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HATCHING SUCCESS AND NESTING DEPTH OF *Chelonia mydas* (FAMILY: *Cheloniidae*) IN EGGS RELOCATION PROGRAMME AT PENANG ISLAND, PENINSULAR MALAYSIA

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ABSTRACT

A study on hatching success and nesting depth of *Chelonia mydas* was conducted at Penang Island, Peninsular Malaysia from 1 December 2009 to 31 December 2010. Twenty nine natural nests were relocated for *ex situ* incubation, and incubated at 55, 65, and 75 cm depth. Two hatcheries plots, one covered (Hatchery A) and another one uncovered (Hatchery B), were prepared. This study found that nest temperature influenced the hatching success, incubation period, and hatchling sizes. High hatching success and less days of incubation period were found in Hatchery B as the plot was uncovered and exposed to sunlight, thus had higher temperature than covered Hatchery A. The mean nest temperature in Hatchery A was 28.0°C, which was lower than Hatchery B, 29.5°C. There was a significant difference in nest temperature at 55, 65, and 75 cm nesting depths in both plots ($p<0.01$). As the nesting depth increased, the nest temperature also increased. The mean hatching success was 50.5% in Hatchery A and 59.8% in Hatchery B. The mean incubation period was 54.9 days in Hatchery A and 50.7 days in Hatchery B. There was a linear relationship between nesting depth and hatching success, $p<0.01$. Due to warmer temperature, Hatchery B produced hatchlings with bigger sizes. In conclusion, nest temperature does affect the hatching success ($p<0.01$) and morphological characteristics of hatchlings ($p<0.01$). Eggs should be incubated in an uncovered plot with nesting depth of 55 cm for a maximum hatching success.

Key words: Hatching success, hatchling, nesting depth, nest temperature, relocation

ABSTRAK

Kajian dijalankan di Pulau Pinang, Semenanjung Malaysia dari 1 Disember 2009 hingga 31 Disember 2010. Dua puluh sembilan sarang telah dipindahkan untuk pengeraman secara *ex situ*, dan dieramkan pada kedalaman 55, 65, dan 75 cm. Dua kawasan penetasan, plot tertutup (kawasan penetasan A) dan plot terbuka (kawasan penetasan B) disediakan. Keputusan menunjukkan suhu sarang memberi kesan kepada keberjayaan penetasan, tempoh pengeraman, dan saiz anak penyu. Tingginya keberjayaan penetasan dan kurangnya tempoh pengeraman untuk sarang-sarang eksperimen dalam kawasan penetasan B adalah disebabkan plotnya yang terbuka, dan terdedah kepada suhu yang tinggi daripada cahaya matahari. Purata suhu sarang adalah 28.0°C di kawasan penetasan A kurang daripada Hatcheri B, iaitu 29.5°C. Terdapat perbezaan signifikasi untuk kedalaman suhu sarang di 55, 65 dan 75 cm di kedua-dua kawasan penetasan A dan B ($p<0.01$), menunjukkan suhu sarang akan semakin meningkat dengan meningkatnya kedalaman sarang. Suhu tanah ini diambil pada 5 cm dari permukaan tanah. Purata keberjayaan penetasan adalah 50.5% di kawasan penetasan A (plot tertutup) dan 59.8% di kawasan penetasan B (plot terbuka); min tempoh pengeraman adalah 54.9 hari di kawasan penetasan A dan 50.7 hari kawasan penetasan B. Terdapatnya perkaitan liner diantara kedalaman sarang dan keberjayaan penetasan, $p<0.01$. Disebabkan terdedahnya pada suhu yang tinggi, Hatcheri B menghasilkan saiz anak penyu yang lebih besar. Kesimpulannya, suhu sarang mempengaruhi keberjayaan penetasan ($p<0.01$) dan ciri-ciri morfologi anak penyu ($p<0.01$). Oleh sebab keputusan keberjayaan penetasan di kawasan penetasan A adalah rendah, adalah dicadangkan agar telur diteruskan pengeraman di kawasan penetasan B, dan diselaraskan kedalaman sarang pada 55 cm untuk keberjayaaan penetesan yang maksimum.

Kata kunci: Keberjayaan penetasan, anak penyu, kedalaman sarang, suhu sarang, dipindahkan

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INTRODUCTION

In Malaysia, the greatest current threats to marine turtle populations are habitat degradation, accidental and opportunistic turtle capture by fishermen with fish trawls, and direct capture of nesting female turtles and consumption of their eggs (Hamann *et al.*, 2006). Additionally, disturbances from the unregulated eco-tourism industry also affect the marine turtle populations (Yeo *et al*., 2007). One of the major causes of marine turtle population decline is a long history of eggs exploitation (Chan, 2006). Eggs poaching in Penang Island has decreased since the establishment of the Kerachut Turtle Conservation Centre in 1995 and enforcement of the strict laws of the Penang State Government (Fisheries Methods of Turtle and Eggs, 1999; Wildlife Ordinance, 1997) that have reduced night disturbance and thus protected the vegetation habitat of nesting site. Look at Department of Fisheries Malaysia (2014) for laws and ordinances of sea turtles in Malaysia. Other than Penang, the Terengganu State Government also has banned egg exploitation and sanctioned the commercial sale of leatherback turtle eggs in the market.

