SPLITTING THE EGGS METHODS: COMPARISON OF EGG SURVIVORSHIP BETWEEN STYROFOAM AND OPEN AREA NESTS FOR PENANG ISLAND TURTLE CONSERVATION

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ABSTRACT

This research paper reports the observation of successful hatching comparing Styrofoam and Open Area nest. Splitting the eggs method was studied using green turtle (*Chelonia mydas*) clutch size whereby they were divided equally and incubated in two nest plots (Styrofoam and Open Area). The aim of this study is to test the rate of hatching success between the two nest plots, as well as to measure the hatchling morphological characteristics. Results showed that eggs incubated in Open Area nest produced slightly higher hatching success (81.16%), than Styrofoam nest (74.31%). Mean incubation period of Open Area nest was 54.1 days, while in Styrofoam nest was 56.3 days, as low temperature slows down the time of hatching. Hatchling from Open Area nest had larger straight carapace length measurement (mean HSCL=45.89 mm) than hatchling from the Styrofoam nest (mean HSCL=45.23 mm). In contrast, emergence success in Styrofoam nest shows better hatchling emergence. This research is important, as we need to know the rate of hatching success when incubated either in Open Area or Styrofoam nest. Splitting the eggs method shows that Open Area nest produced a good rate of hatching success and hatchling morphological characteristics. It is recommended to further this study to test the hatchling physical performance between Styrofoam and Open Area nest, as high energy reserved within individual's hatchling body does impact their longer survival to face the ocean's predator and also for a greater fitness.

Key words: Styrofoam nest, hatching success, incubation period, temperature, emergence success

INTRODUCTION

The green turtles (*Chelonia mydas*) are widely distributed in Penang Island and has been the more abundant species as compared to the least abundant olive ridley turtles. To protect these species, Kerachut Turtle Conservation Centre was established in Penang Island. Through the establishment of Kerachut Turtle Conservation Centre by Penang Department of Fisheries, successful conservation efforts to preserve marine turtles have been made in recent years. Relocation of nest (*Ex-situ*) has been performed to maintain the hatching success of marine turtle, as *In-situ* programmes are not encouraged to be done in Penang Island. The reasons are, nest; are at risk as they are exposed to land predators (e.g., crab, monitor lizard), exposed to human poachers, exposed to tidal wash during the high tide and Kerachut Turtle Conservation Centre has limited number of staff to patrol the beach length of Kerachut and Teluk Kampi to protect from disturbances. *Ex-situ* method has been conducted in many Malaysian states [i.e., Melaka (Mortimer *et al*., 1993), Terengganu (Aini Hasanah & Nik Fadzly, 2015)] due to the above reasons.

One of the *Ex-situ* methods recommended by Mortimer (1999) is by splitting the clutch size into smaller sizes and incubating it in the Hatchery. The rational of splitting the eggs method was decide to performed in Penang Island was to observe the hatching success, as many conservation programmes

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in Malaysia have used this method (Rusli *et al*., 2016) with recommendation from the Department of Fisheries as it is believed to be able to increase the rate of hatching success (Mortimer, 1999; Mortimer *et al*., 1994). In addition, eggs incubated inside the Styrofoam box is also able to produce higher rates of hatching success (Mortimer, 1999; Aini Hasanah & Nik Fadzly, 2015).

This method was designed by splitting the clutch size into two equal numbers of eggs, and incubated at two different plots (Styrofoam and Open Area nest) at equal nest depth of 65 cm. The reason we chose 65cm depth because this is the common range of natural nesting depth dug by green turtle in most areas (Cheng *et al*., 2009). The objectives of this study are to investigate; 1) the difference in terms of hatching success, emergence success, and incubation periods between nests in Styrofoam and Open Areas, 2) which nest either from Styrofoam or Open Area nest produces healthier hatchling (in terms of larger and heavier hatchling sizes) and 3) to investigate the effect of temperature on hatchling morphological characteristics in both nests. This method was a first batch trial in Penang Island, hence, we decided to investigate the comparison results of eggs survivorship and hatchling morphological characteristics between Styrofoam and Open Area nest as an initial study. Study was performed within the breeding season of green turtle in Penang Island (from March-August 2014) with full co-operation from the staff of Kerachut Turtle Conservation Centre and Department of Fisheries.

