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# ARTIFICIAL INCUBATION AND EMBRYONIC DEVELOPMENT OF OLIVE RIDLEY TURTLE EGGS (*Lepidochelys olivacea* Eschscholtz)

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## ABSTRACT

Studies on artificial incubation and embryonic development of olive ridley turtle took place at Phuket Marine Biological Center during 1980-1985. The eggs of ridley turtle (*Lepidochelys olivacea*) were collected from the nesting beaches. Using the styrofoam box incubation technique the eggs were incubated at atmospheric temperature conditions. The embryonic development of eggs was observed by removing eggs from the nest. The different stages of developing embryos were recorded daily under a microscope. After 10 days incubation, head and eyes were prominent. The carapace was distinguished on the 23rd day, the complete characters were developed after 35 days. The hatchling was 38.6 mm in carapace length, 33.9 mm in carapace width and about 16.8 g in weight. The hatching success in styrofoam box incubation was 69.7%-83.2%, the incubation period was 58 - 64 days at an average temperature of 26.6 - 30°C. The hatching success in styrofoam boxes was not significantly different from hatching of eggs reburied in sand of the nesting beach (hatch rate was 58.0% - 83.9%).

## INTRODUCTION

The west coast of Thailand constitutes one of the most important remaining sea turtle nesting grounds. The olive ridley turtle (*Lepidochelys olivacea*) is the most abundant species while green turtle, hawksbill turtle and leatherback turtle occur occasionally. Unfortunately the population of sea turtle in Thailand has declined markedly. The decline is due to the over-harvest of eggs, the incidental capture by fishing gear and the intentional hunting for meat or carapace. In recent years, fishing restrictions in near shore have been enforced by the Department of Fisheries, and protection of turtle eggs has been increased by authorities patrolling nesting beaches during nesting seasons. The Southern part of Thailand, especially Phuket Province, is a tourist area and sea turtle eggs have become one of the favourite food items of foreign tourists. Sea turtle eggs fetch high prices, so the poachers have become a big problem for conservation of sea turtle in southern Thailand. To solve this problem, the nesting beaches have been patrolled and protected against poachers, eggs have been collected and reburied in safer places.

Recently, artificial incubation of sea turtle eggs has been widely used. Incubation in styrofoam boxes offer better protection against predators and poachers than leaving eggs in sand. Even wire netting around the nest cannot prevent natural predation or destruction from taking place. Occasionally nets are penetrated by borrowing animals, or the nest may be destroyed by unexpected heavy rain and be washed away, including eggs deposited in the sand. The hatching rates of eggs incubated in styrofoam boxes are good. Comparisons have been made with Atlantic olive ridley in Mexico and the results showed 10 % improvement over eggs left in sand (Marquez, 1978). Experiments with styrofoam box incubation with Kemp's ridley turtle in Mexico gave excellent result: about 95% hatching success (Woody, 1981). In Surinam, hatching success of green turtle in styrofoam boxes was 80-92% which is similar to the best hatching (85%) obtained from various samples left in sand. Leatherback clutches in styrofoam boxes hatched with a success of 54% while the best result in sand was 50% (Schulz, 1975). Other methods in artificial incubation encompass vermiculite and sponge as moisture control instead of sand. The results were satisfactory with good hatching rates (at temperature 29.25 °C) and an average hatching of 82 % (McLean *et. al.*, 1983). The incubation time of green sea turtle in styrofoam boxes displayed variation from 45 to 60 days with a mean of 52 days (Simon, 1975). Comparisons of hatching success showed that hatching rates in polystyrene incubators are higher than eggs reburied in other beach sites (Wyneken, *et. al.*, 1988).

