

## Chapter 2

### Literature Review

#### 2.1 Softshell turtle

General cladistic classification of softshell turtles is:

Kingdom Animalia

Phylum Chordata

Subphylum Vertebrata

Class Reptilia

Order Chelonia (Testudines)

Suborder Cryptodira

Family Trionychidae

Phyletic relationship of softshell turtles and other living families of turtles is shown in figure 2-1.

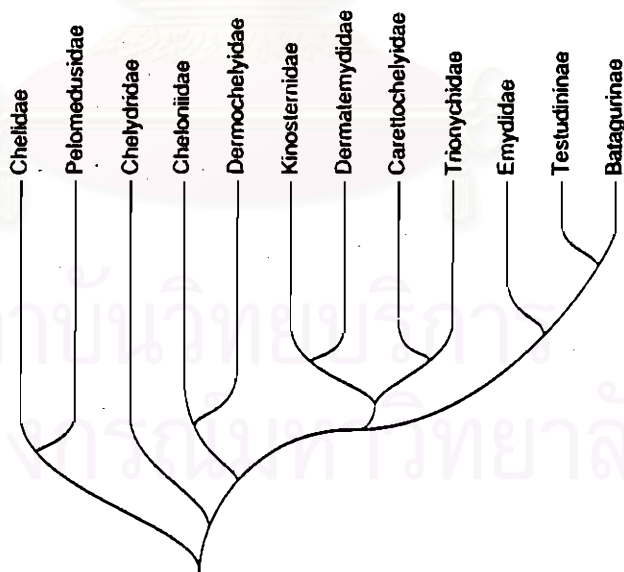


Figure 2-1 Dendrogram of presumed phyletic relationships among the living families of turtles (Zug, 1993).

The softshell turtle of the family Trionychidae is an old and very distinctive group of aquatic Testudines. In nearly all other families of turtles the body is surrounded by a bony shell, formed from dermal bone with participation of the ribs and certain bones of the pectoral girdle, cover by large epidermal scutes. In Trionychids the bony shell is strongly reduced and large area are formed by cartilage. The dorsal carapace lacks the ring of peripheral bones (except *Lissemys*), and costal plates are short with the rib ends projecting out of them. The ventral plastron are reduced to a trellislike frame as in sea turtles. The carapace and plastron are connected only by cartilaginous and ligamentous connective tissue and there is no bony connection as occurs in other turtles. This construction allows considerable volume changes inside shell, necessitated by flexion and retraction of the long neck and large head. The surface of the shell is a thick, leatherly skin with no hint of epidermal scutes. This specialized flexible shell is reported to originated with contribution to three aspects of softshell turtle behavior i.e. to facilitate greater swimming speed by allowing longer strokes of the legs, to increase burrowing efficiency, and to allow increased speed and range of striking and snapping at preys and predators (Pritchard, 1979; van Dijk, 1992; Zug, 1993).

Head of softshell turtles typically ends in a protruding snorkellike snout. Trionychids can lie hidden on the bottom and extend their neck until only the tip of the snout projects above the water surface. They lack cloacal bursae and hence do not indulge in anal respiration. Air breathing occurs via the tube-nose, and is supplemented by pharyngeal respiration. It is possible that they conduct a fair amount of underwater respiration through the skin (Pritchard, 1979; van Dijk, 1992; Zug, 1993).

All species of softshell turtle are predominantly carnivorous. Preys are captured by both foraging and ambush. Juveniles eat small invertebrate preys and with growth tackle ever larger prey. Several species develop broad crushing surfaces on their jaws as a result of feeding predominantly on freshwater mussels and snails. Carrion is eaten whenever encountered e.g. the large softshell turtles are the main agents of disposal of all the incompletely cremated corpses and dead cattle that are entrusted to the great rivers of India. (van Dijk, 1992; Zug, 1993)

The distribution of the family is roughly similar to that of the Emydidae. Softshell turtles are abundant in temperate eastern North America and tropical Southeast Asia. They are also found on the more western islands of

Indonesia, one species is found in the Middle East, and there are several in Africa (Pritchard, 1979).

The living members of the family are universally accepted to form two natural monophyletic within a monophyletic Trionychidae. The subfamily Cyclanorbinae is characterized by cutaneous femoral valves which cover the hind limbs when withdrawn, and various skeletal features. Cyclanorbines include only three genera(four species), and show no apparent differences in general behavior and ecology from the Trionychines. The subfamily Trionychinae is characterized by the absence of peripheral bones, a very short bridge between carapace and plastron, a greatly reduced plastron, fusion of hyo- and hypoplastral bones occurring in old adults, and other less obvious osteological features. The Trionychines, although geographically more widespread, are not particularly diverse(18 species); their greatest diversity is in southern Asia and southeastern North America (van Dijk, 1992; Zug, 1993; Webb, 1997).