MATERIALS AND METHODS

Study sites

Penang (GPS coordinate: N 5° 15’ 47.9442", E 100° 29’ 4.6356"), is a state in Malaysia (Southeast Asia) located on the northwest coast of Peninsular Malaysia. Within the Penang Island, there is a Penang National Park located in Telok Bahang, which is a remote town area located southwest of Penang Island, also locally known as Fisherman village. Penang National Park was gazetted by the Federal Government of Malaysia (Putrajaya) on the 10th April 2003, and the land area is approximately 1,181.949 ha and the sea area is 1, 381.014 ha (Taman Negara Pulau Pinang, 2016). Kerachut Turtle Conservation Centre is located within Penang National Park and was established in 1995. The location of Penang Island is labeled in Figure 1. The total length of Kerachut is 558 m. Teluk Kampi is the nearest and adjacent to Kerachut, and the distance is approximately 300 m from Kerachut.

Splitting the eggs methods

Ten (10) nests were collected from both Kerachut and Teluk Kampi between March till July 2014 comprising of five nests from turtle MY3423/MY3424 and another five nests from turtle MY3911/MY3912. The reason only 10 nests were used in this study is because splitting the eggs method was not recommended as recent study found out it could reduce the hatchlings energy rate (Rusli *et al*., 2016). Thirty (30) eggs were randomly collected from each nest and the egg’s diameter was

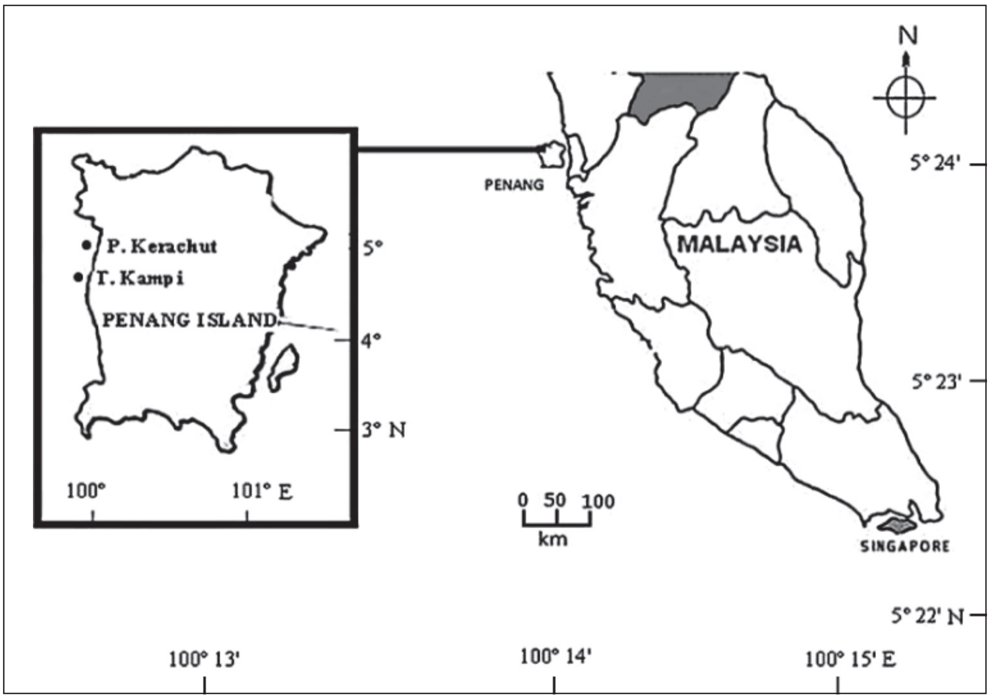

Fig. 1. Location of surveyed sites, Kerachut and Teluk Kampi.

measured using Vernier slide calliper, ±0.1 mm, and weight using an electronic weighing balance, A3360-LT5001 Smith model, ±0.1 g. Clutch size was split equally and 50% eggs were incubated in Styrofoam box, and another 50% eggs were incubated in Open Area nests. For clutch size that had odd numbers, half clutch size had an extra egg. In both nest plots, eggs were arranged inside the nest at 65 cm depth, and at the same nest width.

a) Styrofoam nest

The size of one Styrofoam box was [90 cm (length) × 50 cm (width) × 60 cm (height)]. Two Styrofoam boxes were placed vertically on top of each other to incubate eggs at 65 cm nest depth. The top Styrofoam box were cut at the pedestal, and combined with the below Styrofoam box using toothpicks. The below Styrofoam box was pierced with 0.5 cm diameter holes at base, and the space between holes was 5 cm to facilitate drainage of water (Mortimer, 1999). The sides of two Styrofoam box were pierced with 0.5 cm, 5 cm space between the holes. A total of 18 to 28 holes at all sides of each Styrofoam box were needed for enough air ventilation, smooth water flow and to control humidity of the sand from extreme condition.