The embryonic development has been studied with loggerhead turtle and green turtle. During artificial incubation, sea turtle eggs can be removed immediately after oviposition (or within 3 hours) without harm (Miller and Limpus, 1983). In reptiles, the embryo becomes attached to the inside of the egg shell at an early development stage. If the egg is turned over, the embryo will have the yolk resting on top of it and no more development will take place (Bustard, 1973). Embryonic development in loggerhead turtles has been divided into thirty-one stages, with the first six occurring preovipositional. The last seven to thirty-one were post-ovipositional stages (Dodd, 1988 and LeBuff, 1990). The development of embryos in green turtle has been studied long time ago, with five different stages reported (Parker, 1880). In Thailand, the artificial incubation has been done by removing eggs to rebury them in a safer place. The styrofoam incubation method has scarcely been used. But studies of incubation and development of eggs have been conducted in nature. By observing the green sea turtle eggs at Kram Island, Chonburi Province many stages of embryonic development were reported. The hatching averaged 70% with an average incubation period of 52 days (Penyapol, 1957). The success of natural hatching of olive ridley turtle along the west coast of Thailand averaged 70-80% (Phasuk and Rongmaungsart, 1973). In the Gulf of Thailand, hatching success of hawksbill turtle eggs in boxes was 83-96% (58-60 days) while hatching was 82-87% when incubated in sand. The development of embryos in the boxes were lower than those left in sand (Ganjanamavint and Rongmaungsart, 1987).

On most nesting beaches of southern Thailand, it is impossible to leave the eggs incubated in nature because too many people living nearby. Hence the styrofoam method of incubation is suitable because it is inexpensive, the hatching success is good, and it is easier to put the eggs in a styrofoam box than to remove all eggs and dig another hole in the sand. Because of lack of information about reproductive biology of olive ridley turtle in Thailand, studies of artificial incubation of olive ridley turtle eggs have been conducted by removing the eggs, reburying them in a safe place and comparing the result with the styrofoam incubation method. Observation of hatching and development of eggs were conducted at Phuket Marine Biological Center during nesting season from 1980 to 1985. The purpose of this study is to increase the knowledge of reproductive biology of sea turtle in order to improve the culture technique in hatcheries with a view to support efficient conservation in future.

## MATERIALS AND METHODS

### The move and rebury method

Artificial incubation of olive ridley turtle (*Lepidochelys olivacea*) eggs was observed during the nesting season 1980-1982. Olive ridley turtle eggs were sampled at random from three nesting beaches: Ko Phrathong, Thaimaung beach and Niyang beach.

In 1980, the total number of eggs was 3137 in 29 nests collected at Thaimaung and 1856 eggs in 18 nests collected from Niyang beach.

In 1981, 2771 eggs in 26 nests were collected from Ko Phrathong nesting area, 4574 eggs in 44 nests were collected from Thaimaung beach and 2929 eggs in 28 nests from Niyang beach.

In 1982, a total of 1100 eggs in 10 nests were collected from Ko Phrathong beach, 58 nests with 6020 eggs from Thaimaung beach and 14 nests with 1480 eggs from Niyang beach.

The eggs were removed from original nests and reburied in safer places within the same beach. Eggs were carefully placed into holes dug in the sandy beaches. They were placed in a pile like in the original nest, then the nests were covered with sand.

The hatcheries were surrounded with nylon net to protect eggs from the predators and unauthorized collection of hatchlings. The incubation processes were carefully observed.

**Styrofoam box incubation method**

The styrofoam box incubation method has been used for comparison of the hatching success of *Lepidochelys olivacea* in Thailand. A total number of 631 olive ridley turtle eggs were collected from Niyang beach and separated into six boxes for incubation in 1980. In 1981, 471 eggs were incubated in 8 boxes. In 1982, 393 eggs in 8 boxes.

Styrofoam boxes measuring 30x50x30 cm were used. The bottoms of the boxes were covered with about five centimetre of moist sand, preferably from the beach. 50 to 100 eggs were stacked in the middle of the box, which was filled with sand. The covering of sand prevents dehydration of the uppermost eggs and keeps them warm, especially at night. The surface sand was moistened when necessary by spraying with fresh water or seawater, normally once a week. Moderate variation of moisture does not influence development of the eggs, according to Schulz (1975). He reported that sea turtle eggs incubated at a moisture from 2-8% showed no significant difference in hatching success. The temperature in clutches was measured by thrusting thermometers vertically into the sand until the egg clutches were reached. They were left in situ. Recording of sand temperature was carried out four times daily at about 6.00 am, 12.00 am, 06.00 pm and 12.00 pm, to make sure that the measurements would cover the range of temperature change within 24 hours.

