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Temperature-Dependent Sex Determination of Green Turtle, *Chelonia Mydas*, in the Andaman Sea

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ABSTRACT
A study on the effect of incubation temperature on sex determination of green turtle, *Chelonia mydas*, was conducted at Hu-Yong Island, a member of the Similan Islands National Park, in the Andaman Sea. Five nests were collected in March 2009 and relocated to the nesting area. Incubation temperatures from each nest were recorded every 30 minutes using temperature data loggers fixed into the middle of each nest. Sexes of dead hatchlings were determined using a tissue histological method. The results showed that the incubated temperature of all nests ranged between 27.96 – 35.86 ^{O}C, while the mean temperatures taken during the middle third period of each nest ranged from 29.11 ± 0.41^{O}C to 30.67 ± 0.73^{O}C. The warmest incubation temperature (30.67 ± 0.73^{O}C) produced 94.12% female turtles, while the coolest incubation temperature (29.11 ± 0.41^{O}C) produced 63.64% females. Thesex ratio of the green turtle at Hu-Yong Island during dry season was 4 females to 1 male.

KEYWORDS: *Chelonia mydas*, sex determine, the Andaman Sea

INTRODUCTION

Sea turtles are amniotic vertebrates which lack heteromorphic sex chromosomes (Kuchling, 1998). Their sex determinations are temperature-dependent. Sex of the offspring is determined by the incubation temperature during their embryonic development (Benton, 1997; Godfrey and Mrosovsky, 2006; Yntema and Mrosovsky, 1980). It is evident that warmer, incubated temperatures produce greater proportions of female sea turtles. A study of green turtles from Pahang, Malaysia, showed that more than 80% of males were produced at 27.2-28.2^{o}C, while the higher temperatures of 32-33^{o}C produced a 100% female birth rate (Abdullah and Ismail, 2004). The pivotal temperature of loggerhead turtles at Southern Kyparissia Bay, Greece, was reported to be at the average temperature of 29.7±0.2^{o}C (Rees and Margaritoulis, 2005). They also reported that incubation temperatures higher than 30.35^{o}C resulted in 100% female births, while a 100% male birthrate occured when the incubation temperature was lower than 28.3^{o}C.

In nature, the temperature of the nest was influenced by many factors including latitude, beach orientation, depth of nest, monsoon season, shading, etc. (Tatsukawa et al., 2001; Matsuzawa et al., 2002; Godley, et al., 2001; Rees & Margaritoulis, 2005) so that the sex ratio of the wild sea turtle population would have wide temporal and spatial variations accordingly. The current issue of global climate change might be the cause of higher air temperature in some locations. It is possible that sea turtle communities will be at risk of having only a female population left, which might lead to their disappearing from some areas and the extinction of some species. Therefore, study of the relationship between the incubation temperature and sex determination of sea turtles is in need.

Chelonia mydas, *Eretmochelys imbricata*, *Lepidochelys olivacea*, and *Dermochelys coriacea* are the four species of sea turtle which nest in Thailand. The Gulf of Thailand is the nesting site of two species; *C. mydas* and *E. imbricata* while four species were found in the Andaman Sea. *C. mydas* is the dominant nesting sea turtle in Thailand. Hatching success of green turtles using a Styrofoam box incubation method were 70-83 %. (Kittiwattanawong, 2004), while the relocated nest method at Hu-Yong Island provided a hatching success of 40±32% (mean±S.D) (Kittiwattanawong, 2008). Tatsukawa et al. (2001) suggested that the pivotal temperature of the green turtle in Thailand was 28 ^{o}C. Kittiwattanawong (2008) used air and nest temperatures to predict female ratio in both populations in Thailand (Hu-Yong population and Khram population), and reported that the proportion of female hatchlings was high in February-June (> 50%) in February-July and March-July for Hu-Yong's and Khram's populations respectively, while the pivotal temperature of 29.2 ^{o}C was used.

