CHAPTER III

MATERIALS AND METHODS

Specimen collection

Specimens of *M. subtrijuga* were collected in August, 1992 at the start of the breeding season. Thirty male and thirty female turtles were collected randomly in rice fields at Rangsit area, Pathum Thani Province. Among turtles collected were both subadults and adults. Adult and subadult can be separated based on the complete development of testis and ovary (Figure 3) and may be distinguished by the reddish brown color of the shell. Very young turtles whose sex could not be identified were excluded from the study. Eggs of *M. subtrijuga* were collected randomly during December 1991 and January 1992 at Tharang District, Phetchaburi Province. Both study areas are located in the central plain of Thailand and have lowland water bodies, such as ponds, canals, wet rice fields and swamps, which are the species's favoured habitat (Figure 4a-b).

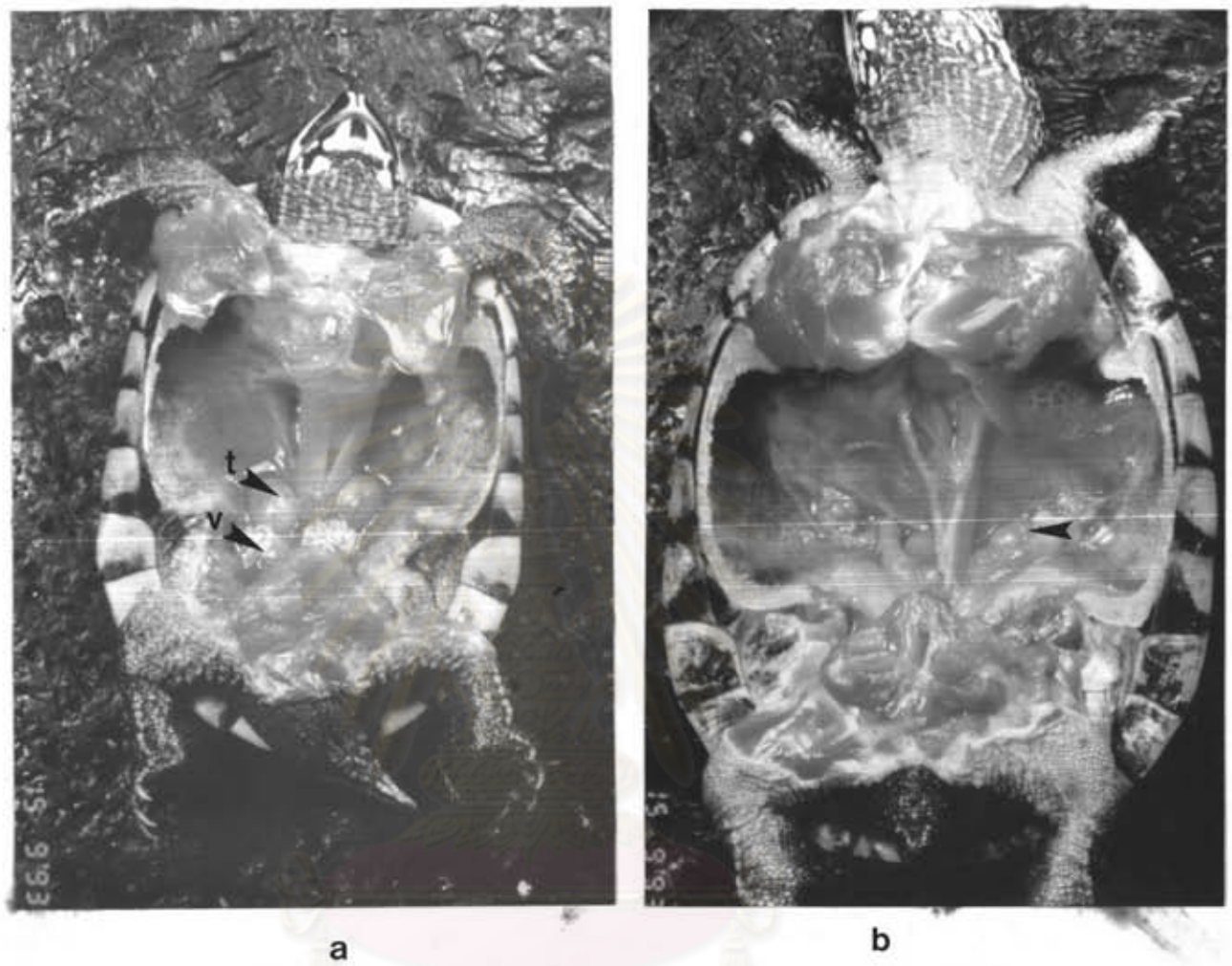

Figure 3. Sex organs of *M. subtrijuga*: (a) testes [t] and vas deferens [v] in adult male (b) ovaries in adult female

a ➤

b ➤

Figure 4. Habitat of *M. subtrijuga*: (a) Rangsit area, showing wet rice paddies after rice harvesting (b) Tharang district, showing planted rice paddies, and a small irrigation waterway.