## 2.2 *Amyda cartilaginea*

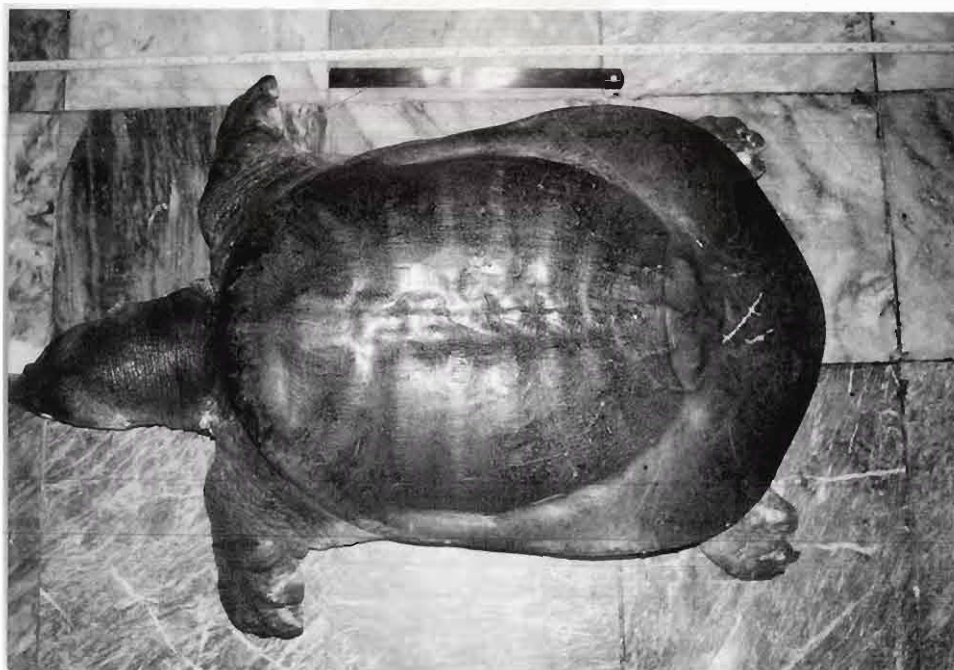


Figure 2-2 *Amyda cartilaginea* (sampled specimen BP0051096).

Biology and status of *Amyda cartilaginea* have been currently reviewed by Meylan, Moll and van Dijk (1995). The followings are partly extracted from their work.

*A. cartilaginea* is a large softshell turtle with a bony disc length to 403 mm and a total carapace length up to 830 mm and weight up to 35 kg or in exceptional cases over 70 kg. This species has long epiplastra in contact or narrowly separated from each other on the midline, one neural between the first pair of costals, and a strong median ridge on the symphysis. Carapace and plastron of *A. cartilaginea* is illustrated in figure 2-3.