In recent years, most turtle populations in Asia has declined and some to the brink of extinction (Shanker & Pilcher, 2003). It's importance to produce a maximum hatching success in order to maintain the existence of turtle population in future. At least 70% of the eggs laid should be protected in order to maintain a healthy nesting population (Mortimer, 1999). However, eggs relocation programme can produce a net negative impact on turtle populations due to some serious limitations. For examples, there is limited number of well-trained and reliable staff to keep the effective operation of the hatchery and fish feeding stations are created when hatchlings from a hatchery are released at the same time and place each day. Other than that, in terms of financial limitation, the cost to build hatcheries is very expensive (Mortimer, 1999).

Ideally, sea turtle eggs should be incubated in the natural nest. In case *in situ* protection is impossible, relocation of eggs to a protected hatchery site should be undertaken only as the last solution (Mortimer, 1999). Eggs relocation programme has been conducted at Penang Island since 1995 as *in situ* protection of the natural nests on the beach is impossible due to nest exposure to flooding (Brown & Macdonald, 1995; Hitchins *et al.*, 2004), predation of crabs, feral dogs, and human poachers (Fowler, 1979), nest exposure to high humidity (López-Castro *et al*., 2004), and nest placed too close to the sea.

According to Mortimer (1999), hatching success in hatcheries is usually lower than in the *in situ* nests. This study was conducted in Penang Island to find the best hatcheries that would produce a high hatching success at the relocated nest. Nest needs to relocate as Kerachut Beach and Telok Kampi are located at remote areas and have problem of exposure to human poacher. Others threats are land predators (such as monitor lizards, crabs, and common palm civet), fishermen disturbances (e.g., torch light and noise from boat engine), and nest located too near to the sea thus exposing the nest to high moisture.

Published papers from the past studies such as Sarahaizad *et al*. (2012a, 2012b) focus on the nest distribution, behaviours, ecology, and nest site selection of Green Turtle. Researches on eggs relocation programme and hatching success have not been conducted yet in Penang Island. The issue is, although eggs relocation programme has been introduced at Kerachut Beach since 1995, no testing has been conducted to test the survivorship of eggs from the relocated nests.

There are two hatcheries at Kerachut Beach, and the eggs are incubated at both covered and uncovered plots. However, which plot produces higher hatching success than other plots is still not known. Another question is that: Is hatching success affected by nesting depth? All these questions and issues need to be addressed.

The objective of this study was to determine the best nesting depths (55 cm, 65 cm, or 75 cm) that would produce the highest hatching success. In this study, the eggs were incubated at two hatcheries namely Hatchery A (covered plot) and Hatchery B (uncovered plot) with different exposures to a range of temperature from the sunlight. This study was also aimed at investigating the effects of direct and indirect temperature on hatching success and the effects of hatcheries on the morphological characteristics of hatchlings. Therefore, this study attempted to find (a) the relationship between nesting depth and hatching success, and between nesting depth and incubation period, (b) the relationship between mean sand temperature and nesting depths, and (c) the differences in hatchling sizes produced between Hatchery A and Hatchery B.

Penang Island

Penang (GPS coordinate: N 5° 15' 47.9442", E 100° 29' 4.6356"), which is located on the northwest coast of Peninsular Malaysia by the Malacca, is a state in Malaysia and the name of its constituent island. Penang is the second smallest Malaysian state after Perlis. It is composed of two parts – Penang Island and Seberang Perai on the Malay Peninsula. Penang Island is a tropical island, just like other parts of Malaysia. Penang National Park, which is located at Telok Bahang, comprises Telok Duyung, Telok Aling, Telok Ketapang, Kerachut Beach, Telok Kampi, Pantai Mas, Pantai Acheh, and Muka Head. This park is famously known for the amazing nature and oceans.

Kerachut Beach and Telok Kampi

Kerachut Beach and Telok Kampi have been gazetted under the management of Penang National Park, Malaysia (see Figure 1). The latitude and longitude of Kerachut Beach is respectively 5° 27' 4" N, 100° 10' 58" E and Telok Kampi is 5° 26' 20" N, 100° 10' 46" E. Kerachut Beach and Telok Kampi are adjacent to Telok Duyung, Telok Ketapang, Telok Aling, Telok Kertang, Pantai Mas, and Pantai Acheh, which are located along the bay on the East Coast of Penang Island, famously known as the centre of attraction for tourists who want to experience nature, tropical rainforest, and ocean view. The distance of Kerachut Beach is approximately 1 km from Telok Bahang Jetty. Successful conservation efforts to preserve turtles have been made in recent years since 1995 until present through the establishment of the Kerachut Turtle Conservation Centre by the Penang Department of Fisheries. The department will buy the eggs from the licensed turtle eggs collector and incubate those eggs in the hatchery.

