. The hingeless plastron is narrower and shorter than the carapace, angulate laterally, truncate anteriorly and deeply notched posteriorly. Its hind lobe is shorter than the bridge. The color of the plastron is yellow with a large black blotch on each scute. The scutes are very thin. The head is relatively large. The symphysis of the lower jaw is very heavy and is produced into a strong, blunt hook. When closed, the jaws do not occlude and they are unable to cut. The cheek bar is broad. The supraoccipital crest is large. The jaw-closing musculature is extremely powerful. These adaptations are associated with a diet of hard-shelled molluscs. The head is black with several distinctive white or pale yellow stripes, some continuing onto the neck. The limbs are gray to black with a narrow yellow outer border. The tail is very short.

Sexual dimorphism

Ashley (1962) published that the reproductive anatomy is similar for all chelonian species. Although determination by examination of internal gonads is certain in young specimens, the turtles must be sacrificed. Thus many works have tried to investigate many valid secondary sexual characters. Graham (1979) reviewed secondary sexual characters in turtle as follows :

1. The male preanal tail length is twice as long as the female (eg. *Clemmys insculpta*).

2. The male tail length is longer and thicker than female (eg. *Deirochelys reticularia, Gopherus agassizi, Terrapene carolina* and *Trionyx spiniferus*).

3. The position of male anal opening is posterier to the rear of the carapace margin; in contrast, that of female is anterior to the rear carapace margin (eg. *Chelydra serpentina*, *Deirochelys reticularia*, *Graptemys barbouri* and *Malaclemys terrapin*).

4. The male foreclaws are longer than the female (eg. *Trachemys scripta*).

5. The male hindlimb nails are short, strong and curved but long, straight and slender in female (eg. *Terrapene carolina*).

6. The male inner hindleg surface has a small patch of tilted scales, whereas in the female these scutes are relatively smooth (eg. *Sternotherus odoratus*).

7. The male iris color is different from that of female (eg. red in males of *Terrapene carolina* but yellow-brown in females).

8. The male head is narrow, more pointed but it is blunt and broader in the female (eg. *Malaclemys terrapin*).

9. The length of male carapace is less than that in female (eg. *Graptemys barbouri*, *Malaclemys terrapin*, and *Trionyx spiniferus*).

10. The shape of male carapace is more tapered posteriorly when compared to that in female (eg. *Chelonia mydas* and *Malaclemys terrapin*).

11. The height of carapace is less domed in male than in the female (eg. *Chrysemys picta*).

12. The male relative plastron length is less than that in female (eg. *Terrapene carolina*).

13. The male plastron is concave but it is flat in female (eg. *Clemmys insculta* and *Gopherus agassizi*).

14. The male rear plastron lobe is narrow but wider in the female (eg. *Chelonia mydas*).

Ernst and Barbour (1989) published that the sex determination of *Malayemys subtrijuga* is based on the tail length and tail width. The tail in the male is longer and thicker than in the female.

Growth rate

Agassiz (1857) was the first to study the growth of turtles. He proposed that the growth of 2 species of *Chrysemys* is slow. The females begin to lay eggs when they are 10 to 11 years of age. An adult snapping turtle *Chelydra serpentina* may grow only one inch in 45 years. This supported the public's opinion that turtles grow slowly and have high longevity. This fact is further attested by Flower (1925) who showed that at least four species of tortoise have age records of 100 or more years. Since Agassiz, the work done on growth rate of many species grew rapidly. However, most studies were conducted in captivity.

Pearse (1923) made the first important analysis of growth in turtles living under natural conditions. He showed that *Chrysemys picta bellii* increase their length by 73 % in the second year of their life, by 31 % in the third year, and by 25 % in the fourth and fifth years. He showed correlation of growth with age, but Hubbs (1924) noted that Pearse failed to indicate how age could be determined.

Benedetti (1925) reported that the growth rate of *Testudo graeca* varies with the size of the individual, the sex, and the seasons of the year.

Hildebrand (1932) stated that under similar conditions the diamondback terrapin (*Malaclemys terrapin*), both male and female may show different growth rates. Some reach nearly full size in 5 or 6 years, whereas others require 12 to 15 years or longer to reach full size.

Sergeev (1937) studied the growth of *Emys orbiculalis* and concluded that: firstly, growth is exceedingly variable; secondly, growth is rapid in juveniles but ceased completely in old individuals; and lastly, the attainment of sexual maturity did not result in a slowing of growth. He also first demonstrated the growth rate estimation method by measuring growth rings, plastral laminae lengths, and plastron length.