Two pieces of nylon fabric were cut into square, each to a size slightly larger than the surface area of the bottom of the box. The materials were placed into the pierced box according to order starting from bottom. One piece of nylon fabric, one 10 cm layer of moist beach sand, 3-4 layers of freshly laid turtle eggs, 45 cm of moist beach sand, one piece of nylon fabric, one 10 cm layer of another moist beach sand (Mortimer, 1999) were used. Nest incubation was at 65 cm depth inside the box. Styrofoam nests were labelled with bamboo stakes and incubation date, clutch size, turtle tag number, and nest number was recorded. All the 10 boxes of Styrofoam nest were placed and incubated outdoor, inside the covered hatchery at Kerachut Turtle Conservation Centre. Styrofoam boxes were covered with lids during the whole incubation period, and were sprinkled with water twice a day to avoid dryness. Humidity was not measured for both nest plots, as we had problems analyzing the sand samples (due to boat transportation problems between Kerachut and main land to analyse the sand sample).

By using 2-inch stainless steel dial stem thermometer (Extech instruments corporation USA), three temperature readings were taken at 5 cm nest depth below the sand surface, for the Styrofoam nests. The temperature readings were taken once a week until the end of the incubation process. The top layer of sand and fabric were needed to be removed when the eggs began to hatch (Mortimer, 1999), usually on day 50.

Date of emergence was recorded once hatching began. Hatchlings emergence refers to hatchlings that crawl from nest until they reach the sand surface. Number of hatchling emergence was recorded for day one and day two. Emergence success and incubation period is calculated as follows:

1. Emergence success (%) = (Number of hatchlings leaving the nest/clutch size) × 100 (Hitchins *et al*., 2004).
2. Incubation period (day) = First day of eggs incubation until first day of emergence hatchlings (Booth & Freeman, 2006).

On day three, the nests were excavated to determine the eggs survivorship. Empty eggshell fragments, unhatched eggs and dead hatchlings were separated and counted. The surviving hatchlings inside the nest were counted and put inside the box. Hatching success, survival hatchlings, dead hatchlings and unhatched eggs were calculated according to the formula:

1) Hatching success (%) = (Total clutch size – number of unhatched eggs)/total clutch size × 100 (Miller, 1999; Hitchins *et al*., 2004; Zare *et al*., 2012).
2) Survival hatchlings = Number of empty eggshells –number of dead hatchlings (Chan, 2013).
3) Dead hatchings = Clutch size – (number of live hatchlings + unhatched eggs).
4) Unhatched eggs = Clutch size – number of empty eggshells.

b) Open area nest

Eggs incubated at 65 cm nest depth, and the nests were labelled with bamboo stakes (e.g.; 1A–Styrofoam nest, 1B-Open Area nest). Information including nesting date, nesting location, total eggs, and turtle's tag number were written on the bamboo stakes. Open Area nest were placed in the same row beside the Styrofoam box nest from the same clutch size. Nests were protected with plastic mesh to avoid predator attack (Chan, 2010; Sukarno *et al*., 2007) and were placed around the nest. By using a 2-inch stainless steel dial stem thermometer (Extech instruments corporation USA), three temperature readings were taken at 5 cm nest depth below the sand surface around the nest area. This procedure was repeated once a week till the end of incubation process.

The date of emergence was recorded once hatchling began to emerge. The nest was excavated at day three, and emergence success was calculated according to the above formula. Eggs survivorship (hatching success, survival hatchlings, unhatched eggs, and dead hatchlings) were counted and recorded according to the same formula as mentioned previously.

Hatchling size measurement

A random sample of 30 hatchlings were collected from each Styrofoam and Open Area nests for measurements of HSCL and weight. The HSCL was measured using the Vernier slide calliper, ±0.1 mm (Wood *et al*., 2014). The hatchling weight was measured using the electronic weighing balance, ±0.1 g.