In order to compare the hatching success of both incubation methods (move and rebury incubation and styrofoam box incubation), the statistical ANOVA was applied.

**Embryological study**

Embryological studies were carried out in connection with the styrofoam incubation method. Olive ridley turtle eggs were collected and transported to the Phuket Marine Biological Center laboratory. Unhatched eggs were taken carefully out of the incubating boxes every day and examined for the presence of embryos. Embryos at various stages were collected from the nests and preserved in 10 % formalin. Measurements, drawings and photographs were made under a dissecting microscope at 6-50x magnification.

## RESULTS

**Hatching successes**

The hatching success of reburied eggs varied from 58 - 85.7% for three years experiments. The results are shown in Table 1. Total number of eggs incubated in 1980 were 4993, of which 3678 hatched. The average hatching period was 52 days and the average hatch success 73.66%. In 1981, 10,274 olive ridley turtle eggs were collected. Of these 7424 hatched. Incubation period averaged 56 days and hatching success was 72.26%. In 1982, the hatching success was 81.9% (range from 80.5-85.7%): 7295 hatchlings from 8906 incubated eggs and an average incubation time of 55 days (range 52-58 days).

Results of the styrofoam box incubation are shown in Table 2. Number of eggs per box ranged from 100 to 115 in a total of six boxes in 1980. The average hatching success was 69.7% (range from 50 to 85.2 %) with a mean incubation period of 64 days. The temperature at the nest clutches measured during incubation periods, averaged 26.6° C.

In 1981, the number of eggs was reduced to 50 - 65 eggs per box. The average hatching success was 82.2% (range 76.9-93.4%) with a mean incubation period of 60 days at an average temperature of 29° C.

A total of 326 hatchlings developed from 393 eggs incubated in styrofoam boxes in 1982. The average hatching success was 83.2% (range from 62 -95.35%), average hatching period was 58 days and average temperature was 30.1° C.

**Table 1.** The move and rebury incubation method of olive ridley turtle eggs.

| Year | Nesting area | No. of eggs | No. of hatchlings | Hatch period (average) | % hatched |
|---|---|---|---|---|---|
| 1980 | Ko Phrathong | - | - | - | - |
| | Thaimaung | 3137 | 2346 | 52 | 74.8 |
| | Niyang | 1856 | 1332 | 52 | 71.8 |
| 1981 | Ko Phrathong | 2771 | 2040 | 52 | 73.5 |
| | Thaimaung | 4574 | 2895 | 55 | 63.3 |
| | Niyang | 2929 | 2489 | 60 | 58.0 |
| 1982 | Ko Phrathong | 1100 | 911 | 52 | 83.9 |
| | Thaimaung | 6020 | 4961 | 57 | 82.4 |
| | Niyang | 1480 | 1191 | 55 | 80.5 |