MATERIALS AND METHODS

Green turtle (*Chelonia mydas*) eggs collected from December 2009 to December 2010 (13 months) were relocated and incubated in two *ex situ* plots, Hatchery A (covered plot) and Hatchery B (uncovered plot). All 29 nests were reburied from the natural nests and transferred to Hatchery A (n=14 clutches) and Hatchery B (n=15 clutches) and incubated at three depths namely 55, 65, and 75 cm.

Eggs relocation programme, 2001–2009

The record of eggs located, eggs relocated, hatched eggs of relocated nests, and hatching success from 2001–2009 were obtained from the record of Penang Department of Fisheries (secondary data). The secondary data were needed to show the recent record of eggs collected and the hatching success from the establishment of Kerachut Turtle Conservation Centre. Intensive nocturnal surveys and morning track counts were performed (Wang & Cheng, 1999).

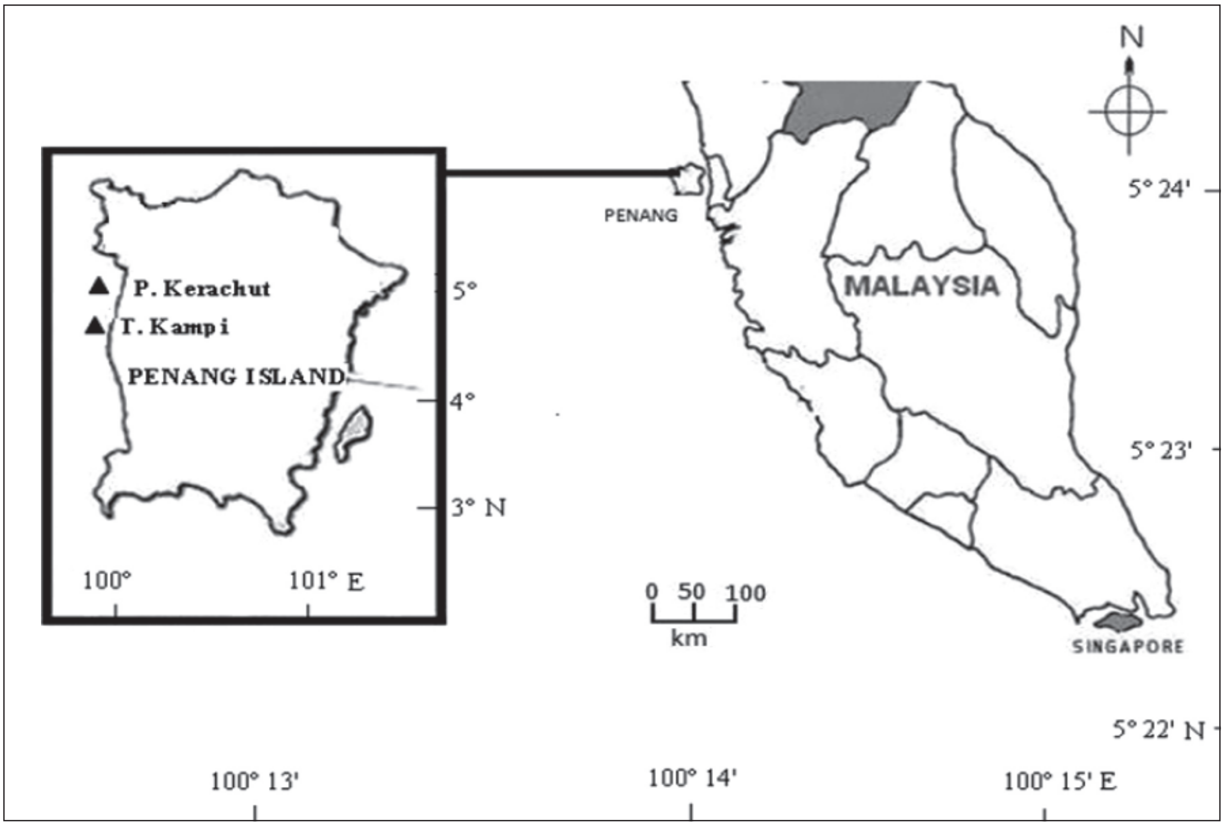

Fig. 1. Map of Penang Island situated in the northern region of Peninsular Malaysia. The surveyed beaches are Pantai Kerachut and Telok Kampi.

Field surveys

Two types of surveys were conducted from 1 December 2009 to 31 December 2010. Intensive nocturnal survey was performed. The tracks were counted, and nests were verified at night, followed by morning track counts (Wang & Cheng, 1999). If heavy rainfall or rough seas occurred on the night before where nests were hard to be identified during nocturnal surveys, morning track counts were performed to check for any overlooked landing. Morning track counts were performed between 0800 hrs to 0930 hrs in the next morning.