Shell morphology and tail width

Measurements of external morphology of collected turtle samples were made on the carapace length, carapace width, plastron length, height, tail width. Plastron midline length of the gular, humeral, pectoral, abdominal, femoral and anal scutes were measured (Figure 5-7).

Sexual dimorphism

Comparisons to see whether the collected turtle samples were sexually dimorphic were studied by using methods as follows.

1. Measured means of shell morphology and tail width of adult males (n=14) and adult females (n=25).

2. Determined mean ratios carapace length with characters of shell morphology and tail width between males (n=30) and females (n=30).

3. Regression analysis performed for : carapace length equals independent variable, shell morphology and tail width equal dependent variables between males (n=30) and females (n=30).

T-tests were performed to test the differences between sexes on the above comparisons.

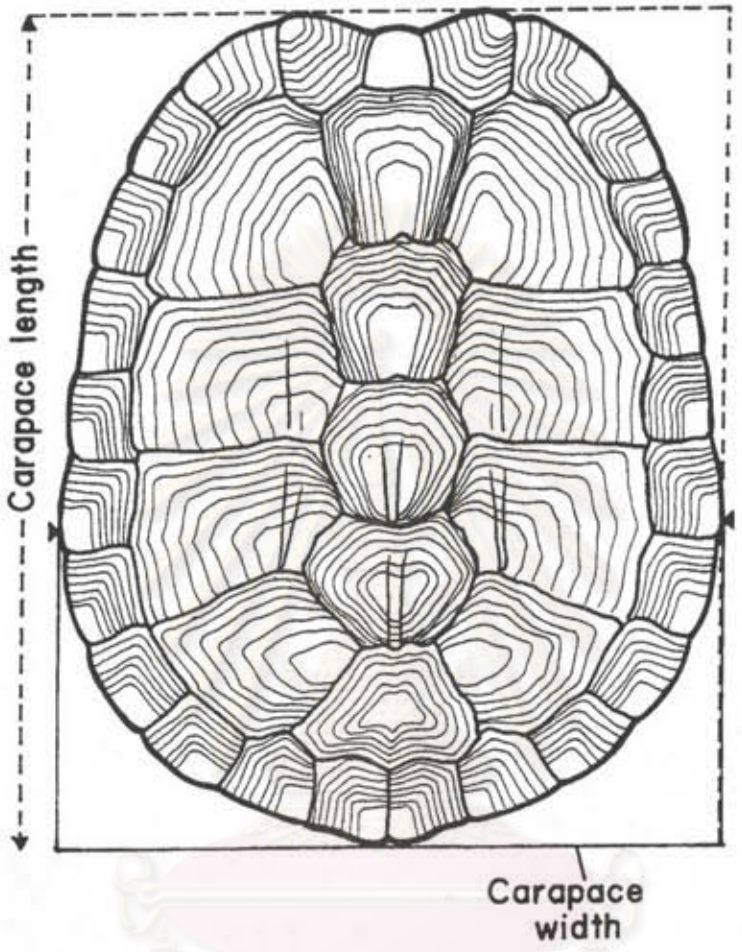

Figure 5. Carapace length and carapace width of *M. subtrijuga* (Drawing from specimen no. CUMZ (R) 1994-03-22,3)