Statistical analysis

Independent sample *t*-test was done using SPSS 17.0 version was used to compare the mean for two continuous variables (Pallant, 2002). In this case, independent sample *t*-test was used to test the incubation period between Styrofoam and Open Area nest plot. Pearson's correlation analysis was used to find a significant relationship between two continuous variables (Pallant, 2002). In this case, Pearson's correlation analysis was used to measure; 1) between eggs diameter and eggs weight 2) between incubation period and sand temperature. Chi-square test was used to discover if there is a relationship between pattern of emergence hatchlings with two categorical variables (2-days of emergence hatchlings and nest plots).

RESULTS

Eggs diameter and weight

Clutch size collected on Penang Island ranged from 94 to 155 eggs. Mean eggs diameter ranged from 39.05 to 40.98 mm, and mean eggs weight ranged from 32.93 to 38.67 g (n=10 nests). Overall mean eggs diameter was 39.71 mm and overall eggs weight was 35.14 g. There was a significant correlation between mean eggs diameter and mean eggs weight of 10 nests collected, Pearson's rank correlation coefficient (r) = 0.688, $p < 0.05$.

Hatching success and incubation period

For both nest plots, eggs were incubated from March to July 2014 and hatchling hatched between May-September 2014. Styrofoam nests took a slightly longer duration for eggs to hatch than eggs inside the Open Area nests. The incubation period of Styrofoam nests ranged from 53 to 61 days, while incubation period of Open Area nests ranged from 52 to 56 days (Table 1). There was a significant difference of incubation period for Styrofoam nests (M=56.30, SD=2.50) and Open Area nests [M=54.10, SD=1.29; $t(18)$=2.48, p<0.05, n=10], means that

Table 1. Total number of eggs, incubation date, hatching date, and incubation period between Styrofoam and Open Area nests

Turtle Tag	Nest No.	Nest type	Eggs	Sum of Eggs	Incubation date	Hatching date	Incubation period (days)
MY3423/	1A	Styrofoam	69	138	30/3/2014	25/5/2014	56
MY3424	1B	Open Area	69		30/3/2014	25/5/2014	56
	2A	Styrofoam	72	143	1/4/2014	25/5/2014	55
	2B	Open Area	71		1/4/2014	26/5/2014	56
	3A	Styrofoam	65	129	15/4/2014	8/6/2014	54
	3B	Open Area	64		15/4/2014	8/6/2014	54
	4A	Styrofoam	62	129	10/5/2014	6/72014	57
	4B	Open Area	67		10/5/2014	3/7/2014	54
	5A	Styrofoam	78	155	10/5/2014	3/7/2014	54
	5B	Open Area	77		10/5/2014	3/7/2014	54
MY3911/	6A	Styrofoam	63	126	14/5/2014	6/7/2014	53
MY3912	6B	Open Area	63		14/5/2014	5/7/2014	52
	7A	Styrofoam	52	104	31/5/2014	26/7/2014	56
	7B	Open Area	52		31/5/2014	23/7/2014	53
	8A	Styrofoam	48	96	23/6/2014	21/8/2014	59
	8B	Open Area	48		23/6/2014	15/8/2014	53
	9A	Styrofoam	63	126	28/6/2014	25/8/2014	58
	9B	Open Area	63		28/6/2014	21/8/2014	54
	10A	Styrofoam	47	94	3/7/2014	2/9/2014	61
	10B	Open Area	47		3/7/2014	27/8/2014	55

incubation period shows a difference between both nest plots. In addition, hatching success in Open Areas ranged from 61.7% to 100% (mean=79.94%), and in Styrofoam nests ranged from 40.43% to 100% (mean=72.45%).

Eggs survivorship

Open Area nests produced better survival hatchlings, Pearson's rank correlation coefficient (*r*) = 0.930, $p < 0.001$ than Styrofoam nests. Styrofoam nests produced survival hatchling that ranged between 34.04 to 98.61% (mean=69.68%), less than survival hatchling emerging from Open Area nests (ranged=51.06 to 97.18, mean=77.29% in Table 2). Result of hatching success between Styrofoam and Open Area nest is shown in Table 3. Mean of unhatched eggs in Styrofoam nests was 26.38%, while in Open Area nests was 19.86% (Table 2). Furthermore, mean dead hatchlings in Styrofoam nests was 2.77%, while in Open Area nests was 2.86%. Overall eggs survivorship result is shown in Table 3.