Kerachut Beach and Telok Kampi were surveyed every two hours every night by walking along the beach length between 1900 hrs to 0600 hrs. We (including staff of Kerachut Turtle Conservation Centre) walked along the sandy beach using minimal light to verify any emergence tracks. Once a turtle had emerged from waters, the source of light was switched off to avoid disturbances. Nest was verified after the turtle had finished nesting and compacted the sand down. Then, the number of clutch size at the nesting site was recorded, relocated, and incubated to the hatchery of Kerachut Turtle Conservation Centre.

Relocated nest

Eggs need to relocate when nest is placed too close to the tide line, or else these eggs would be swiped away by waves of high tide or rain, and the nest could be exposed to high moisture. On the other hand, if nest is located too far from the tide line, the eggs are exposed to very low moisture and hatching success might thus decrease. In this study, eggs were counted and relocated by carefully excavating the nest as soon as the turtle had returned to the sea (Hays & Speakman, 1993).

To relocate eggs from their natural nests to the experimental sites, a large plastic bucket was used to fill in the eggs, and we were required to wear gloves during eggs relocation process for hygiene purpose. Initial handling should be completed within three hours of eggs deposition to ensure maximum eggs hatches (Parmenter, 1980). This procedure was unlikely to affect the survival of the eggs adversely. Previous studies show that careful excavation and handling of sea turtle eggs shortly (three hours or less) after they are laid do not induce egg mortality (Parmenter, 1980; Harry & Limpus, 1989).

Once the nests were identified, the eggs must be handled with extreme care to prevent horizontal and vertical rotation of eggs (Chan, 1989). Development of eggs may suspend from frequent motion, and this factor may prevent the attachment of the embryonic membranes (Parmenter, 1980). Then, the bucket full of eggs was filled with sand to maintain the temperature. Eggs from one clutch were immediately transferred for incubation. The method of eggs incubation was done as explained by Mortimer (1999).

Hatchery A and Hatchery B

In order to test the effects of different temperature exposure on the hatching success, two different hatcheries plots were set. Hatchery A was an original site for incubation with an area of 10 m x 5 m and fully covered with a black net. The purpose of the black net was to control or decrease the sunlight exposure to the nests incubated in the hatchery. On the other hand, Hatchery B had no net cover, was directly exposed to sunlight, and thus had warmer temperatures than Hatchery A. In addition, Hatchery A was an old plot used for eggs incubation since 2001 and Hatchery B was a rather new plot (since 2008).

The number of eggs incubated per clutch depended on the natural clutch size deposited by mother turtles. It ranged from 48 to 144 eggs per clutch. After the green turtle had finished nesting, eggs were reburied and transferred to the hatcheries for further incubation. Hatchery A hosted 14 clutches, and Hatchery B had 15 clutches. In both plots, clutch sizes were incubated under depths of 55 cm, 65 cm, and 75 cm and a diameter of 20 cm according to the natural depths. According to Booth and Freeman (2006), 40 to 100 cm is the range of natural green turtle nesting depths.

Four clutches were incubated at 55 cm depth, and five clutches were incubated under each depth of 65 and 75 cm in Hatchery A (n=14). In Hatchery B, five clutches were incubated under each depth of 55, 65, and 75 cm (n=15). The eggs were left incubated until emergence of hatchlings. Incubation period was then recorded per clutch (from the first day of eggs incubated until the first day of emergence of hatchlings). The number of healthy hatchlings emerges was recorded in order to calculate the hatching success. Hatching success was calculated using the formula as follows:

> Hatching success (%) = total healthy hatchlings/total clutch size incubated x 100 (Chen & Cheng, 1995).

Nest temperature

The nest temperature was monitored every two hours for whole incubation period at nesting depth 55, 65, and 75 cm at both plots. Temperature logger model DS1921G was placed at the middle of each nest together with the eggs, and left for depleting data until hatchlings emergence occurred (Booth & Freeman, 2006; Rusli *et al.*, 2011). As the temperature logger can monitor for a maximum time of 45 days, two temperature loggers (±1°C) were placed in the middle at each nest. After hatchlings had emerged and left the nest, temperature logger was excavated and plugged in into USB reader, and data were read from the computer.

Sand temperature

Sand temperature of Hatchery A and Hatchery B was recorded using a soil thermometer (±1°C). The sand temperature was recorded at three occasions as follows: (a) 1950 hrs and 2130 hrs on 15 April 2010; (b) 1440 hrs and 1700 hrs on 16 April 2010, and (c) 0125 hrs and 0315 hrs on 17 April 2010 at depths of 5 cm from sand surface at both plots. Refer to Hays and Speakman (1993).

Morphological characteristics of hatchlings

The morphological characteristics of 50% of healthy hatchlings from each clutch (total 6 clutches) were determined by measuring the hatchling straight carapace length and hatchling weight (Chen & Cheng, 1995). Hatchling straight carapace length was measured using a vernier slide calliper (mm), and hatchling weight was measured with a spring balance model Acculab VI-400 (0.1 g).