**Table 2**. Olive ridley turtle eggs incubated in styrofoam boxes.

| Year | No. of boxes | No. of eggs | No. of hatchlings | Hatch period (average) | % hatched | Average temperature |
|---|---|---|---|---|---|---|
| 1980 | 1 | 100 | 50 | 64 | 50.0 | 27.0 |
| | 2 | 101 | 56 | 66 | 55.4 | 27.0 |
| | 3 | 105 | 84 | 65 | 80.0 | 26.7 |
| | 4 | 105 | 67 | 64 | 63.8 | 26.7 |
| | 5 | 105 | 85 | 61 | 81.0 | 25.9 |
| | 6 | 105 | 98 | 63 | 85.2 | 26.5 |
| 1981 | 1 | 65 | 59 | 54 | 90.8 | 29.3 |
| | 2 | 65 | 54 | 59 | 67.7 | 29.4 |
| | 3 | 61 | 57 | 60 | 93.4 | 28.8 |
| | 4 | 55 | 43 | 60 | 78.2 | 28.9 |
| | 5 | 50 | 34 | 61 | 68.0 | 28.9 |
| | 6 | 65 | 50 | 61 | 76.9 | 28.8 |
| | 7 | 55 | 50 | 61 | 90.9 | 29.3 |
| | 8 | 55 | 50 | 61 | 90.9 | 28.8 |
| 1982 | 1 | 50 | 47 | 62 | 94.0 | 29.2 |
| | 2 | 43 | 41 | 62 | 95.4 | 29.2 |
| | 3 | 50 | 40 | 70 | 80.0 | 29.0 |
| | 4 | 50 | 47 | 62 | 94.0 | 29.2 |
| | 5 | 50 | 42 | 55 | 84.0 | 30.5 |
| | 6 | 50 | 40 | 46 | 80.0 | 31.3 |
| | 7 | 50 | 31 | 55 | 62.0 | 31.0 |
| | 8 | 50 | 38 | 54 | 76.0 | 31.3 |

The hatching success with styrofoam box incubation of the three years showed nonsignificant difference between locations and among years (two way ANOVA, $P > 0.05$).

The hatch success in reburied incubation from three locations and three years observation showed nonsignificant difference between three locations ($P > 0.05$), but significant difference between the years ($P < 0.05$).

Comparing between styrofoam box and reburied incubation, the results from 25 nests of olive ridley turtle eggs (9 from Ko Phrathong, 9 from Thaimaung and 7 from Niyang observation in 1982) were considered to represent the reburied incubation method. The results showed nonsignificant difference between these two methods ($P > 0.05$).

**Embryonic development**

When the fertilized egg develops to the oviposition stage, the embryonic disk containing the gastrula and nearby membrane begins to rise to the upper part of egg. Then it adheres to the membrane which lines the inner shell wall. The yolk producing membrane is separated from the yolk, and embryo from the albumen. When adhesion to the shell membrane has occurred, a white spot develops on the top of the egg at the location where the membrane has adhered. After completion of this process the egg colour becomes chalky white. Embryonic development in olive ridley from artificial hatching can be described as follows:

**Age 12 days** : Head-fold with prominent optic vesicles, lens visible, beating of heart in abdomen portion, columella auris distinct (Fig. 1).

**Age 15 days** : More than thirty vertebrals visible, columella auris clear, eyes with big lens, pharyngeal cleavage evident, maxillo-palatine fold is seen, heart beating, small limbs form buds, tail process extends beyond base of hindlimbs (Fig. 2).

**Age 20 days**: Vertebrals difficult to see, limb buds forming prominent buds, pharyngeal clefts obscured, oral space is wider, tail long and curled, at this stage not so much developed as at the age of 15 days (Fig. 3).

**Age 23 day** : The embryo is fairly well developed, carapace evident as a marginal ridge, inframarginal invisible, pharyngeal clefts closing, limbs now free, elongate, thick fins separated from body, fins without pigment, tail longer than hindlimbs, brain growing and swelling to lobes, the eyeballs very large. The head and tail curled round for sustaining yolk. The body divided into four regions namely, head, neck, trunk and tail (Fig. 4).

**Age 25 days** : Carapace visible with scales obscured, inframarginal scales poorly developed, limbs well developed with 2 digital ridges visible, fore flippers growing into angles, eyeballs large with well developed eyelids which cover eyeballs, columella auris less prominent. Snout stronger and the lower jaw almost completely developed (Fig. 5).

**Age 27 days**: The embryo similar to 25 days, annular eyelid more distinct and covers more of the eyeball. Eyes and carapace slightly pigmented, claws on first digit. Plastron scutes recognizable.