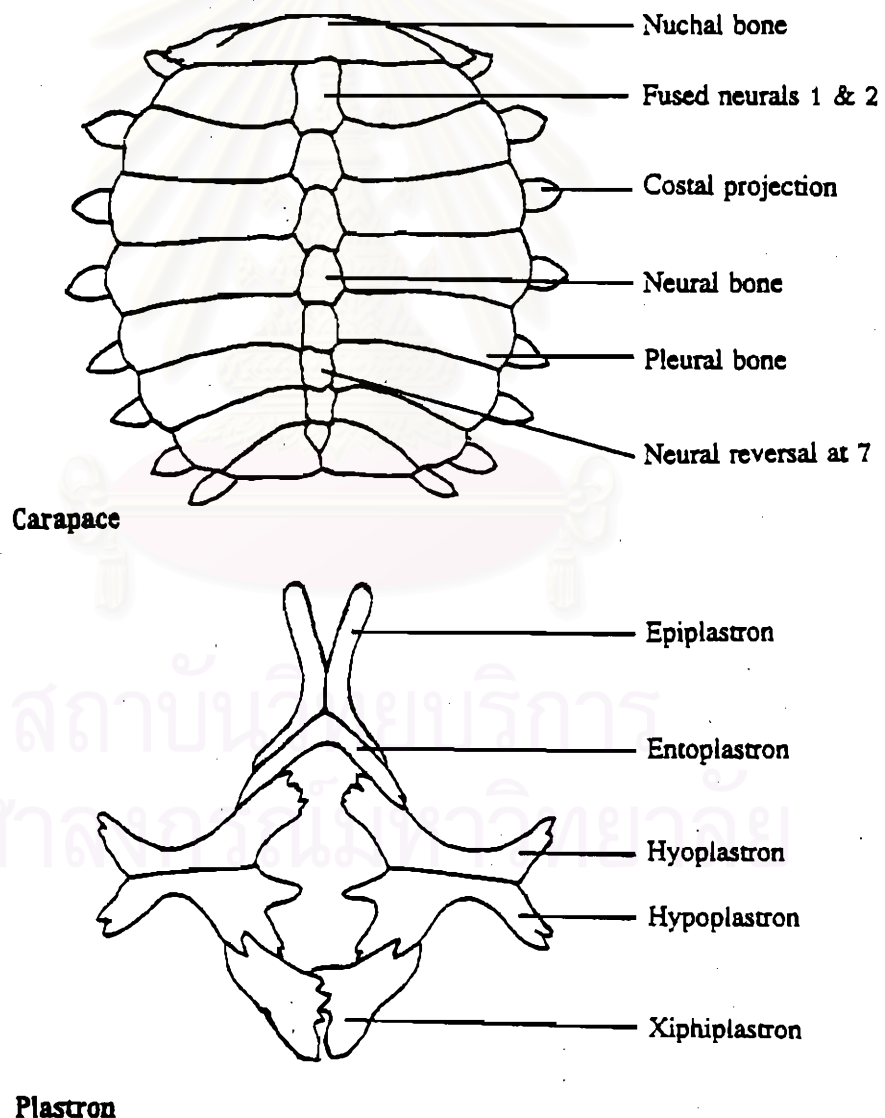


Figure 2-3 Carapace and plastron of *Amyda cartilaginea* (Modified from Wachira Kitimasak, 1996).

This softshell turtle species is found in southern and eastern Myanmar, possibly extending to extreme southwestern Yunnan, all of Thailand and Cambodia, the Mekong drainage area of Laos, central and possibly south Vietnam, the Malay Peninsula and the continental shelf islands of Sumatra, Bangka, Java, Balitung, Lombok, and Borneo. Its suggested occurrences in northern Vietnam, the Molluccas or Timor remain unconfirmed. The distribution map of this species is shown in figure 2-4. The occurrence on the western Thailand which was not presented in this figure should be noted (Kumthorn Thirakhupt and van Dijk, 1994).

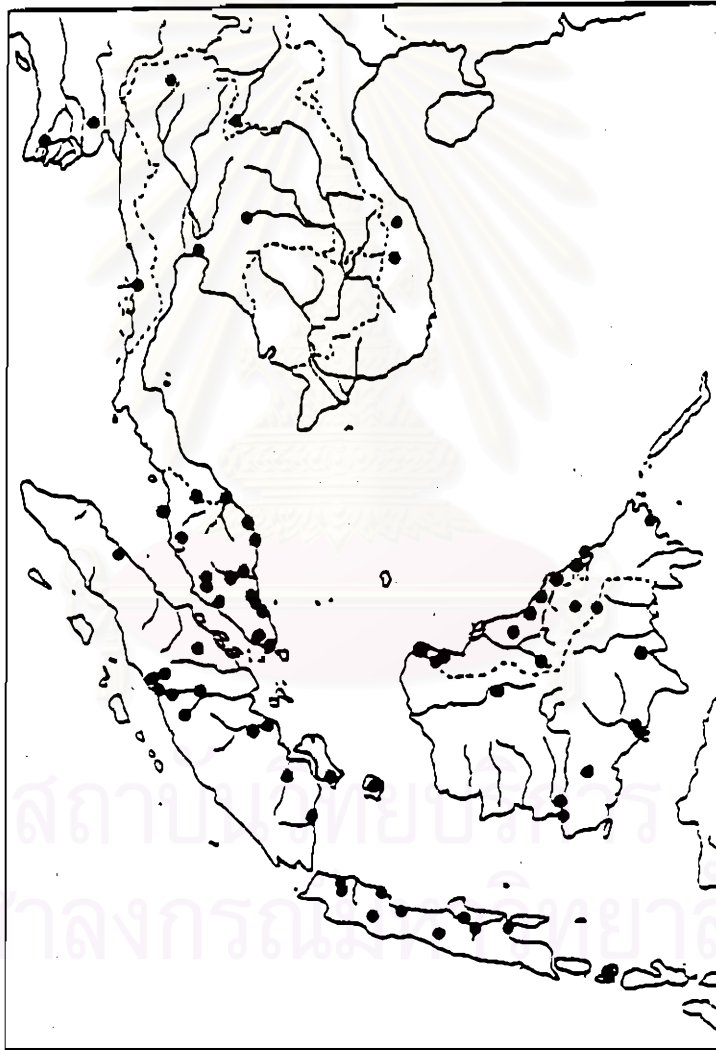


Figure 2-4 Distribution of *Amyda cartilaginea* (Iverson, 1992).