Emergence success

Emergence success on day-1 was 45.72% (283 hatchlings) and on day-2 was 16.48% (102 hatchlings). Lesser number of emergence successes was obtained in Open Area nests, where the emergence success on day-1 was 36.56% (227 hatchlings) and in day-2 was 10.63% (66 hatchlings in Figure 2). The pattern of hatchlings emergences (Figure 2) was not uniformly distributed ($\chi^2 = 13.33$, $df = 1$, $P < 0.001$). This shows that hatchling's emergence between Styrofoam nest and Open Area nest is not synchronized for day 1 and day 2.

Table 2. Eggs survivorship of 10 experimental nests between Styrofoam and Open Area nests

Turtle Tag	Nest no.		Eggs	Sum of eggs	Survival hatchling	(%)	Dead hatchling	(%)	Unhatched Eggs	(%)
MY3423/	1A	Styrofoam	69	138	38	55.07	0	0	31	44.93
MY3424	1B	Open Area	69		40	57.97	0	0	29	42.03
	2A	Styrofoam	72	143	71	98.61	1	1.39	0	0
	2B	Open Area	71		69	97.18	2	2.82	0	0
	3A	Styrofoam	65	129	29	44.62	8	12.31	28	43.08
	3B	Open Area	64		44	68.75	4	6.25	16	25
	4A	Styrofoam	62	129	48	77.42	0	0	14	22.58
	4B	Open Area	67		53	79.10	1	1.49	13	19.4
	5A	Styrofoam	78	155	73	93.59	2	2.56	3	3.85
	5B	Open Area	77		69	89.61	2	2.6	6	7.79
MY3911/	6A	Styrofoam	63	126	53	84.13	1	1.59	9	14.29
MY3912	6B	Open Area	63		57	90.48	1	1.59	5	7.94
	7A	Styrofoam	52	104	31	59.62	1	1.92	20	38.46
	7B	Open Area	52		40	76.92	0	0	12	23.08
	8A	Styrofoam	48	96	33	68.75	0	0	15	31.25
	8B	Open Area	48		35	72.92	0	0	13	27.08
	9A	Styrofoam	63	126	51	80.95	1	1.59	8	12.7
	9B	Open Area	63		56	88.89	2	3.17	5	7.94
	10A	Styrofoam	47	94	16	34.04	3	6.38	25	53.19
	10B	Open Area	47		24	51.06	5	10.64	18	38.3
	Total		1240		930		34		270	

Styrofoam nests		**Open area nests**	
Mean survival hatchling (%):	69.68±20.05	Mean survival hatchling (%):	77.29±14.19
Mean dead hatchling (%):	2.77±3.64	Mean dead hatchling (%):	2.86±3.17
Mean unhatched eggs (%):	26.38±17.51	Mean unhatched eggs (%):	19.86±13.21

Table 3. Result of eggs survivorship and nest success between Styrofoam and Open Area nests

Nest Type	Eggs	Sum of Eggs	Eggs survivorship	Total	%
Styrofoam	619	1240	**Eggs survivorship**		
			Survival hatchling	443	71.57
			Dead hatchling	17	2.75
			Unhatched eggs	153	24.72
			Nest success		
			Hatching success	460	74.31
			Emergence success	398	64.3
Open Area	621		**Eggs survivorship**		
			Survival hatchling	487	78.42
			Dead hatchling	17	2.74
			Unhatched eggs	117	18.84
			Nest success		
			Hatching success	504	81.16
			Emergence success	390	62.8