DEFINITION OF TERMS

Relocated nests (ex situ): Relocated nests (Chan, 2010; Chen & Cheng, 1995; Fowler, 1979; Van De Merwe, 2006) or translocated nests (Hitchins *et al.*, 2004) are defined as nests that contain eggs taken out from their natural nesting site and reburied to a safer place.

Hatching success: Defined as the number of healthy hatchling hatched from a clutch.

Healthy hatchlings: Defined as the surviving hatchlings emerged from a clutch.

Incubation period: Defined as the length of time (in days) for eggs to incubate plus the time for hatchlings to emerge from the nests (Fowler, 1979). Once eggs are incubated, the important data that need to be recorded are as follows: 1) location of natural nest collected; 2) date and time of nests located; 3) clutch size; 4) adult's tag number; 5) nest number; 6) species; and 7) incubation types (*in situ* or *ex situ*) Other information that needs to be recorded in book is the expected date of hatching.

Morphological characteristics of hatchlings: Defined as the hatchling straight carapace length (mm) and hatchling weight (g).

Statistical analysis

The results were analysed using Microsoft Excel and SPSS version 18. The relationship between two parameters was analysed by either performing a linear regression or correlation analysis method (Sokal & Rohlf, 1982). Linear regression was performed to test should there be a strong linear relationship between nesting depth and hatching success. Spearman's Rank Order Correlation was used to calculate the strength of the relationship between two continuous variables (Pallant, 2002). These continuous variables were nesting depths and incubation period. The independent sample *t*-test was used to compare the mean score on some continuous variables for two different groups of subjects (Pallant, 2002). Independent sample *t*-test was conducted to determine should there be a statistically significant difference between total healthy hatchlings for Hatchery A and Hatchery B, and between hatchling straight carapace length (HSCL) and hatchling weight (HW).

RESULTS

Eggs records from 2001 to 2009

Eggs located refers to original nests (*in situ*) that we did not transfer, while eggs relocated refers to eggs that we transferred to another place for incubation. The recent records from 2001 to 2009 were important to show the fluctuation of eggs located, relocated, and hatching success. A total of 80.8% nests were relocated to a hatchery at Kerachut Turtle Conservation Centre (see Table 1). From 2001 until 2009, the number of eggs located ranged from 3,985 to 7,974; eggs relocated ranged from 3,341 to 7,442; and hatched eggs of relocated nests ranged from 1,667 to 4,528. Under the protection of the Kerachut Turtle Conservation Centre, each nest encountered on the beach was highly protected from predator threat, and eggs poaching on the beach was also minimised. The highest percentage of eggs located was in 2009 with 14.5% of the total number of eggs; the highest percentage of eggs relocated was also in 2009 with 14.6% of the total number of eggs; and the highest percentage of hatched eggs was in 2001 with 14.9% of the total number of eggs. In terms of hatching success from 2001 until 2009, it ranged from 46.8% to 75.9% of the total number of eggs. Overall, a total of 55,106 eggs were located; 51,057 eggs were relocated; and 30,317 healthy hatchlings were successfully produced in the 9-year period under the relocation programme. Therefore, 92.7% of the eggs were relocated in the 9-year period, and Kerachut Beach had produced 59.4% hatchings from overall eggs relocated.

Hatching success

The mean of hatching success in both hatcheries decreased as there was an increase in nesting depth. Hatchery B produced higher hatching success than Hatchery A. At 55 cm depth, the hatching success in Hatchery A was 71.8%; at 65

Table 1: Record of located and relocated Green Turtle (*Chelonia mydas*) nests from 2001 to 2009 at Penang Island, Peninsular Malaysia

Year	Total nests located	Total eggs located	Total nests relocated	Total eggs relocated	Hatched eggs of relocated nests	Hatching success (%)
2001	66	6937	53	6922	4528	65.4
2002	39	3985	24	3341	1667	49.9
2003	47	5193	35	4124	2700	65.5
2004	62	7040	50	6469	3417	52.8
2005	42	4282	37	4031	1885	46.8
2006	71	7786	55	7036	4079	58.0
2007	62	7396	52	7279	4202	57.7
2008	44	4513	38	4413	3351	75.9
2009	73	7974	65	7442	4488	60.3
Total	506	55106	409	51057	30317	

cm depth, the hatching success was 50.6%; and at 75 cm depth, the hatching success was 28.2%. In Hatchery A, the total number of healthy hatchling was 725 hatchlings from 1435 eggs incubated, thus the hatching success in the Hatchery A was 50.5% (see Table 2a).

Mean hatching success in Hatchery B at 55 cm depth was 80.8%; at 65 cm depth, the hatching success was 67.4%; and at 75 cm depth, the hatching success was 31.4%. In Hatchery B, the total number of healthy hatchling was 1116 hatchling from 1867 eggs incubated, thus the percentage of hatching success in Hatchery B was 59.8% (see Table 2b).