*A. cartilaginea* appears to be very generalised in habitat preferences. In northern and western Thailand, it inhabits quiet areas of streams up to altitude of 400-600 m. The species is quite susceptible to death by dehydration, limiting its occurrence in seasonal water bodies. It appears most abundant in lowland lentic



waters such as swamps, ponds and oxbow lakes adjacent to large rivers. In Thailand, where these areas were long ago converted to agricultural land and settlement areas, the species inhabits rivers, reservoirs, ponds, canals and ditches.

Little objective data are available about activity cycles; the animals spend long periods buried in bottom substrate and can be active at any time of day or night, although activity seems to peak at dawn and dusk. Basking was observed once in Bangkok. Wirot Nutaphand (1979) wrote that the animals like to spend long periods buried ashore, but these may be animals burying themselves in shallow water before water levels dropped.

*A. cartilaginea* is reported to feed on a variety of aquatic arthropods, molluscs, fish and amphibians. It is also reported as opportunistic omnivore eating fruits and seeds.

This softshell turtle species is a popular food animal and is widely and intensively hunted. At present live animals, frozen whole or butchered animals, and prepared softshell turtle curry are all available in the Bangkok weekend market. Several upmarket restaurants serve softshell turtles dishes; for chauvinistic reasons, wild *A. cartilaginea* is preferred over cultured Chinese softshell turtle. Its curio value and perceived wholesome effects sustain a demand for softshell turtles for consumption. In rural areas, softshell turtles and other wild animals remain an important protein source for local people. Several softshell turtle populations are in decline, yet the species still appears moderately abundant because several populations occur inside protected areas.

Internationally, this species was listed as a threatened species (vulnerable) according to the IUCN red list of threatened animals (1996). It is not listed on any CITES appendix. In Thailand it is protected animal under the Wild Animal Protect Act of 1992.

### 2.3 Sexual dimorphism

Sexual dimorphism is a condition in which the males and females in a species are different in morphological traits such as coloration, size or other features. Presumably the dimorphism in some species reflects factors important in social interactions, survival, or reproduction (Bury, 1979).

Three major hypotheses have been proposed to explain sexual differences in organisms: 1) the female fecundity hypothesis: females are larger because larger body size is associated with increased number or size of eggs, 2) the competition avoidance hypothesis: differences in head and mouth size and differences in microhabitat usage result in decreased intersexual competition for resources, and 3) the sexual selection hypothesis: males are larger because large male size is favored in male-male disputes over breeding territories (Darwin, 1889; Slatkins, 1984; Shine, 1989, 1990).

Graham (1979) reviewed and provided a compilation of criteria for sex determination of selected turtle species as followings.

1. *Chelydra serpentina*: preanal tail length of male is greater than 86 % of posterior plastral lobe length while female is less than 86 %. Anal opening of male is posterior to rear carapacial margin while female is anterior.
2. *Sternotherus odoratus*: male has a small patch of scales on inner hindleg surface while female is relatively smooth. Male has longer and stouter tail ending in a blunt nail. Male has broader skin area between plastral seems.
3. *Clemmys insculpta*: preanal tail length of male is twice as long as that of female. Male has concave plastron and prominent forelimb scales while female has flat and reduced.
4. *Terrapene carolina*: male usually larger in plastron length and has red iris while female has yellow-brown. Posterior plastral lobe is concave in male and flattened in female. Male has longer and stouter tail and shorter, stronger and curve hindlimb nails.
5. *Malaclemys terrapin*: male is smaller with more point behind carapace. Anal opening of male is posterior to rear carapacial margin while female is anterior. Head shape of male is dorsal outline narrower and more pointed.
6. *Graptemys barbouri*: male is smaller with longer and thicker tail. Anal opening of male is posterior to rear carapacial margin while female is anterior.
7. *Chrysemys picta*: male is smaller in shell dimensions with elongated foreclaws. Anal opening of male is posterior to rear carapacial margin while female is anterior.