**Age 30 days**: Carapace well developed with prominent, pigmented plastron scutes. Head bone prominent, columella auris almost disappeared. Annular eyelids well developed and cover most of the eyeball. Two claws distinct on each fore flipper, one claws on hind limbs and tail reduced to be shorter than hind limbs (Fig. 6).

**Age 35 days**: Pigments prominent on dorsal part, the ventral still white, plastron scutes distinct. Carapace scales very distinct with presence of ridges at the marginal scutes, vertebral scutes with prominent keels. Cranial distinct, scales on head well defined, columella auris totally disappeared. Two claws on both fore and hind limbs, only few scales on posterior border of fore flippers. The tip of the digits form into points along the margin of each limb. The eyelids annular with round notches on upper and lower lids. Volume of yolk nearly the same as volume of embryo (Fig. 7).

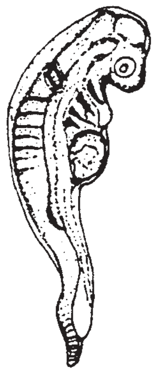

**Figure 1.** Age 12 days

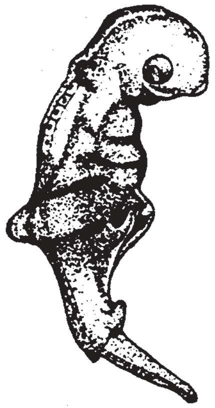

**Figure 2.** Age 15 Days

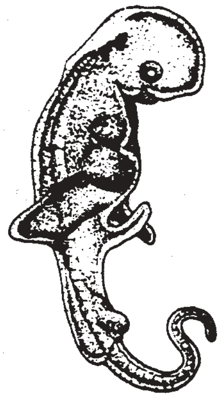

**Figure 3.** Age 20 days

**Figure 4.** Age 23 days

**Figure 5.** Age 25 days

**Figure 6.** Age 30 days

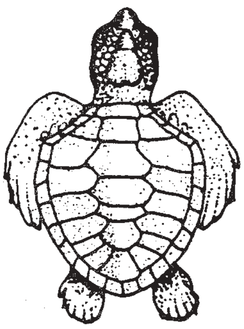

**Figure 7.** Age 35 days

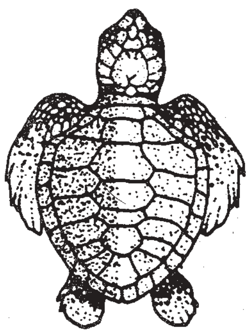

**Figure 8.** Age 40 days

**Table 3.** Growth measurements of olive ridley turtle embryos in various developing stages.

| Age (day) | Total length (mm) | Carapace length (mm) | Carapace width (mm) | Head length (mm) | Head width (mm) | Fore flipper (mm) | Hind flipper (mm) | Weight (g) |
|---|---|---|---|---|---|---|---|---|
| 23 | 19.0 | 10.0 | 5.5 | 7.0 | 6.6 | 4.6 | 3.2 | 0.37 |
| 24 | 20.7 | 11.0 | 7.0 | 8.4 | 7.7 | 5.0 | 3.6 | 0.50 |
| 25 | 21.8 | 12.6 | 7.9 | 8.7 | 8.0 | 5.4 | 4.0 | 0.59 |
| 27 | 23.7 | 14.0 | 10.0 | 10.0 | 8.4 | 5.6 | 4.8 | 0.62 |
| 28 | 23.7 | 12.8 | 9.4 | 9.4 | 8.0 | 6.9 | 5.2 | 0.66 |
| 29 | 27.1 | 16.6 | 12.3 | 11.0 | 8.8 | 8.2 | 5.6 | 1.10 |
| 30 | 29.7 | 18.2 | 14.0 | 11.3 | 8.9 | 8.3 | 5.8 | 1.47 |
| 31 | 31.5 | 19.4 | 14.5 | 12.0 | 9.0 | 9.0 | 6.6 | 1.60 |