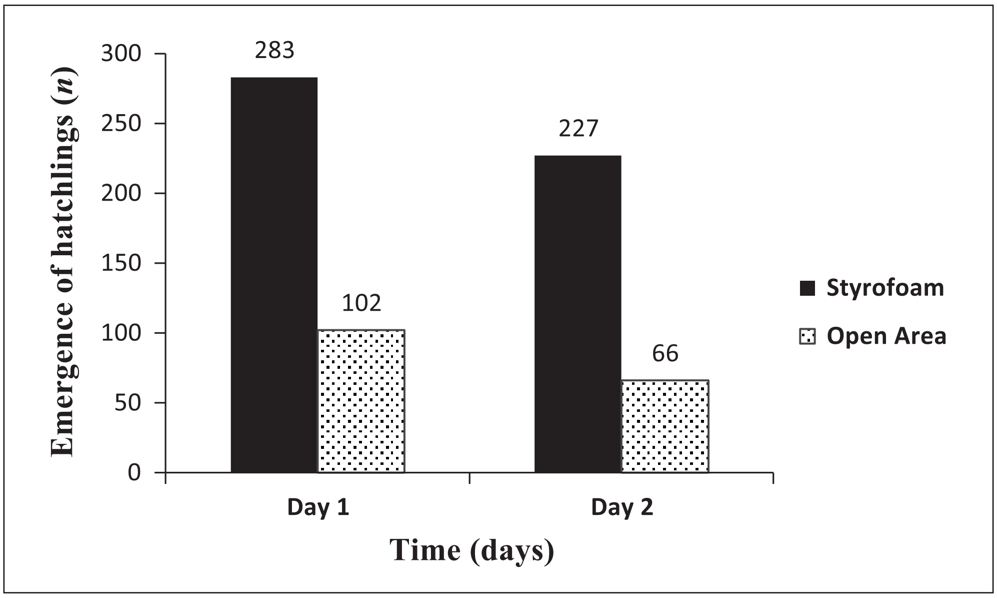

Fig. 2. Comparison of emergence success (day 1 and day 2) between Styrofoam and Open Area nest.

Hatchling morphological characteristics

In Styrofoam nests, mean HSCL ranged between 43.75 to 47.38 mm (overall mean=45.23±1.10) and hatchling weight ranged between 19.51 to 22.05 g (overall mean=20.19±0.70). Meanwhile, mean HSCL for hatchling produced in Open Area nests ranged between 44.62 to 47.57 mm (overall mean=45.89±1.10) and hatchling weight ranged between 19.40 to 22.32 g (overall mean=20.88 ±0.87) (Table 4). Open Area nests produced larger sizes and heavier hatchlings than hatchlings from Styrofoam nests. As presented in Table 5, Open Area nests had higher sand temperature in compared to Styrofoam nests, probably relating with the size of hatchlings.

Table 4. The measurement of HSCL and hatchling weight produced from Styrofoam and Open Area nests

Nest Type	*n*	HSCL (mm)		Hatchling weight (g)	
		Mean±SD	Mean range	Mean±SD	Mean range
Styrofoam	150	45.23±1.10	43.75–47.38	20.19±0.70	19.51–22.05
Open Area	150	45.89±1.10	44.62–47.57	20.88±0.87	19.40–22.32

Table 5. Temperature and incubation period between Styrofoam and Open Area nests

Nest Type	Temperature (ºC)		Incubation period (days)	
	Mean±SD	Range	Mean±SD	Range
Styrofoam	28.95±0.69	26.2–31.9	56.3±2.37	53–61
Open Area	29.78±0.37	27.4–33.0	54.1±1.22	52–56

Temperature

Overall mean temperature in Styrofoam nests was 28.95ºC, while the overall mean temperature in the Open Area nests was 29.78ºC (Table 5). There is a significant correlation between incubation period and mean temperature of Styrofoam nests, Pearson's rank correlation coefficient (r) = -0.705, $p < 0.05$, n=10.

DISCUSSION

Styrofoam nests produced lower hatching success because they were exposed to lower temperature, than Open Area nests. According to Booth (2006), the nests temperature throughout the incubation process influences the rate of embryo development. Eggs will only develop between 25°C and 34°C nest temperature (Bustard & Greenham, 1968). High temperature increases the metabolic process of embryonic eggs [George *et al*., 1994; Booth *et al.,* 2004; Booth, 2006; Booth & Evans 2011) and leads to faster egg development and produces a higher hatching success. More tissues are synthesised at higher temperature (Booth, 1998; Booth *et al.*, 2004; Booth & Evans, 2011). As the heat is generated by the embryo, it continues to grow and maintains the tissues (Booth *et al.*, 2004). This explains the reason why hatching success is decreased in Styrofoam nests whereas low temperatures are slowing down the metabolic rate and high temperature activates the metabolic rate. The production of hatching success also depends on the optimal sand temperature. Hatching success will decrease as nest temperature exceeds 34°C (Maulany *et al.*, 2012) due to exposure to the overheated environment. Nonetheless, eggs in natural nests are also able to survive short periods of exposure to temperatures as low as 18°C and as high as 45°C (Georges *et al.*, 2005).