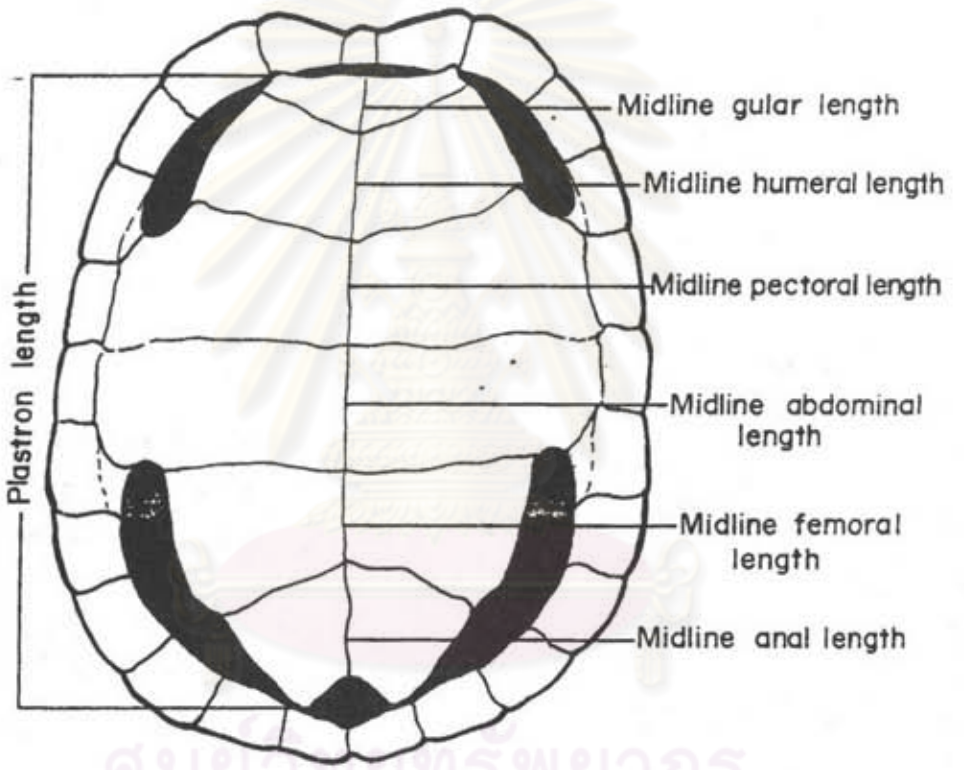

Figure 6. Plastron length and midline length of plastron scutes (gular, humeral, pectoral, abdominal, femoral and anal) of *M. subtrijuga* (Drawing from specimen no. CUMZ (R) 1994-03-22,3)

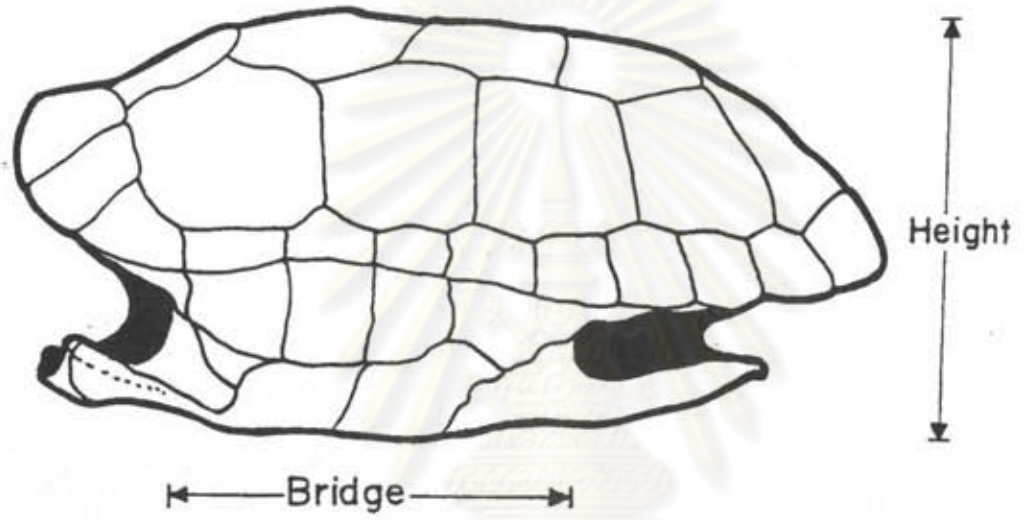

Figure 7. Height and bridge of *M. subtrijuga* shell (Drawing from specimen no. CUMZ (R) 1994-03-22,3)

Growth rate

Growth rate was estimated by modifying the summation method proposed by Cagle (1946). By this method, plastron midline length of each age class was estimated by combining the median border lengths of growth rings formed in the same year. Carapace length at each year was estimated from the constructed plastron midline length and carapace length using linear regression analysis. In this study, the relationship of plastron midline length and carapace length of males (n=20) and females (n=20) was seperately determined (Figure 8).