| 32 | 34.2 | 21.6 | 16.1 | 13.2 | 9.3 | 11.0 | 7.3 | 2.03 |
| 33 | 36.3 | 23.3 | 16.2 | 11.7 | 10.0 | 12.5 | 8.1 | 2.26 |
| 34 | 37.5 | 23.8 | 17.5 | 12.8 | 9.8 | 13.8 | 9.0 | 2.40 |
| 35 | 39.7 | 24.1 | 19.0 | 12.7 | 10.0 | 14.9 | 9.7 | 2.73 |
| 36 | 43.0 | 27.1 | 20.0 | 13.6 | 10.0 | 17.3 | 10.0 | 3.29 |
| 37 | 41.1 | 25.7 | 20.1 | 13.2 | 9.4 | 16.3 | 10.7 | 3.37 |
| 38 | 46.2 | 29.8 | 22.5 | 15.7 | 10.5 | 19.7 | 12.0 | 5.01 |
| 39 | 48.8 | 30.0 | 23.3 | 16.2 | 11.4 | 21.2 | 12.6 | 5.59 |
| 40 | 51.6 | 31.6 | 24.9 | 16.7 | 11.8 | 23.4 | 13.5 | 6.80 |
| 41 | 54.3 | 33.1 | 26.5 | 17.2 | 12.3 | 25.7 | 14.5 | 8.00 |
| 43 | 54.4 | 33.9 | 28.0 | 17.6 | 13.0 | 29.0 | 17.2 | 9.00 |
| 45 | 61.6 | 38.5 | 31.0 | 18.6 | 14.2 | 30.4 | 19.7 | 9.50 |
| 48 | 59.0 | 36.7 | 29.8 | 18.0 | 14.0 | 35.0 | 20.0 | 11.14 |
| 50 | 59.0 | 35.0 | 32.0 | 19.5 | 14.5 | 34.2 | 21.0 | 13.30 |
| 52 | 59.3 | 38.1 | 32.6 | 18.3 | 14.2 | 37.3 | 20.5 | 12.85 |
| 53 | 61.1 | 38.6 | 33.9 | 18.6 | 14.7 | 37.8 | 21.6 | 16.80 |

**Age 40 days** : Embryo perfectly developed, body complete in all parts. The neck much shorter relative to the last stages, but covered with very thick skin. Volume of yolk much smaller than volume of embryo. Scales on head and flippers clear, the colour of embryo dark dorsally and more light at plastron (Fig. 8).

**Newly hatched turtle**: After 53 days on average the turtle hatched. The newly hatched turtle has a small amount of egg yolk attached to umbilical cord, the baby turtle absorbed its preserved food within a few days. After a few days the yolk has been used or withdrawn into abdomen leaving an umbilical scar. The newly hatched olive ridley turtle has black color, measurements of body parts were as follows: total length (straight length) 61.1 mm, carapace length 38.6 mm, carapace width 33.9 mm, head length 18.6 mm and body weight 16.8 g. Details of measurements are shown in Table 1.

## DISCUSSIONS AND CONCLUSIONS

### Artificial incubation

Only small numbers of olive ridley turtle remained to lay eggs in the nesting areas along the west coast of Thailand. It is impossible to leave