The duration of the incubation period is influenced by temperature (Hays *et al*., 2002). Open Area nests show lesser incubation period than Styrofoam nests. Open Area nests were directly exposed to the sun resulting in a high metabolic heating towards eggs development (Eckert *et al.*, 1988) and the tissue synthesis was greater at higher temperatures, thus a shorter time was taken for the eggs to develop (Booth, 1998; Booth *et al.*, 2004). Therefore, high incubation temperature leads to a shorter incubation period duration (Glen *et al.*, 2005). Hence, Open Area nests took fewer days for the eggs to hatch. As the Styrofoam nests is covered with lid during the incubation process, the maximum temperature recorded was 31.9ºC, which occurred on 3rd July 2014, and the maximum temperature recorded in Open Area nests was 33.0ºC, recorded on the same date. Lower temperature slows down the egg metabolic process, thus resulting in a longer time for eggs to hatch.

Factors affecting hatching success and emergence success include the temperature during incubation (Glen *et al*., 2005; Wood *et al*., 2014), and hatching locomotors performance (Booth *et al*., 2004). In the present study, eggs incubated in both Styrofoam and Open Area nests at the same nest depth of 65 cm shows that Styrofoam nests had a better emergence success. Thermal gradients within nests relates to the asynchronous emergence, since the incubation duration of marine turtle eggs is related to temperature, eggs hatch quicker when the temperature is higher (Houghton & Hays, 2001). Furthermore, high temperatures inhibit digging activity (Rusli *et al*., 2016). Digging activity can resume after the sand cools later in the afternoon or at night and this is the reason why most marine turtle hatchling emergence events occurs at night or during cool cloudy days (Bustard, 1967; Gyuris, 1993). Hence, this might explain the reason Styrofoam nest had a better emergence success, as the nest plot was covered and had cooler sand surface.

Nest temperature influenced body size of green turtle hatchlings (Booth & Evans, 2011). According to Glen *et al.* (2005), hatchling size is also affected when the eggs are incubated under high temperatures. High temperatures may give the embryo the chance to channel excess energy towards its size (Foley, 2000; Booth, 2006). When eggs are exposed to high temperatures during the incubation period, the metabolic rate process is increased and become faster (Eckert *et al*., 1988); George *et al*., 1994; Booth *et al*., 2004) and eggs have higher chances in producing larger sizes hatchlings. Furthermore, when exposed to high temperatures, body tissue will generate more actively during the embryonic development stage (Booth, 2006; Booth & Evans., 2011). Therefore, eggs incubated in Open Area nests have longer HSCL and heavier hatchling weight than hatchlings from Styrofoam nests.

This current results represent a result from the conservation programme currently performed at Kerachut Turtle Conservation Centre in Penang Island. However, recent findings advice that it's not recommended to split the clutch size during incubation process as it will reduce the energy reserved when hatchlings enter the sea (Rusli *et al*., 2016). The result assumed that an increase in group size from 10 to 60 hatchlings caused an approximately 50% decrease in time taken to escape the nest and reduces the mean metabolic rate during this time, resulting in decreased energy expenditure during nest escape (Rusli *et al.,* 2016). Hatchlings entering the sea with larger energy reserves are presumably able to survive longer before finding food and therefore escaping the nest in larger cohorts probably results in hatchlings with greater fitness (Rusli *et al.,* 2016). Besides, hatchlings aggregation also decreases chance of predation (Unglaub *et al*., 2013). This result may have implications for conservation management of marine turtles in Malaysia as currently splitting the eggs method was actively performed in many Malaysian states (including Penang Island). Hence, a continuous study of splitting the eggs method in Styrofoam and Open Area nest is advisable performed at Kerachut Turtle Conservation Centre as we need to investigate the hatchlings energy reserved for this method in Penang Island. The outcomes of hatchlings energy reserved and hatchlings physical performance will give an impact to the sustainable of hatchlings population in Penang Island.

CONCLUSION

In conclusion, even splitting the eggs method in Open Area nest have produced a good rate of hatching success, and larger sizes of hatchlings morphological characteristics, it's advisable this study is further continued to test the hatchlings physical performance test (e.g. swimming speed, locomotors performance, and crawling speed test). Strong physical performance ability can affect the hatchlings longer survival in open oceans as they are highly exposed to predators (e.g. fish, eagle) in order to survive.

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