Estimation of age by counting the number of growth rings was based on 3 assumptions (Zug, 1991). First, that the scute growth has a regular, cyclic pattern and each major cycle produces a distinct and visible mark (one annulus growth ring per year). Second, that the cyclic growth pattern is associated with regular climatic events, therefore each scute mark represents a specific interval of time. It is also assumed that only one growth cycle occurs in one year. Finally, it assumed that the scute marks remain visible through the life of the animal, or if not, the number of marks lost can be estimated accurately. In this study, twenty turtle samples of each sex met the assumtions and were used to study the growth rate. Since growth rings after 5 years of *M. subtrijuga* are not clear and are sometimes invisible, only growth rings from hatchling year to 5 years old were compared in this study.

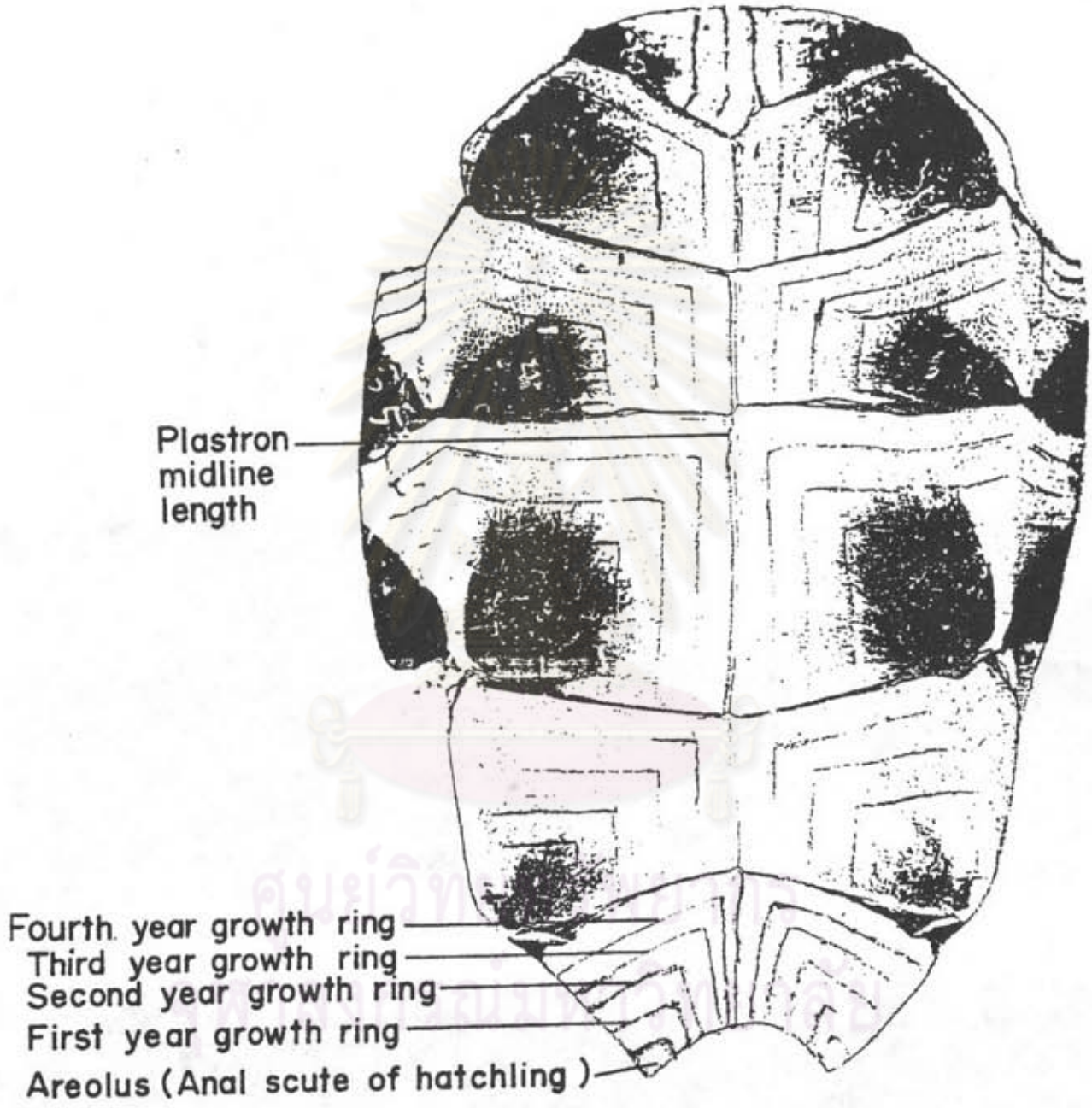

Figure 8. Plastron of *M. subtrijuga* (Female, CUMZ (R) 1994-08-25,1, 175 mm CL), showing plastron midline length and growth rings on the scutes.