There was a linear relationship between nesting depth and hatching success in Hatchery A, $y = -2.18x + 191.9$, $R^2 = 0.749$, $n = 14$, $p < 0.01$, and Hatchery B, $y = -2.47x + 220.4$, $R^2 = 0.738$, $n = 15$, $p < 0.01$. Independent sample *t*-test was conducted to compare the total healthy hatchlings for Hatchery A and Hatchery B. There was no significant difference in scores for Hatchery A (mean = 51.79, SD = 28.68) and Hatchery B [mean = 74.40, SD = 30.77; $t(27) = 2.04$, $p > 0.05$].

Incubation Period

Table 2a and 2b show the mean of incubation period for 29 clutches incubated in Hatchery A and Hatchery B. Hatchery B took lesser day than Hatchery A for the eggs to hatch. Mean incubation period at the three nesting depths in Hatchery A was 55.1 days (see Table 2a) and in Hatchery B was 50.7 days (see Table 2b).

The mean of incubation period for 15 clutches incubated in Hatchery B showed that uncovered plot took lesser time than Hatchery A for the eggs to hatch. High nests temperature, 29.5°C and sand temperature, 28.88°C in Hatchery B (see Table 3) gave a better metabolism for eggs to hatch. Thus, the Hatchery B produced higher hatching success than Hatchery A and reduced the time of incubation period. The mean nests temperature in Hatchery A, 28.0°C was lower than Hatchery B.

There was no significant correlations between nesting depths and incubation period for clutches incubated in Hatchery A, with its Spearman's rank correlation coefficient (r) = 0.075, $p > 0.05$, $n = 14$. There was also no significant correlations between nesting depths and incubation period for clutches incubated in Hatchery B, with its Spearman's rank correlation coefficient (r) = 0.210, $p > 0.05$, $n = 15$. Therefore, incubation period did not correlate with nesting depth.

Nest temperature

Table 3 presents the result of sand temperature for six clutches incubated in Hatchery A and Hatchery B. The mean of sand temperature for the three clutches incubated in Hatchery A was 28.0°C, and 29.5°C for the three clutches in Hatchery B. An independent sample *t*-test was conducted to compare the reading of sand temperature for the six clutches in Hatchery A and Hatchery B. There was a significant difference in scores for temperature monitoring every two hours at the three different nesting depths in Hatchery A (mean = 28.03, SD = 1.13) and Hatchery B [mean = 29.50, SD = 0.71; $t(2775.46) = -46.74$, $p < 0.01$, $n = 3775$].

Morphological characteristics of hatchlings

An independent sample *t*-test was conducted to compare the mean of hatchling straight carapace length (HSCL) and hatchling weight (HW). There was a significant difference in sizes of HSCL between Hatchery A (mean = 45.81, SD = 1.14) and Hatchery B [mean = 46.70, SD = 1.41; $t(255.40) = -7.22$, $p < 0.01$]. There was also a significant difference in HW measured between Hatchery A (mean = 20.08, SD = 1.35) and Hatchery B [mean = 20.92, SD = 1.46; $t(229.00) = -4.41$, $p < 0.01$].

Table 2: Hatching success (%) and incubation period (days) for 29 relocated nests (*ex situ*) of Green Turtle (*Chelonia mydas*), incubated in Hatchery A and Hatchery B at three nesting depths (cm)

a) Hatching success and incubation period of clutches in Hatchery A

No.	Nesting depth (cm)	Date of incubation	Total eggs	Total healthy hatchlings	Hatching success (%)	Mean	Incubation period (days)	Mean
1	55	21/2	85	61	72		56	
2	55	21/3	120	83	69		51	
3	55	20/4	144	108	75		52	
4	55	20/5	113	80	71	71.8	52	52.8
5	65	10/5	139	71	51		63	
6	65	30/4	104	57	55		60	
7	65	27/4	129	55	43		54	
8	65	10/6	103	55	53		59	
9	65	16/6	78	40	51	50.6	57	58.6
10	75	13/1	66	31	47		47	
11	75	30/6	124	19	15		55	
12	75	28/6	48	8	17		48	
13	75	5/7	88	11	13		55	
14	75	15/7	94	46	49	28.2	62	53.4
	Mean incubation period = 55.1 d Hatching success = 725/1435×100 = 50.5%							

b) Hatching success and incubation period of clutches in Hatchery B

No.	Nesting depth (cm)	Date of incubation	Total eggs	Total healthy hatchlings	Hatching success (%)	Mean	Incubation period (days)	Mean
1	55	17/1	122	102	84		51	
2	55	15/2	137	99	72		49	
3	55	9/3	121	96	79		49	
4	55	15/3	114	103	90		52	
5	55	24/4	84	66	79	80.8	50	50.2
6	65	27/1	144	76	53		48	
7	65	15/2	137	116	85		53	
8	65	19/2	135	76	56		53	
9	65	15/3	114	83	73		50	
10	65	29/3	144	101	70	67.4	48	50.4
11	75	23/1	144	72	50		52	
12	75	4/3	124	37	30		45	
13	75	21/3	120	43	36		58	
14	75	30/3	114	36	32		53	
15	75	20/5	113	10	9	31.4	50	51.6
	Mean incubation period = 50.7 d Hatching success = 1116/1867×100 = 59.8%							