8. *Deirochelys reticularia*: male is smaller with longer and thicker tail. Anal opening of male is posterior to carapacial margin while female is anterior.
9. *Gopherus agassizi*: male is larger than female with longer gular projections and longer and thicker tail. Plastron of male is concave while female is flattened.
10. *Chelonia mydas*: male carapace is tapering more posteriorly while female is more rounded posteriorly. Rear plastral lobe of male is narrower. Tail of male is vertically prehensile, tipped with a heavy nail, and extends far beyond rear of carapace while female barely reaches rear edge of carapace.
11. *Trionyx spiniferus*: male seems to be smaller with longer and thicker tail with anal opening near tip. Carapace pattern of male retains the juvenile dark ocelli or white dots while female loses juvenile pattern and develops mottled lichenlike markings on the carapace.

Berry and Shine (1980) reviewed data on sexual size dimorphism, reproductive behavior, and habitat types in turtles and reported that patterns of sexual size dimorphism are correlated with habitat type and male mating strategy that:

1. In most terrestrial species, males engage in combat with each other. Males typically grow larger than females.
2. In semiaquatic and bottom-walking aquatic species, male combat is less common, but males often forcibly inseminate females. As in terrestrial species, males are usually larger than females.
3. In truly aquatic species, male combat and forcible insemination are rare. Instead, males utilize elaborate pre-coital displays, and female choice is highly important. Males are usually smaller than females.

In terms of sexual selection theory, males are larger than females when large male size evolves as an adaptation to increase success in male combat, or to enable forcible insemination of females. In contrast, males are usually smaller than females where small size in males evolves to increase mobility, or because selection for increased fecundity may result in increased female size (Berry and Shine, 1980).



Gibbons and Lovich (1990) investigated sexual dimorphism in turtles with emphasis on *Trachemys scripta* and found that females attain larger body sizes than males in all populations. Other reported sexually dimorphic traits are longer precloacal length, elongated foreclaws, longer snout and melanism in males.

Lambert (1993) studied sexual dimorphism of *Geochelone sulcata* in Mali, and found that sexual dimorphism is more strongly marked by gular extension which is according to males combat. There is a significant correlation between rear aperture diameter and carapace length in male, but not in female.

Dunaway (1994) investigated morphometric variations between male and female *Terrapene carolina* in Alabama, and indicated that the posterior portion of the carapace of females is higher than the anterior portion, while in males, the anterior portion is higher than the posterior portion.

Mushinsky, Wilson and McCoy (1994) studied growth and sexual dimorphism of *Gopherus polyphemus* in central florida and proposed that the best indicator of sex is the size, shape and depth of plastral concavity. While anal notch and anal width are also dimorphic but less distinct. This study also mentioned diminished sexual dimorphism due to rapid growth and abrupt attainment of sexual maturity in this population.

Lambert (1995) investigated sexual dimorphism of *Geochelone pardalis* in Somaliland and showed that males tend to be more elongated and slightly narrower carapace. Rear aperture diameter decreases exponentially in male but increases exponentially in female.

In *Amyda cartilaginea* males show longer heavier tails and seems to reach larger sizes than females. Smith (1931 cited in Meylan, Moll and van Dijk, 1995) wrote that the plastron is white in males and grey in females. While other sexually dimorphic characters have not been recorded (Wirot Nutaphand, 1979; van Dijk, 1992; Meylan, Moll and van Dijk, 1995).

#### 2.4 Annual reproductive cycle

General reproductive biology of turtle base on macro- and microscopic changes of gonad have been thoroughly reviewed by Moll (1979). In

temperate zone, patterns of reproductive cycle of males and females could be summarized as followings.

**Males:** Spermatogenesis begins in the spring, peaks in late summer, and ends in the fall as spermatozoa leave the testes to overwinter in epididymis. Spring is the peak period for breeding in most species, although fall and continuous breeding may also occur. Endocrine cells activity peaks during gametogenic quiescence, rapidly decreases as spermatogenesis is renewed, and is minimal at the peaks of spermiogenesis.

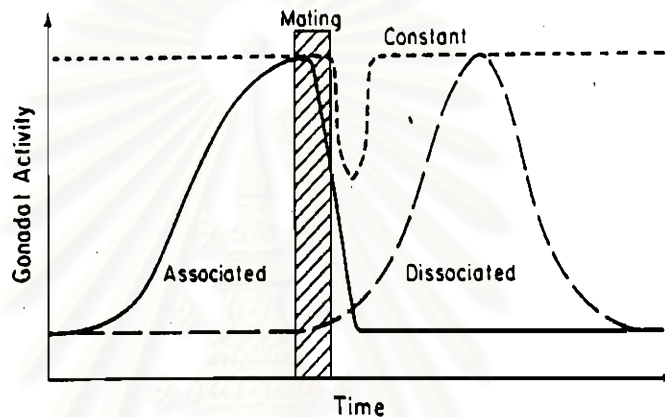
**Females:** Generally, females have an annual cycle but in some species cycles of 2 to 4 years are also evident. The female cycle consists of 4 phases including 1) follicular enlargement resulting from vitellogenesis begins in late summer or fall until completion of the second phase in spring; 2) ovulation and intra-uterine period; 3) nesting period - nesting seasons commonly fall between late April and late July but in lower latitude, nesting often begins earlier and extends over a greater period; and 4) latent period.