Hu-Yong Island is a part of the Similan Island National Park in Phang-Nga province which is closed during May-October due to the turtle hatching protection program operated by the Third Naval Area Command. Therefore, the island is a perfect area for the study of green turtle nesting and the effect of incubation temperatures on their sex determination, without human disturbance. One further objective of this study is to develop a method for studying sex-temperature relationship by using dead specimens.

MATERIALS AND METHODS

Study site and nesting data collection

This study was conducted at Hu-Yong Island (8^o 28.8'N, 97^o 38.4'E), which is a part of the Similan Islands National Park, in the Andaman Sea on the west coast of Thailand. Nighttime beach patrolling was performed for a period of 3 hours at high tide, in March 2009. Information of nester, date, time, and clutch size were recorded. The clutches were relocated to the prepared sand bed on the following day. Depths of the relocated nests were between 60-80 centimeters.

Temperature data

Temperature was recorded in the 5 relocated nests during the incubation period, using temperature data loggers (HOBO Pendent Temp, accuracy ±0.47 °C). The loggers were embedded in the middle of each nest and set for every 30-minute record. The nests were influenced by sunlight in the morning, but around 2.00 pm those nests were in the shade from the tree.

Study on sex determination

Hatchlings were transferred to Phuket Marine Biological Center for separate, captive rearing in five ponds. Dead hatchlings with a known incubation temperature and incubation period were collected for sex determination. The gonad and paramesonephric duct along with kidney of the specimens (N=99, aged <1 year after hatched) were dissected and preserved in 10% buffered formalin. The Gonads were embedded in paraffin for thin-section histology. The cross- section tissues (5 µm) were stained with hematoxylin and eosin, based on Humason (1972) and Bancroft (1967). The sex was examined under a compound microscope. Criteria for sex determination were based on Yntema and Mrosovsky (1980), Miller and Limpus (1981) and Merchant-Larios et al. (1989). The female hatchling ovaries exhibited a distinct cortex (C), an unorganized medulla without tubule (M), and tuniga albuginea (A) (Fig. 1A). The testes of the males lacked a cortex (C) and had an organized medullary region with developing seminiferous tubules (M) (Fig. 1B). The paramesonephric duct of the females were with complete lumen (L), epithelium columnar (E), and long thin stalk (S), (Fig. 2A). But the paramesonephrict duct of the males is undeveloped and is without lumen, inner epithelium and long thin stalk (Fig. 2B)

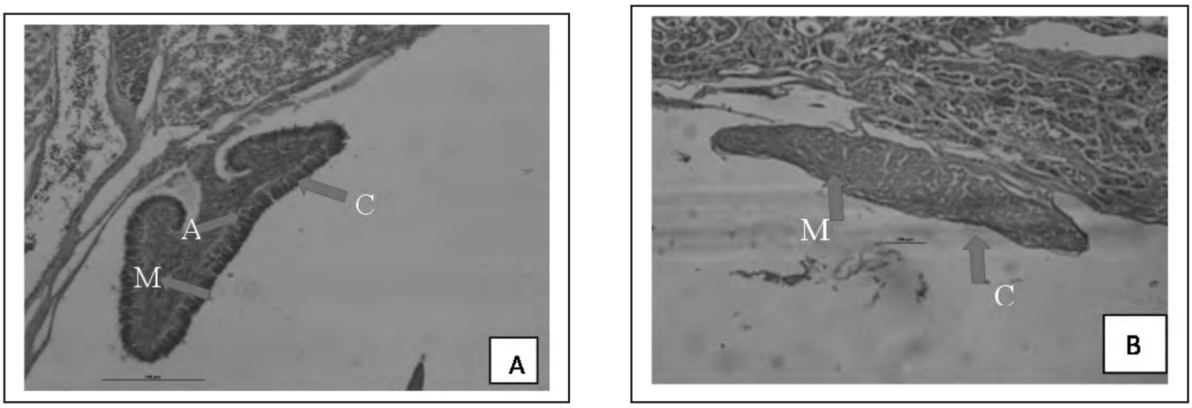

Fig.1. Histology of cross–section of gonad showing distinction between ovary and testis of green turtle (A) ovary showing thick cortex and dense medulla without tubule (B) testis showing thin cortex and medulla with tubule