Hatchlings produced in Hatchery B had a greater value of HSCL (mean = 46.70, SD = 1.41) and HW (mean = 20.92, SD = 1.46) compared to hatchlings produced in Hatchery A with HSCL (mean = 45.48, SD = 1.14) and HW (mean = 20.08, SD = 1.35) (see Table 4). The uncovered plot and direct exposure under the sun in Hatchery B could the factors for the difference. Higher temperature, 29.5°C in Hatchery B (see Table 3) was suggested to increase the metabolic process, thus the eggs had greater sizes in Hatchery B, compared to 28.0°C for the mean nests temperature and 28.64°C for the sand temperature in Hatchery A (see Table 3b).

DISCUSSION

Eggs relocation is a common strategy for conservation of declining reptilian populations around the world (Pfaller *et al.*, 2008). In this study,

Table 3: a) Mean nest temperature (°C) for six Green Turtle (*Chelonia mydas*) clutches incubate in Hatchery A and Hatchery B for the whole incubation period b) Mean sand temperature (°C) of Hatchery A and B recorded at 5 cm from sand surface at three occasions

a) Mean nest temperature (°C) for the whole incubation period

Plots	Nesting depth (cm)	Mean nest temperature (°C)	Range (°C)
Hatchery A	55	27.1	26.0-29.5
(covered)	65	28.3	27.0-29.0
	75	28.6	27.0-30.0
Mean		**28.0**	
Hatchery B	55	29.0	28.0-30.0
(uncovered)	65	29.7	28.5-30.5
	75	29.8	28.5-31.0
Mean		**29.5**	

b) Mean sand temperature (°C) at 5 cm of sand surface

Plots	Date	Time	Mean sand temperature (°C)	Range (°C)
Hatchery A	15/04/2010	1950-2130 h	28.94	28.1-29.6
(covered)	16/04/2010	1440-1700 h	29.22	29.3-30.4
	17/04/2010	0125-0315 h	27.77	27.2-28.4
Mean			**28.64**	
Hatchery B	15/04/2010	1950-2130 h	27.89	26.8-28.7
(uncovered)	16/04/2010	1440-1700 h	31.11	29.8-32.8
	17/04/2010	0125-0315 h	27.63	26.5-38.4
Mean			**28.88**	

Table 4: Morphological characteristics of Green Turtle (*Chelonia mydas*) hatchling from Hatchery A (covered plot) and Hatchery B (uncovered plot)

Site Measurements	Hatchery A		Hatchery B	
	mean (SD)	*n*	mean (SD)	*n*
Straight carapace length (mm)	45.48 (1.14)	96	46.70 (1.41)	135
Weight (g)	20.08 (1.35)	96	20.92 (1.46)	135

along the 13-month observations, turtle nests laid at Kerachut Beach and Telok Kampi were relocated due to high exposure to human poacher, located at an open beach, and exposure to predators (e.g., crabs, feral dogs, common palm civet, and monitor lizards). In addition, the original nests were located too close to the tide line, thus were exposed to waves. Besides that, there was a lack of staff to patrol Telok Kampi and Kerachut Beach.

Eggs incubated at 55 cm nests depth had higher rate of hatching success (71.8–80.8%) than 65 and 75 cm, and eggs incubated in uncovered plot in Hatchery B had higher rate of hatching success and larger sizes of hatchlings (59.8%) than eggs incubated in covered plot in Hatchery A. Temperature affected the hatching success and hatchling sizes (discussed at hatching success section next). Thus, in order to reach high survival of relocated nests, the Kerachut Turtle Conservation Centre should continuously incubate eggs at 55 cm depth and in uncovered plot.

In improving the number of hatching success in Hatchery A, the sand at the hatchery should be replaced with the new fresh sand. Due to the long period of 9 years, the sand should have been contaminated with the bacteria from the past incubation, and the sand have never been replaced previously.

To enhance the Kerachut Turtle Conservation Centre's programme, it is suggested that more research should be conducted at the Kerachut Beach to look into the factors that could affect the hatchability of green turtle's eggs. It is suggested that more experiment should be conducted at Kerachut Beach with the focus on the hatching rate and mortality of eggs incubated in Styrofoam box, *in situ*, and by splitting the eggs clutches. According to Mortimer *et al.* (1994), splitting eggs clutches and burial in separate nests will improve the hatching success. In addition, eggs clutches incubated in Styrofoam box enjoy high rate of hatching success (Mortimer, 1999).