Diets

Diets of *M. subtrijuga* were investigated by analysis of the contents left in the guts of only 5 males and 5 females turtles, since many of the collected samples had empty guts. The contents were removed from the digestive tracts and preserved in 70% alcohol for later identification.

Reproductive biology

In this study, reproductive biology includes the following aspects : clutch and egg size, incubation period and hatching success, and hatchling survival rate.

1. Clutch size and egg size

Field surveys during nesting time were conducted in an area of about two square kilometers at Tharang distrinct, Phetchaburi Province, with assistance from villagers (Figure 9 a-f). Clutch size was determined by counting the number of eggs found in a nest (Figure 10 a-d). Eggs were removed from the nests and transported to the laboratory at the Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok and were measured using a vernier caliper and an electronic balance. The differences between means of egg length and egg weight from different clutches were analyzed using ANOVA.

Figure 9. Photographs of egg collection at Tharang district, showing: (a) searching for egg nets (b) egg nest (c) opening of an egg nest (d) soil lid (e) portion of eggs in a nest (f) taking eggs from a nest

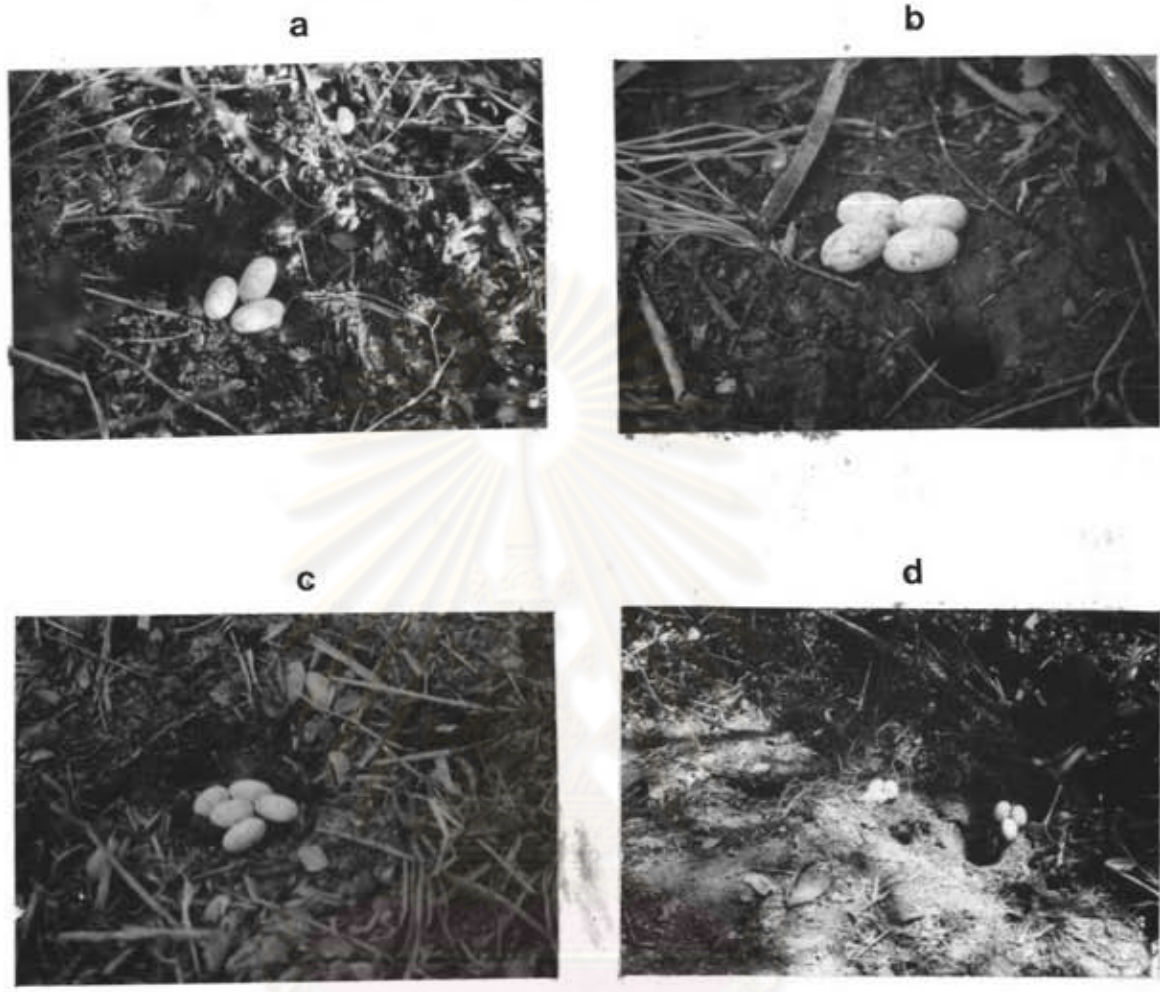

Figure 10. Photographs (a-d): showing number of eggs, ranging from 3-6 eggs per clutch