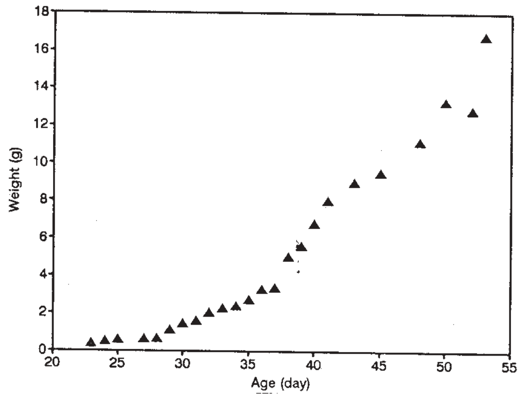


**Figure 9.** Increase in weight (g) of the embryo as a function of age in days.

turtle eggs incubated in nature because there are too many poachers looking for them. Therefore, artificial incubation of turtle eggs is needed, both reburied in nature and incubated in styrofoam boxes. The eggs were collected and transported to hatcheries as soon as possible because the transportation can cause lower hatching percentage. Schulz (1975) reported that the sea turtle eggs can be removed within 3 hours without harm. Before the oviposition stage the embryo has not adhered to the membrane of the inner shell wall. Hence it is possible to remove the eggs to new hatching sites. When the embryo reaches post-oviposition stage adhesion of the embryo to the shell membrane occurs. If not maintained in this position, damage may occurs and the embryos fail to develop. Development of the embryo depends on temperature. The eggs can be transported longer time at lower temperature. The hatching success of loggerhead turtle eggs was significantly reduced with increasing temperature when the eggs had been rotated 180°. -24 hours after eggs were deposited, hatching success was 0% when the eggs were rotated at a temperature of 29°C. The hatching rate was 40% when rotated at 27.5°C, 50% 24.5°C, and 100% hatched when eggs were rotated at 14°C. In addition, the hatching ratio decreased when the eggs were rotated at longer time intervals (48 hrs, 72 hrs, 96 hrs), no eggs survived inversion after 72 hours at temperature of 29°C, 27.5°C and 24.5°C (Miller & Limpus, 1983). Therefore, reduction of temperature can be used for long distance transport of eggs in order to reduce the mortality.

During collection of sea turtle eggs, some clutches were found many hours after they had been laid. Such eggs must be carefully kept in correct position when moved into styrofoam boxes.

The results of hatching success with styrofoam box incubation in 1980 was an average of 69.7% (range from 50-85.2%), the average hatching period was 64 days. It seems that the hatching success was lower than in 1981 and 1982, but statistical multiple range ANOVA analysis of the experiments showed no significant difference in hatching success between the three experiments ($P > 0.05$). This may be because so few samples were analyzed (6 clutches in 1980, 8 in 1981 and 8 in 1982), the variance is probably too large (13.3) and it can cause erroneous conclusions.

The results of statistical ANOVA showed nonsignificant difference ($P > 0.05$) of hatching success in comparisons between reburied and styrofoam box incubation. However, the average hatching success (Table 1 and 2) was slightly better in styrofoam box incubations. It is speculated that the reason could be related to environmental conditions such as small burrowing predators, penetration of rainwater and/or infection by bacteria during incubation period of the relocated material. Wyneken *et. al.* (1988) reported that the hatching success in artificial incubators (average 83%) was significantly higher than found with the reburied incubation method (average 71%) using the Chi-square test ($X = 11.13$, $P < 0.01$). Temperature is an important factor which influences the incubation of sea turtle eggs. No hatch success was obtained with eggs incubated at temperatures lower than 24°C or higher than 36°C, but the temperature can vary from 26°C to 32°C without too much effect on hatching success (Heads & Haute, 1979). In consequence the incubation in this study has occurred within the acceotable range of temperature. Moreover, temperature has pronounced effect on the change of sex in sea turtle. Incubation of loggerhead turtle eggs at constant temperatures of 26, 28, 30, 32 and 34°C, resulted in 0% females at 28 and 26°C, while 50% females hatched at 30°C and 100% females at 32 and 34°C incubation temperatures (Haute, 1983; Heave & Haute, 1979)

The temperatures during incubation in styrofoam boxes ranged from 25.9 to 27.0°C in the experiment in 1980 (Table 2), this might have caused a lower number of hatching of females. Because of awareness of the effect of low temperature, the incubation boxes were kept under shelter outside the room and allowed to receive sunlight in early morning and evening (experiments in 1981-1982). The incubation temperature was raised (range from 28.8 to 31.3°C), which should result in appropriate sex ratios. In nature, about 60% loggerhead turtle are females (Mrosovsky, 1983). At an incubation temperature at about 31°C, about 60% females and 40% male should develop (Haute, 1983). Therefore, during the styrofoam box incubation the sex ratio can be regulated. This method is more safe from damage by conditions in nature (predators, storm tides and poachers).