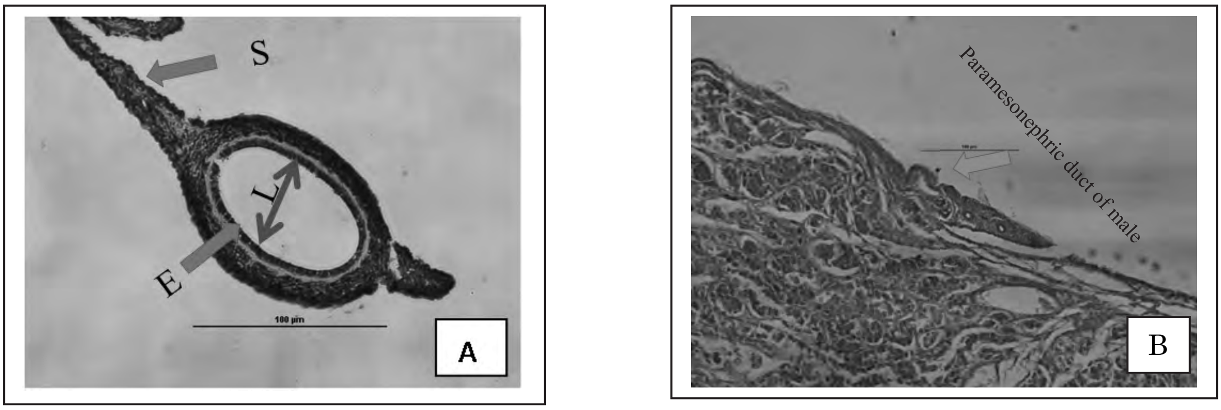

Fig.2. Histology of cross–section paramesonephric duct showing distinction between female and male green turtle (A) female paramesonephric duct showing complete lumen, columnar epithelium and long thin stalk (B) male paramesonephric duct showing incomplete lumen without columnar epithelium and without long thin stalk

Data analyses

-The correlations between sex and incubation temperature, both of each incubation week and middle third incubation temperature were analyzed using the Pearson correlation.

- Estimated sex ratio of each nest

The sex ratio of each nest was predicted by using the equation of (Godley, 2002) then compared with the sex ratio between pivotal temperature at 28.8°C (Godley, 2002) and 29°C with root mean square error.

$$\text{Proportion female} = \frac{1.0}{1+\text{EXP}\left(\frac{-(T-Tp)}{0.6}\right)}$$

Where: T = mean temperature in the middle third of incubation
Tp = estimated pivotal temperature

- Root mean square error

Root mean square error value was used to determine accuracy of estimated pivotal temperature based on the percentage of females in this study.

$$\text{RMSE} = \sqrt{\frac{\sum(obs-predict)^2}{n}}$$

Where: obs = sex ratio from observation
predict = sex ratio which is predicted using estimated pivotal temperature
n = number of sample
RMSE = root mean square error

- The lowest mean square error value was used to discover the estimated pivotal temperature so as to investigate the relationship between middle third incubation temperature and percentage of resultant females.

RESULTS

Sex and Middle Third Incubation Temperature $T_{(m)}$

Data on the nesters, $T_{(m)}$ and sex of the hatchlings are presented in Table 1. We examined 99 samples. $T_{(m)}$ of 5 nests ranged between 27.96-33.95 °C and mean of the $T_{(m)}$ were 29.11±0.41°C, 29.84±0.88°C, 29.89±0.70°C, 30.19±0.36°C and 30.67±0.73 °C.

The histological analysis of 99 green turtle hatchlings revealed the differences between males (Fig.1) and females (Fig.2). Since the incubation period was 8 weeks, the average middle third incubation period was determined to be between days19 to 36.

The warmest $T_{(m)}$ (30.67±0.73 °C) produced 94% females and the coolest $T_{(m)}$ (29.11±0.41 °C) produced 64% females (Table 1). The correlation co-efficiency (R^2) of the regression between sex and $T_{(m)}$ was 0.7. Therefore, it can be concluded that the percentage of females increased with the rising of $T_{(m)}$ (Fig.3).