Liu and Hu (1940) concluded that the growth of *Chinemys reevesii* is rather rapid and constant during the first five years but irregular and slow afterwards. They suggested that this slowing of growth may be due to the attainment of sexual maturity.

Graham (1979) suggested that the absolute and estimated determinations are the two fundamental kinds of growth analysis. Absolute determination is by the capture-recapture method where turtles will be captured, measured (carapace or plastron length), marked, released, recaptured and remeasured. This method not only can be performed on captive as well as wild animals but also can reflect growth under artificial conditions. This measurement is the most exact method of assessing growth because it lacks much of the error inherent in growth estimation techniques. However, this method may fail to capture some initially measured turtles and it takes much time. Moreover, the individuals are not released on the same day and recaptured on a single subsequent day. Therefore, exact growth comparison among individuals is quite difficult. The other growth rate determination is the growth rate estimation method.

Cagle (1946) proposed that median border lengths of the growth rings on all plastral laminae can be summed for a given age class (growth ring number) to estimate plastron midline length. Growth estimation by summation method has been performed on *Pseudemys scripta* (Cagle, 1946), *Chelydra serpentina* (Gibbons, 1968 a), *Clemmys guttata* (Graham,1970), and *P.rubriventris* (Graham, 1971b).

Some turtle species are well suited for growth study because both their age from growth rings and their body size from plastron length can be accurately established. Cagle also suggested the use of plastral laminae in growth studies because plastral growth rings are formed more in a plane than are carapacial growth rings.

Presently, there are no published growth studies on *M. subtrijuga*.

Age determination

In general, age determination of turtle specimens is difficult and often highly subjective. However, Zug (1991) reviewed some useful methods for age determination as follows :

1. Mark-Release-Recapture

This method is reviewed by Swingland (1978) and Fermer (1979). Each hatchling turtle will be uniquely marked. Upon recapturing, these marked turtles can be aged accurately since their emergence dates are known. Examples were studied in *Chrysemys picta* (Tinkle et al., 1981), and *Clemmys guttata* (Ernst, 1976).

2. Captive Rearing

Animals in captivity usually grow more rapidly. Thus, in order to study growth and age as close as possible to wild animals, the animals's captive environment should be set to match the natural environment. This method was tried in determining age of *Malaclemys terrapin* (Hildebrand, 1929) and *Emydura krefftii* (Banks,1987)

3. Body Length or Mass

When captured, turtles are weighed and measured for carapace length or plastron length. These data can be divided into size classes. From the size class the relative age can be roughly estimated. This method is appropriately used to examine "age" aspects of population within a short time but the percentage of accurancy is uncertain. Generally, the body length is more suitable than the mass because the latter can vary from several factors such as the recency of feeding and drinking water and from season of the year. Examples were studied in *Malaclemys terrapin* (Hurd et al., 1989) and *Trachemys scripta* (Gibbons et al.,1980).

4. Scute Growth Zones

Turtles display patterns of scute growth, except trionychids, carettochelyids and dermochelyids, which do not have scutes. Each scute develops from the epidermal layer covering the entire shell. The growth ring counts should always be taken from the same scute on each turtle to avoid increasing sampling error. This principle assumes that only one scute layer and growth line is formed each year, hence the number of scute layers is equal to number of years of life. Therefore, the inherent assumptions are as follows: First, scute growth shows a regular pattern. Second, the growth is directly associated with regular climatic events so that each scute mark represents a specific interval of time. Finally, either the scute marks remain visible through the turtles's life, or the number of marks lost can be accurately estimated. Scute layers are the most useful age determination criterion, because workers can estimate the age upon first capture without killing the animal. Examples were studied in *Chrysemys picta* (Sexton, 1959), *Trachemys scripta* (Cagle,1946), and many others.

5. Coloration changes

Color patterns are also different between sexes in some species when they reach maturity, for example the blackening of head and carapace color in male *Batagur baska*. (Moll, 1980)

There are no published studies on age determination in *M. subtrijuga*.

Diets

The three basic methods to sample ingested food items from turtles are as follows:

1. Sacrifice the turtle and investigate food items in the digestive tract. This method yields the best quantitative results because it can provide exact data on the kinds, the amounts, and ultimately the nutritional quality of food resources.