In situ incubation should be the priority in order to maintain the natural incubation. Thus, beach has to be protected from animal disturbances. This can be done by increasing the number of well-trained staffs to monitor the beach. According to Mortimer (1999), the predators disturbing the nesting and hatching areas at the beach are usually species introduced by human or conditions created by human. Such conditions can occur when human refuses to provide food sources for the predators or when human has eliminated the predators' natural enemies.

There was a linear relationship between nesting depth and hatching success. According to Van De Merwe *et al.* (2005) and Glen *et al.* (2005), nests with deeper depths prolong the hatchlings emergence and have higher risks of higher number of dead hatchlings.

However, the results showed that nests incubated at 75 cm depth had lower percentage of hatching success as compared to nests incubated at 55 cm depths (Table 2). This finding is similar to Leh (1994) at Talang-Talang Islands, Sarawak.

High hatching success in Hatchery B could be related with the plot that was exposed directly under the sun thus having a mean sand temperature of 28.88°C (see Table 3b). The high temperature in Hatchery B increased the metabolic process of embryonic eggs (George *et al.*, 1994). This factor thus caused faster egg development and produced a higher hatching success than eggs incubated in Hatchery A. As supported by Eckert *et al.* (1988), the rate of embryo development is influenced by the nests temperature throughout the incubation process. More tissues are synthesised as the embryo is developed. As the heat is generated by the embryo, it continues growing and maintaining the tissues (Booth, 1998).

It is also suggested that higher hatching success in Hatchery B than Hatchery A could be related with sand hygiene and moisture content for eggs incubation. During nest excavation, the sand was dirtier and wetter as it increases the nesting depth in Hatchery A, and a few egg shells from previous incubation were also found (Personal observation). Compared to the sand in Hatchery B, the plot in Hatchery A was newer and the sand was cleaner. As supported by McGhee (1990), moisture content could influence the rate of hatching success and affect the successful development of turtle eggs.

Moreover, eggs incubated in Hatchery B had lesser days of incubation period than eggs incubated in Hatchery B. The reason was because the nests incubated in Hatchery B were exposed directly to the sun and thus had a high metabolic heating (Eckert *et al.*, 1988). Therefore, it took lesser time for the eggs to hatch. According to Hays *et al.* (2002), the duration of incubation period is influenced by temperature, and high incubation temperature can cause short incubation duration (Glen *et al.*, 2005). Other than that, Booth (1998) explains that the tissue synthesis is greater at higher temperature, and thus shorter time is taken for the eggs to develop. This high temperature factor explains the reason incubation period in Hatchery B was lesser than incubation period in Hatchery A. This finding is similar with the study by López-Castro *et al.* (2004) in the research on Olive Ridley Turtle and with the study by Chan *et al.* (1999) on hawksbill sea turtle at Pulau Gulisaan, Sabah.

Sea turtle's nest temperature depends on the complex interactions between chemical, physical, and biological factors, which vary between the beaches (Van De Merwe *et al.*, 2006). Bustard and Greenham (1968) suggest that green turtle eggs will only develop between 25°C and 34°C nest temperature. However, for the hatching success above 60%, an optimal temperature range of between 27°C and 32°C is required (Bustard & Greenham, 1968). In this study, the sand and nest temperatures observed at both plots were well in the optimal range for green turtle's eggs development.

Size and growth rates are influenced by incubation temperature (Booth *et al.*, 2004). Hatchling size is also affected when the eggs are incubated under high temperature (Glen *et al.*, 2005). In this study, hatchlings produced in Hatchery B had longer hatchling straight carapace length (HSCL) and higher hatchling weight (HW) than hatchlings produced in Hatchery A due to exposure to high temperature (mean nest temperature = 29.5°C). Thus, high temperature may give the embryo the chance to channel excess energy towards its size (Foley, 2000).

Moreover, hatchling sizes measured at Kerachut Beach were quite similar with the sizes of hatchling in the research performed at Wan-An Island, Taiwan. The study recorded that the mean hatchling straight carapace length of the green turtle was 46.9 mm, and the mean hatchling weight was 22.7 g (Chen & Cheng, 1995).

CONCLUSIONS

The hatching success from the uncovered plot in Hatchery B produced better hatching success than the covered plot in Hatchery A. Therefore, it is recommended for eggs to be incubated in an uncovered plot as the direct exposure under the sun and high temperature has positive effects on the embryonic growth, increases eggs metabolic processes, and increases the number of hatched eggs.

In terms of nesting depth, eggs incubated at 55 cm nesting depth produced higher hatching success compared to eggs incubated at 65 cm and 75 cm nesting depth. Therefore, relocated nests produce a better hatching success when incubated at 55 cm nesting depth than in deeper depth.

As a recommendation, a research on *in situ* nests should be conducted to compare the result of hatching success with the results of relocated nests. Aspects such as eggs survivorship, hatchling morphological characteristics, and hatchling sexes could also give important results to define the differences of hatching success and hatchling health. Another recommendation is to widen the study on the ecological factors of relocated nest, such as sand particles and sand moisture content.

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