2. Incubation periods and hatching success

Incubation periods and hatching success were studied under laboratory conditions at the Department of Biology, Faculty of Science, Chulalongkorn University (Figure 11 a-c). Eggs from each clutch were incubated in plastic boxes. A substrate medium was made of a moist mixture of sand and coconut-husk-fluff in 1:1 proportion. Eggs were buried completely in the medium. The boxes were covered with plastic lids to maintain humidity. All eggs were weighed once a week for the first 3 weeks to assess their weights. Air temperature and relative humidity in the containers were recorded every day (Figure 12 a-c). The difference between incubation periods and between hatching success with different clutch sizes were analyzed using ANOVA.

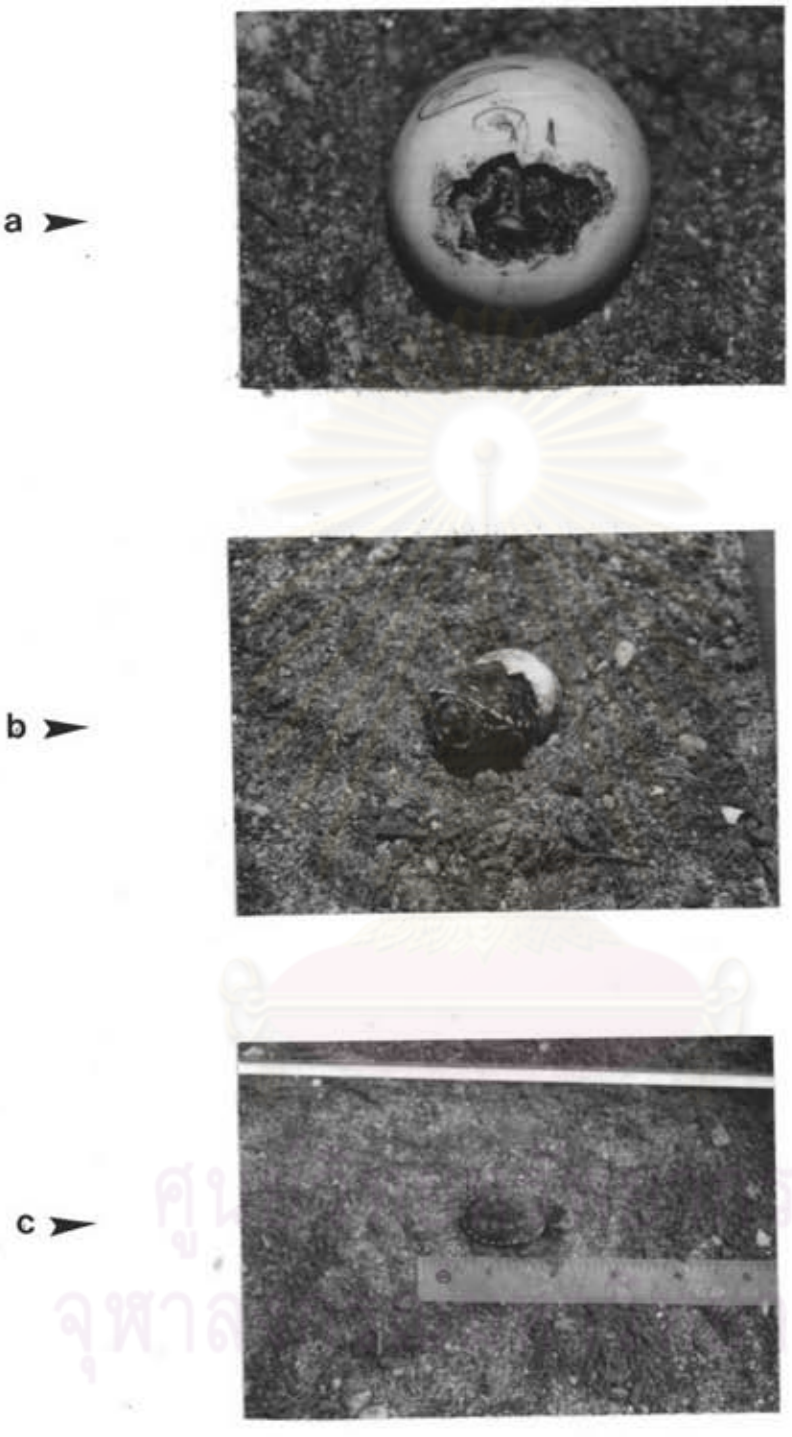

Figure 11. A newborn of *M. subtrijuga*: (a and b) emerging from egg shell (c) size of a hatchling