Table 1: Data on nesters, Middle Third Incubation Temperature $T_{(m)}$, and sex of the hatchlings.

Nester Name	**Middle Third Incubation Temperature (^{O}C)**			**N. of samples**	**N. of Female**	**N. of Male**	**% of female**
	Min	**Max**	**Mean ± SD**				
Seitongyoi 9 March	27.96	33.64	29.11± 0.41	11	7	4	64
Komala11 March	28.26	33.64	30.19±0.36	8	8	0	100
Unknown12 March	28.26	35.86	29.89±0.91	28	19	9	68
Seitongyoi20 March	28.16	33.95	30.67±0.73	17	16	1	94
Komala 23 March	28.16	33.54	29.84± 0.88	35	26	9	74
			Total	**99**	**76**	**23**	**80**

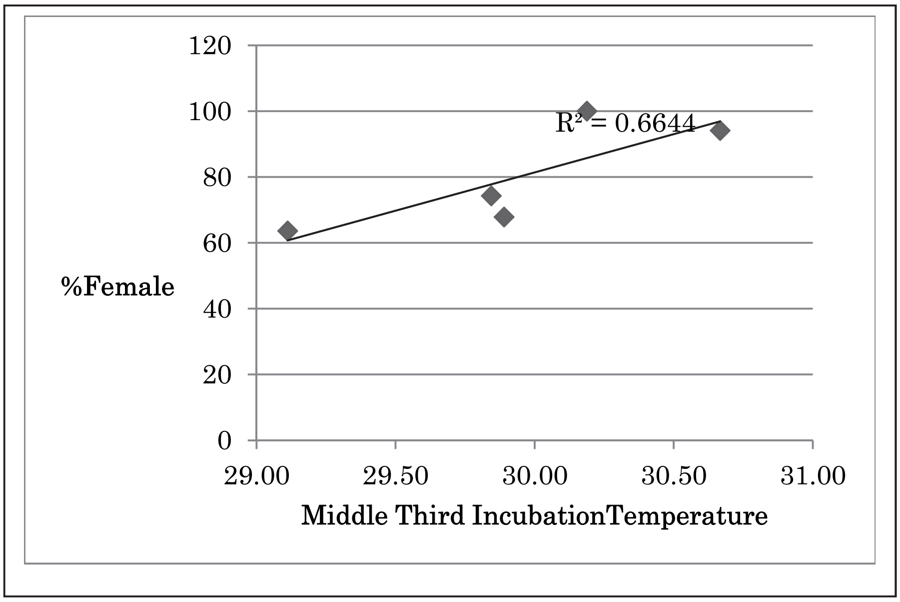

Fig. 3 Relationship between middle third temperature $T_{(m)}$ and percentage of females taken from 5 nests during March 2009.

Sex and weekly incubation temperature

There was a strong correlation between sex and incubation temperature in the first 4 weeks (Table 2).

Table: 2 Pearson correlations between incubation temperature and percent female

1st Week MS 16	2nd Week MS 19/20	3rd Week MS 22/23	4th Week MS 24	5th Week MS 26	6th Week MS 27	7-8th Week MS 29
0.88	0.83	0.96	0.96	0.44	0.55	0.67

*MS=Millor stage (by Gomuttapong et al., 2011)

Sex ratio estimation

The present study did not necessarily derive an absolute pivotal incubation temperature. Nevertheless, sex ratio must be predicted by equation. The table 3 shows the calculated percentage of females depending on different pivotal temperatures. The sex ratio of unknown pivotal temperatures from this study is more closely related to the estimated pivotal temperature at 29 ^{o}C than 28.8^{o}C (Godfley, 2002).

Table: 3 Value of root mean square error for accuracy of sex ratio prediction between estimated pivotal temperatures at 28.8 °C and 29°C.