2. Examination of fecal material. Folkerts (1968) suggested that this method has the advantages that the animals can be marked and release for further studies, space and preservatives are saved, and collection of fecal material is less time-consuming than dissection. Moreover, microscopic inspection of the feces can yield a reasonable species list of food items. However, the fecal remains of food items are often difficult to identify and it is difficullt to reconstruct the original meal

quantitatively from the post digestive remnants found in the feces. By this way, it allows multiple samples to be taken from individual turtles over their lifetimes.

3. Stomach flushing techniques. The idea of this method is to displace the stomach contents out through the esophagus with water injected from a continuous pump syringe into the stomach by way of a flexible tube inserted through the mouth and esophagus. Food items can be readily sorted, identified, and measured. This method allows multiple samples to be taken from individual turtles over their lifetimes and can be integrated with other nondestructive techniques for further studying. This technique was used by Legler (1977) on *Elseya dentata* and several other species. However, Legler also discussed that success of this method is inversely proportional to size of turtle because the volume of water injected with a hand operated syringe may be insufficient for a large animal. However, Parmenter (1980) solved this volume problem by using tap water and a vacuum pump in a laboratory setting.

Natural diets

It was reported that the kind of natural diet depends on seasonal changes. Parmenter (1980) and Schubauer and Parmenter (1981) studied a South Carolina population of *Trachemys scripta scripta* where the summer diet include vegetation and a substantial quantity and variety of animal prey. In contrast, the winter diet was composed entirely of aquatic vegetation. A similar shift from summer omnivory to winter herbivory has been supported in kinosternids (Mahmoud, 1968a). The kind of natural diet also depends on the body size. As *Trachemys scripta* increase in body size from juveniles to adults, the diet composition changes from a fairly balanced mixed of plant and animal matter to one dominated by vegetation. (Marchand, 1942; Clark and Gibbons, 1969; Moll and Legler, 1971; and Hart, 1983).

Ernst and Barbour(1989) reported there are three food habit types for emydids; tentative herbivores such as *Callagur borneoensis*, and *Cuora amboinensis*; tentative omnivores such as *Cyclemys dentata*, and *Melanochelys trijuga*; tentative carnivores such as *Siebenrockiella crassicollis*.

The natural diet of *Malayemys subtrijuga* was reported as carnivorous by feeding on snails, shrimps, crabs, fishes, insects and earthworms (Smith, 1931; Taylor, 1970; Pritchard, 1979; Nutphand,1979; Geissler and Jungnickel, 1989).

Reproductive biology

1. Clutch size

Clutch size is the number of eggs laid in a nest. Clutch sizes in turtles range from one egg in several testudinid (e.g., *Chersina angulata*) to about 150 in *Chelonia mydas* in Surinam (Pritchard, 1979) and Yemen (Frazier,1971). Dunham, Morin and Wilbur reported that the maximum number of eggs a female can carry is limited by the space avaiable in her body cavity and the size of individual eggs.

The findings of the clutch size of some freshwater turtles occurring in Thailand were as follows (Table 1).

Table 1. The clutch size of some freshwater turtles occurring in Thailand

Species	Eggs/clutch	References
Batagur baska	20	(Maxwell, 1911)
	30 - 50	(Nutphand, 1979)
	26	(Moll,1984)
	13 - 34	(Ernst and Barbour,1989)
Callagur borneoensis	12	(Ernst and Barbour,1989)
Cuora amboinensis	3 - 5	(Nutphand,1979)
	1 - 4	(Paull et al,1982)
	2 - 4	(Ernst and Barbou, 1989)
Cyclemys dentata	2 - 4	(Theobald,1868; Smith, 1931; Nollert,1987)
	10 - 20	(Nutphand, 1979)
	2 - 3	(Ernst and Barbour,1989)
Hieremys annandalii	4	(Ernst and Barbour,1989)
Melanochelys trijuga	3 - 8	(Ernst and Barbour,1989
	3 - 7	(Das, 1991)
Siebenrockiella crassicollis	1 - 2	(Ernst and Barbour,1989)
Trachemys scripta	2 - 25	(Ernst and Barbour,1989)
Malayemys subtrijuga	5 - 10	(Nutphand, 1979)

2. <u>Egg size</u>

Moll (1979) reported that egg sizes range from approximately20 mm diameter spherical eggs in *Trionyx sinensis* up to the 60 mm spherical eggs of *Dermochelys coriacea* and *Geochelone elephantopus* or the 70 mm oblong eggs of *Batagur baska*. One of the longest recorded is a 76 x 39 mm egg from a *Rhinoclemys funerea* (Moll and Legler, 1971).