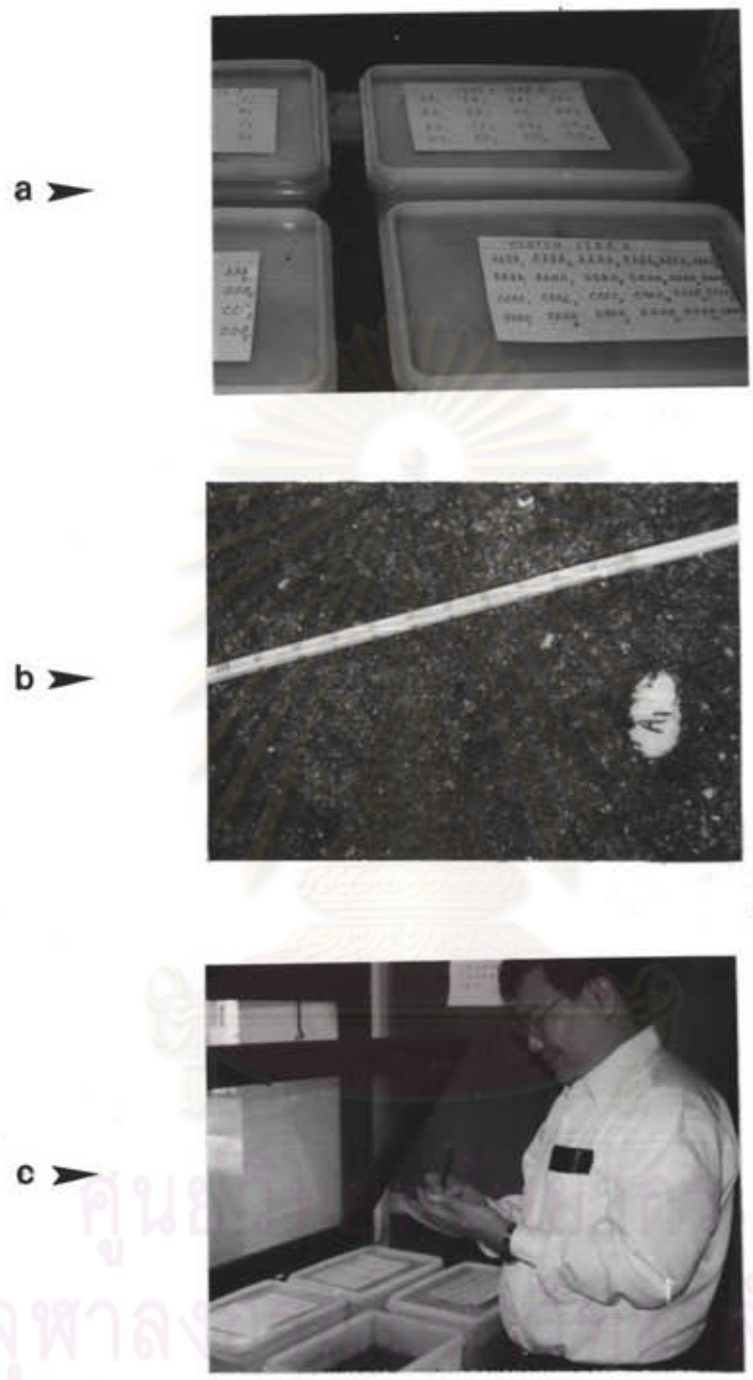

Figure 12. Incubation technique: (a) Eggs were incubated in plastic boxes. (b) Marked eggs were covered in the medium. (c) Air temperatures in the plastic boxes were recorded.

3. Hatchling survival rate

Survival rate of hatchlings in the laboratory was observed for 5 months after hatching. During observation, freshwater snails (*Filopaludina sumatrensis* and *Brotia costula*), small shrimp (*Macrobrachium lanchesteri*) and, small fish (*Oreochromis nilotica*) were provided for feeding (Figure 13).