**Embryonic development**

During the oviposition stage, when adhesion of the embryo has occurred, the egg shell becomes chalky white, starting at the position of adhesion and moving down until the whole egg colour has changed. The adhesion process in loggerhead eggs started after about 24 hrs with chalk-white on top of the egg until the egg was completely white within ten days (LeBuff, 1990). This can be used to estimate the age of clutches by visual observation of the shell color. Consequently, if a clutch was found with half of the eggs chalk-white in colour, it should be about six days old. This can be used to maximize handling success of sea turtle eggs.

Developing embryos could clearly be seen when they were about ten days old. Intentions by authorities to help in conservation of sea turtles must be coupled with knowledge of sea turtle biology and life cycle. The knowledge of critical periods within total incubation, the temperature effects on sexual differentiation may be used to the advantage of conservation programmes. Styrofoam boxes are an unnatural way of incubating sea turtle eggs but may well have advantages, especially the possibility of temperature control in order to keep appropriate sex ratios. Besides, the styrofoam box incubation results in good hatching success and hence, it is beneficial to sea turtle conservation in Thailand.

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## REFERENCES

Bustard, R. 1973. *Sea turtles.* Natural history and conservation. Taplinger Publishing Company, New York. 220 pp.

Dodd, C. K. Jr. 1988. Synopsis of biological data on the loggerhead sea turtle, *Caretta caretta* (Linnaeus 1758). Fish and Wildlife Service, Biological Report, 88(14): 110 pp. Ganjanamavint P. & S. Rongmaungsart, 1987 (Thai). Comparative studies on embryonic development of hawksbill turtles (*Erethmochelys imbricata* Rüppell) in natural environment and in foam boxes. *Thai Fisheries Gazette*, **40**(5): 509-533.

Marquez, R. M. 1978. The Atlantic ridley in Mexico, 1978 season and conservation program. *Marine Tu Newsletter.* **9**:2.

McLean, K. P. Dutton, C. Whitmore and N. Haute, 1983. A comparison of three methods forincubat turtle eggs. *Marine Turtle Newsletter*, **26**:7-9.

Miller, J.D & C.J.Limpus, 1983. A method reducing movement-induced mortality in turtle eggs. *Mar Turtle Newsletter*, **26**:10-11.

Mrosovsky, N. 1983. *Conserving sea turtles.* The Bristish Herpetological Society. *c/o* The Zoologi Society of London, London. 177 pp.

Parker, W. K. 1880. Report on the development of the green turtle *(Chelone viridis,* Schnied). The Voy of H. M. S. Challenger. *Zoology,* **1**:1-58.

Penyapol, A. 1957. A preliminary study of the sea turtles in the Gulf of Thailand. Hydrographic Departm Royal Thai Navy. 13 pp.

Phasuk, B. & Rongmaungsart, S. 1973. Growth studies on the ridley turtle, *Lepidochelys olivacea* in capt and the effect of food preference on growth, *PMBC Research Bulletin*, **1**:14 pp.

Schulz, J.P. 1975. *Sea turtles nesting in Surinam.* Rijksmuseum van Natuurlijke Historie, Leid Netherlands, 143 pp.

Simon, M. H., Glenn F.U. and Alarn S.P. 1975. The green sea turtle (*Chelonia mydas*): mating, nesting hatching on a farm. *Journal of Zoology*, **177**:411-423.

Woody, J.B. 1981. Kemp's ridley, preliminary 1981 nesting information. *Marine Turtle Newsletter.* 13.

Wyneken, J., T.J. Burke, M. Salmon and D.K. Pedersen, 1988. Egg failure in natural and relocated sea tu nests. *Journal of Herpetology*, **22** (1) :88-96.

Ynterma, C. L. & N. Haute, 1979. Incubation temperature and sex ratio in hatchling loggerhead turtle preliminary report. *Marine Turtle Newsletter*, **11**:9-10.