Reproductive cycle of turtles based on endocrine patterns have been reviewed by Licht (1982) with emphatic commentary that the reproductive cycle depends upon responses to pituitary hormones, and the gonads are also important endocrine organs secreting a series of steroid hormones. Hence detailed information on the nature and levels of circulating pituitary and sex hormones is critical for understanding the true functional status of the reproductive system and the mechanisms regulating reproductive cycles.

Endocrinology of reproductive cycle in reptiles have been reviewed in various aspects by many authors in the Tenth International Symposium on Comparative Endocrinology (Norris and Jones, 1987). The followings are some parts of their works.

Whittier and Crews (1987) reviewed patterns and control of seasonal reproductive cycle in reptiles and suggested that timing of reproduction in a population is determined by 1) when the most offspring survive and 2) when parents, often females, are capable of energetically supporting the production of viable young at the least cost to themselves. Not all aspects of reproduction, including gonadal growth, sex steroid hormone secretion and sexual behavior, are expressed at the same time. In most species that have been studied with respect to the environmental cues and physiological mechanisms influencing reproduction,

these three events are functionally associated. However, many species have a dissociated reproductive tactic in which gonadal activity and sexual behavior are expressed at different times. Other species exhibit a constant reproductive pattern in which gonads are maintained in a near steady state and sexual behavior is expressed in response to particular environmental cues. Diagram of the three aforementioned reproductive patterns is shown in figure 2-5.



**Figure 2-5** Three reproductive patterns exhibited in vertebrates (Whittier and Crews, 1987).

Many species of snakes and freshwater turtles exhibit dissociated reproductive cycles in which gonadal activity and sexual behavior peak at different times of year.

Lofts (1987) reviewed testicular function and stated that in male reptiles testosterone has been identified in the plasma of many species while androstenedione and epitestosterone have also been isolated from testicular tissue of some species. According to histochemical, radioimmunological and ultrastructural techniques the interstitial Leydig cells are identified as the main loci for androgen synthesis. A peak in plasma testosterone levels have been recorded before mating in turtles. In postnuptial species including turtles there is an uncoupling of the spring plasma testosterone peak from the spermatogenic cycle. Sertoli cells are suggested to be loci for intratubular steroids secretion. Furthermore, production of tubular androgens varies seasonally and shows maximum synthesis at times when there is also a high level of spermatogenic activity.

Chieffi and Pierantoni (1987) reviewed regulation of ovarian steroidogenesis and suggested that in female turtle, testosterone seems to play a role primarily as a precursor for estradiol synthesis, estrogen titers correlate well with vitellogenesis, while progesterone appears to have an important role in the ovulatory process.

Ho (1987) reviewed endocrinology of vitellogenesis and stated that estrogen is the primary stimulus to vitellogenesis in female reptiles including turtles.

Nagahama (1987) reviewed endocrine control of oocyte maturation and reported the involvement of progesterone in the process of oocyte maturation in female turtles.

Xavier (1987) reviewed functional morphology and regulation of the corpus luteum and showed that female reptiles exhibit fully developed corpora lutea and function as endocrine glands by secreting progesterone. In some turtles corpora lutea begin to regress before oviposition resulting in plasma progesterone rapidly declines to basal level after a preovulatory or ovulatory surge.

Moore (1987) reviewed regulation of reproductive behaviors and stated that in general testicular steroid hormones (testosterone, dihydrotestosterone and estradiol) activate male reproductive behaviors and ovarian hormones (estradiol and progesterone) activate female reproductive behaviors. Although there is some evolutionary conservatism in the control of reproductive behavior of vertebrates, species specificity is also evident in some reptiles.

Reproductive cycle of turtles in term of endocrinology have been studied in many species of turtles in the followings families.

Family Emydidae (freshwater turtle)

Callard et al. (1978) investigated annual ovarian cycle of *Chrysemys picta* and reported that the females display marked changes in three plasma steroids (testosterone, estradiol and progesterone) during the periovulatory period, increasing sharply prior to or around the time of ovulation during mid-May to mid-June, and falling sharply there after. There are also smaller peaks of testosterone and estradiol during fall period of ovarian recrudescence.