Gutzke and Packard (1985) concluded that large eggs of painted turtles (*Chrysemys picta*) have a significantly higher probability of hatching than do small eggs incubated under various conditions of temperature and moisture. Surface area to volume considerations may account for the difference in hatching success between large and small eggs incubated in dry conditions.

The findings of the egg size of some freshwater turtles occurring in Thailand were as follows (Table 2).

Table 2. The egg size of some freshwater turtles occurring in Thailand

Species	**Egg size (mm)**	**References**
Batagur baska	65.78 x 40.73	(Moll, 1980)
	66-70 x 45	(Ernst and Barbour,1989)
Callagur borneoensis	71.3-76.3 x 36.6-44.5	(De Rooij, 1915)
	68-76 x 36-44	(Ernst and Barbour,1989)
Cuora amboinensis	43 x 32	(Ewert,1979)
	40-46 x 30-34	(Paull et al,1982; Ernst and Barbour ,1989)
Cyclemys dentata	57 x 35	(Ewert,1979)
	57 x 30-35	(Ernst and Barbour,1989)
Hieremys annandalii	50 x 30	(Das,1991)
	57 - 62 x ?	(Ernst and Barbour,1989)
Melanochelys trijuga	47 x 27	(Ewert,1979)
	43-55 x 24-30	(Ernst and Barbour,1989)
Pyxidea mouhotii	53.85 x 30.2	(Das, 1991)
	40 x 25	(Ernst and Barbour,1989)
	51-56 x 25-27	(Tikader and Sharma,1985)
Siebenrockiella crassicollis	45 x 19	(Ewert, 1979; Ernst and Barbour, 1989)
Trachemys scripta	30-42 x 19-29	(Ernst and Barbour,1989)
Malayemys subtrijuga	40-45 x 20-25	(Smith,1931)
	44 x 22	(Ewert,1979)

3. <u>Incubation periods</u>

Ewert (1979) stated that "incubation period" refers to the developmental period from laying to hatching. "Hatching" means the primary pipping of the eggshell. He also discussed that the incubation period varies between species as follows: the short developmental periods, usually less than 100 days in natural nests and less than 70 days at constant 30°c, are characteristic of Emydidae, whereas long developmental periods, exceeding 100 days in nests and more than 70 days at 30°c, typify Kinosternidae and Testudinidae. He concluded that most chelids also seem to have long periods. Furthermore, the relationship between incubation period and nesting habitat stands out. Species that nest on sandbars and beaches appear to incubate in short periods. Roze (1964) said that the selective advantage of a short incubation period is obvious because the risk of death from flooding is high. An interesting phenomenon is the extreme variation in long incubation periods, especially within the same clutch.(Jayakar and Spurway, 1964).

The findings of the incubation period of some freshwater turtles occurring in Thailand were as follows (Table 3).

Table 3. The incubation period of some freshwater turtles occurring in Thailand

Species	**Incubation period (days)**	**References**
Family Emydidae		
Batagur baska	70-112	(Ernst and Barbour, 1989)
Melanochelys trijuga	60-65	(Ernst and Barbour, 1989)
	52-407	(Das, 1991)
Trachemys scripta	65-75	(Ernst and Barbour, 1989)
Cuora amboinensis	74	(Inskeep, 1984)
	79	(Mudde, 1987)
Family Trionychidae		
Amyda cartilaginea	130 - 140	(Bourret, 1941)
	74	(van Dijk,1992)

At present, there is no published information about the incubation period of *M. subtrijuga*'s eggs.

Parasites

A wide diversity of turtle ectoparasites and endoparasites were described by Hunsaker (1966), Telford (1967, 1971), and Murphy (1973a, 1973b). In captivity, the great majority of species of parasites are generally not a problem in well cared turtles. Telford (1971) argued that in nature most parasites exist in a balance with their hosts, becoming a serious health problem only under the unusual conditions and stress of captivity.

Ectoparasites often found on turtles are leeches of several genera (Murphy, 1973b), ticks, especially *Ornithodoros sp.* and *Amblyomma sp.*, and mites, notably the common snake mite, *Ophionyssus natricis*. Endoparasites often found in turtles are protozoa (genus *Entamoeba* and *Endolinax*) and Nematode worms.

Esch et al (199O) presented in a checklist of the helminth parasites of *Trachemys scripta* that trematodes, cestodes, acanthocepala, and nematodes occur.

There are no published studies on parasites in *M. subtrijuga*.