Mean middle third incubation temperature	% Female		
	Pivotal 28.8°C	Pivotal 29°C	Unknown (study)
29.11(n=11)	63	66	64
29.84(n=35)	85	80	74
29.89 (n=28)	86	82	68
30.19 (n=8)	96	88	100
30.67 (n=17)	98	94	94
Root mean Square error	9.78	8.7	

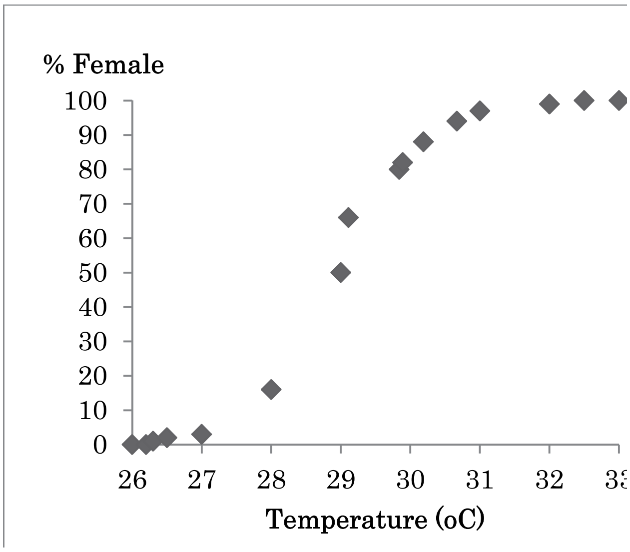

Fig.4. Graph shows sex ratio while using estimated pivotal temperature at 29°c

From Fig. 4 the estimated temperatures to produce 100% female offspring could be inferred as 32.5°C , 100% male at 26.2°C and 50% female/male at 29°C

DISCUSSION

This study showed that the $T_{(m)}$ that produced 100% female offspring should be higher than 30.67 °C in contrast with Broderick, et al. (2000) who have reported that 100% females were produced at 30.6 °C. The finding was in also in contrast to the report of Abdullah and Ismail (2004) which found that the $T_{(m)}$ producing 100% female young was 32 °C.

This study revealed that even at the lowest $T_{(m)}$ (29.11°C) females still made up 64%. Thus, it is likely that the pivotal temperature should be lower than 29.11°C, which is consistent with the studies of (Clint, et al. 2000). However, Abdullah and Ismail (2004) reported the pivotal temperature to be as high as 29.2°C.

Study on the sex ratio of the green turtle in the Gulf of Thailand was carried out by Tatsukawa et al., (2001) and they suggested that the pivotal temperature is roughly 28 °C. Therefore, we used the percentage of females and $T_{(m)}$ of each nest which reported by Tatsukawa et al. (2001) to predict the sex ratio by equation (Godley et al., 2002). We found that the pivotal temperature for green turtles at Kram Island is roughly 30.4°C which is higher than the pivotal temperature of 29°C at Hu-Yong Island as reported in the present study. However, the differences of the pivotal temperatures recorded in the Kram Island population and the Andaman Sea population may be the result of the period during which the experiment was undertaken.

The female: male sex ratio in March 2009 of this study was 4:1. If the sex ratio was calculated using an estimated pivotal temperature of 29°C, then the ratio will be female: male 4.1:0.9. So we concluded that the female ratio of the Andaman Sea is high during dry season, which is concordant with the study of Tatsukawa et al. (2001) who have reported that the sex ratio of females at Kram Island was highest in June (87%) and lowest in December (30.5%)

Therefore, the incubation temperature in summer at Hu-Yong Island is not critical for sex ratio in the wild because males were produced at twenty percent.

Table: 2 correlations between sex and weekly incubation temperature were strong in the first 4 weeks, the temperature may cause alteration of the enzyme aromatase that converts androgen to estrogen, determining the sex of the young (Kuchling, 1998). However, the effect of high temperatures during the early developmental stages should be further studied.

Furthermore, this experience was carried out in March during the peak temperatures of the year in Thailand so, incubation temperatures produced more female than male. Therefore, further study on sex ratio in rainy season is required. Also, the replication of each treatment and data set is quite small in the present study and hence, study should be continued to collect additional data for more accurate results.

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