Lewis, Mahmoud and Klicka (1979) studied seasonal changes in plasma progesterone and estradiol in female *Chelydra serpentina* and showed that among four stages of annual ovarian cycle (hibernation, preovulation, postovulation and vitellogenesis) the highest progesterone level is in luteal stage in June, while estradiol is highest in May immediately before ovulation.

Licht, Breitenbach and Congdon (1985) studied seasonal cycle in testicular activity in *Chrysemys picta* under natural conditions and found that plasma testosterone levels exhibit biphasic cycle with peak in spring and fall. Plasma testosterone is lowest on the day that animals emerge from hibernation at the end of March, then increase rapidly to a transient peak that persist for 2 weeks. Plasma testosterone rise again in September before the onset of hibernation and shortly after the late summer peak in spermatogenic recrudescence.

Mahmoud et al. (1985) investigated ultrastructural changes in testes of *Chelydra serpentina* in relation to plasma testosterone and reported that testosterone level is highest in May and October (mating) and relatively low during the rest of the year. Leydig cells are found to be potentially active throughout the year while Sertoli cells are active only during spermatogenesis from May to October. It was suggested that testosterone of Leydig cells origin is concerned mainly with mating behavior and that of Sertoli cells origin with spermatogenesis and maturation of sperm.

Mahmoud and Licht (1997) studied seasonal gonadal cycle in natural population of *Chelydra serpentina* and reported that in the females testosterone, estradiol and progesterone are highly correlated with follicular growth and vitellogenesis. There is a significant increase in estradiol and progesterone as the ovulation occur. In the males testosterone is significantly correlated with testicular growth and spermiation. Courtship and mating behavior are observed in spring, summer and fall.

#### Family Testudinidae (tortoise)

Casares et al. (1993) measured faecal steroids in *Geochelone elephantopus*, *G. gigantea*, *Testudo graeca*, and *T. hermanni* and found that from August to September, estrone, estradiol and testosterone rise simultaneously in 3 of 6 *Testudo* spp males, while estrone increases during the follicular development in 4



of 6 *Testudo* spp females and 2 of these show simultaneous surges of both estrone and estradiol. From March to May, estrone and testosterone increase in the 4 *Geochelone* spp females. While estradiol surges in 2 *G. gigantea* females between April and May and 1 animal shows a further surge in October.

Rostal et al. (1994) studied seasonal reproductive cycle of *Gopherus agassizii* under semi-natural conditions and reported that male tortoises display a significant rise in plasma testosterone during the summer from May to August which continue into the fall mating period, then decline prior to hibernation. Upon emergence from hibernation in April testosterone levels are significant reduced during the spring mating period (April to May). Female tortoises also show significant rise in testosterone from July to October prior to hibernation. This increase coincides with the onset of fall mating period. The female testosterone levels are highest following emergence from hibernation prior to ovulation in April and May.

#### Family Cheloniidae (sea turtle):

Licht et al. (1979) investigated serum steroids associated with breeding activity in captive *Chelonia mydas* and found that in the males, androgen (testosterone and dihydrotestosterone) levels are lower during mating than in the prebreeding season. In the females, estrogen (estradiol and estrone) levels drop while testosterone peaks in the mating season and boths remain relatively low throughout the nesting season, while progesterone continue to rise progressively during the prebreeding and mating season and upto the time of nesting.

Licht, Rainey and Clifton (1980) studied serum steroids associated with breeding activities in natural populations of *Chelonia mydas* and showed that the mating males display high testosterone level but not much differ from prebreeding males. In the females, there is no different in testosterone level between mating and nesting females but the level drop significantly in the first day following nesting. Progesterone is also similar between mating and nesting females but shows an elevation in 1-2 days postnesting.

Licht et al. (1982) studied changes in steroids in female *Lepidochelys olivacea* and found that progesterone levels markedly increase within a day after

oviposition and then return to near baseline level within 2 to 3 days, while testosterone and estradiol levels show little changes in periovulatory period.

Licht, Wood and Wood (1985) investigated annual and diurnal cycles in plasma testosterone in captive male *Chelonia mydas* and found that testosterone is at nadir in September to November and increase to a peak in April, then begin to decline coincidently with the onset of mating behavior which is peaked in May-June. Spermatogenesis and androgen secretion are not uncoupled hence suggesting that *C. mydas* does not exhibit postnuptial testicular cycle which is typical in many temperate zone turtle. Furthermore it was found that plasma hormones are relative stable over a 24 hours period.