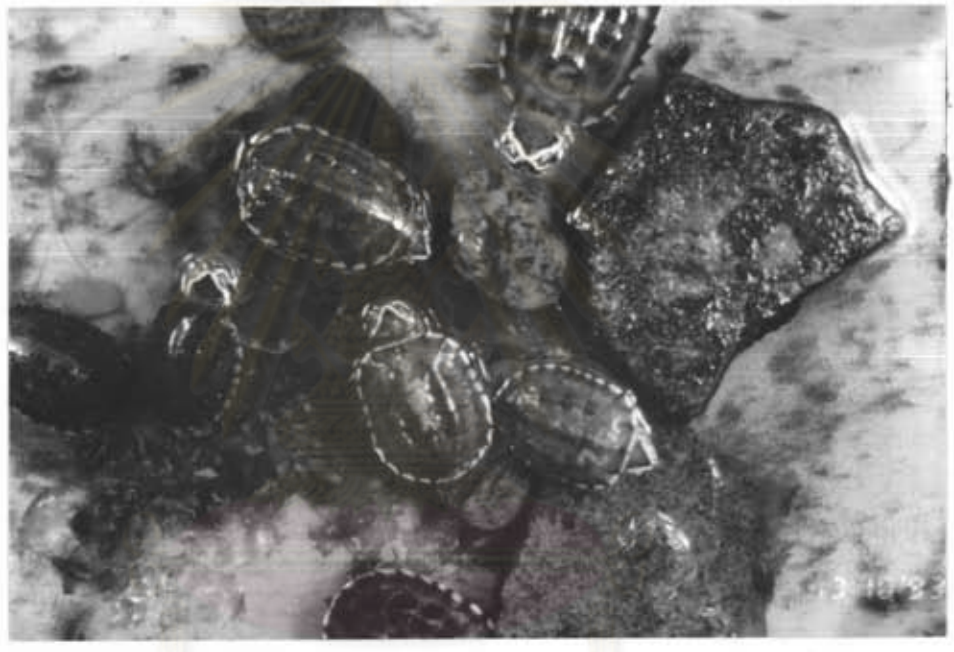

Figure 13. Hatchlings were numbered and put together in a turtorium provided with natural diets.

Parasites

Parasites of *M. subtrijuga* were collected from the same (5 males and 5 females) that were used in the analysis of stomach contents (Figure 14). Ectoparasites from the skin of head, neck, legs, shell, and the rest of the body. Endoparasites from the guts were collected. Specimens were preserved in 70% alcohol and were identified under stereomicroscope at the Department of Parasitology, Faculty of Public Health, Mahidol University.

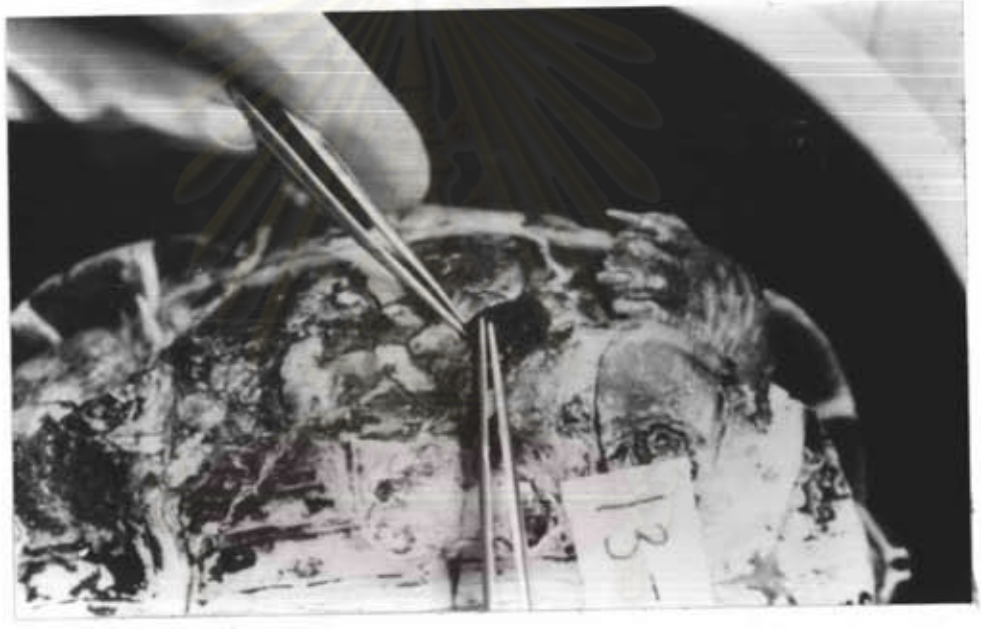

Figure 14. Collecting ectoparasites from the plastron of *M. subtrijuga*