Wibbels (1990) studied seasonal changes in gonadal steroids in *Caretta caretta* and reported that the males show a prenuptial spermatogenic cycle coincident with increased concentrations of testosterone which is high during migration and mating period. In the females, serum estradiol levels increase approximately 4-6 weeks before migration to mating and nesting area suggesting a period of increased vitellogenesis, and then decrease 1-2 weeks prior to migration. Serum testosterone, estradiol and progesterone are elevated during nesting if turtles will nest again, while during the last nesting all steroids are low.

Guillette et al. (1991) investigated changes in plasma estradiol and progesterone during natural oviposition in *Caretta caretta* and reported that plasma steroids concentrations do not vary significantly during nesting with mean concentration of estradiol at 255 pg/ml and progesterone at 395 pg/ml.

Wibbels et al. (1992) studied levels of serum steroids in natural populations of *Chelonia mydas* and *Caretta caretta* and found that after nesting serum progesterone peak within 20 to 50 hours while testosterone decline and estradiol concentrations show no significant changes. The dynamics of progesterone and testosterone concentrations are consistent with the hypothesis that these hormones facilitate specific physiological events during ovulation and egg production.

Whittier, Corrie and Limpus (1997) investigated plasma steroids profiles in nesting *Caretta caretta* and showed that circulating levels of estradiol are mostly undetectable while testosterone and progesterone profiles in the nesting

females are associated with the individual turtles' progression through successive nesting episode, with a marked decline in all steroids by the last nesting episode of the season.

Rostal et al. (1997) studied nesting physiology of *Lepidochelys kempfi* and reported that female serum testosterone and estradiol decline over the course of nesting season while progesterone levels do not fluctuate over the course of nesting season. It was found that serum testosterone is a useful prediction of nesting periodicity in *Lepidochelys kempfi*.

Rostal et al. (1998) investigated seasonal reproductive cycle of *Lepidochelys kempfi* under semi-natural conditions and reported that the males show a pre-nuptial rise in serum testosterone 4 to 5 months before mating period then sharply decline during mating period in March. The females also show pre-nuptial rise in serum testosterone and estradiol 4-6 months prior to mating period and decline during the mating period in April to July. The elevated estradiol correspond with the period of vitellogenesis. It was suggested that *Lepidochelys kempfi* displays a distinct seasonal reproductive cycle in captivity.

#### Family Dermochelyidae (leatherback turtle)

Rostal et al. (1996) investigated reproductive physiology of female *Dermochelys coriacea* and reported that plasma testosterone and estradiol levels correlate well with reproductive conditions while there is no correlation between plasma progesterone level and reproductive conditions. Testosterone declines from 2245 pg/ml at the beginning of nesting cycle to 318 pg/ml at the end of the nesting cycle while estradiol decline from 53.30 pg/ml to 16.50 pg/ml in a similar manner. Endocrine and ovarian patterns of *Dermochelys coriacea* are similar to sea turtle of the family Cheloniidae.

#### Family Trionychidae (softshell turtle)

Licht (1982) studied reproductive cycle of male *Trionyx sinensis* (or *Pelodiscus sinensis* at present) and reported that testosterone is undetectable through most of the year (especially in spring when Leydig cells appear active and breeding is presumed to occur), and plasma testosterone only increases for a brief period in late summer after testes are fully recrudesced.

Sarkar et al. (1996) investigated photothermal effects on ovarian growth and function of *Lissemys punctata punctata*. Although this study was not an direct investigation for seasonal reproductive cycle, it provided experimental data that high temperature have a triggering role on ovarian growth and secretion of estrogen at the early preparatory phase, but once the ovarian function sets in, high temperature seems to have a regressive rather than stimulatory effect on ovary.

In *Amyda cartilaginea*, only general reproductive biology data are available. Wirot Nutaphand (1979) reported that under favorable conditions females can mature in 20 months, they will lay three or four clutches per year, clutch size varying from 7 to 30 eggs. The number of eggs per clutch increase from 6-10 in smaller females to 20-30 in larger ones.

The nesting season of *A. carlilaginea* coincides with the dry and hot seasons in Thailand, from February to July with a peak in March and April. The nesting occurs in the late afternoon or evening, in damp sandy area close to the water. (Sujin Nukwan, Panu Tavarutmaneegul and Anusin Inkuan, 1995; Wichase Khonsue, 1993; Wachira Kitimasak, 1996; Wirot Nutaphand, 1979). The incubation period can be up to 135-140 days, according to Bourret (1941 cited in Meylan, Moll and van Dijk, 1995). Wachira Kitimasak (1996) found that at room temperature in Bangkok hatchling period ranged between 74 and 95 days.

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