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Temperature Influence on Emergence Success and Swimming Speed for *In-Situ* Nesting for *Chelonia mydas* in Penang Island, Malaysia

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Highlights

- Hatching prefers to emerge during night-time rather than day-time.
- Higher sand temperature does influence the increase of swimming speed rate, and lower sand temperature reduces the energy of hatchlings swimming speed.
- Hatching success of in-situ nest does correlate with sand temperature.

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Temperature Influence on Emergence Success and Swimming Speed for *In-Situ* Nesting for *Chelonia mydas* in Penang Island, Malaysia

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Abstrak: Kajian ini dijalankan di Pulau Pinang, Malaysia dari Oktober 2013 hingga Mac 2015. Kajian dijalankan dengan menggunakan kaedah pengeraman sarang *in-situ,* dan telur dieram secara semulajadi di pantai Kerachut. Disebabkan kaedah *in-situ* tidak begitu digalakkan kerana risiko kecurian telur dan gangguan haiwan pemangsa, hanya sembilan telur dibenarkan untuk dieram. Tiga objektif telah dibentuk: pertama, kajian mengenai penentuan pengaruh suhu tanah ke atas keberjayaan penetasan, dan ke atas keberjayaan anak penyu sampai ke permukaan sarang. Kedua, kajian mengenai penilaian pengaruh suhu tanah ke atas kesan tahap kepantasan renang anak penyu, dan ketiga, kajian pantauan ke atas corak keberjayaan anak penyu sampai ke permukaan sarang di antara waktu siang dan waktu malam. Keputusan menunjukkan terdapat signifikasi korelasi di antara keberjayaan penetasan dan suhu tanah, dan di antara keberjayaan anak penyu sampai ke permukaan sarang dan suhu tanah. Di samping itu, terdapat signifikasi korelasi di antara suhu tanah dan tahap kepantasan renang anak penyu. Tambahan, kajian keberjayaan anak penyu sampai ke permukaan sarang menunjukkan bahawa anak penyu lebih terarah untuk muncul pada waktu malam berbanding waktu siang, dan peratusannya adalah 94.18%, 80.67% dan 78.05%, berdasarkan tinjauan dari hari pertama hingga hari ketiga. Purata keseluruhan panjang lurus karapas anak penyu adalah 40.80 mm ± 5.08, lebar lurus karapas anak penyu adalah 31.78 mm ± 4.55, dan berat anak penyu adalah 20.5 g ± 1.22. Untuk tujuan pemuliharaan pada masa hadapan, pertama, kami mencadangkan saiz sampel kajian sarang *in-situ* ditambah untuk tujuan ketepatan keputusan kerana kajian ini memberikan maklumat baru, dan meluaskan kajian dengan menambahkan kajian mengenai ciri-ciri tingkah laku lokomotor anak penyu (tahap kepantasan merangkak dan tahap kepantasan membalikkan sendiri). Kedua, kami mencadangkan kajian mengenai hubung kait di antara suhu air persekitaran dengan tahap kepantasan renang anak penyu.

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Kertas ini memberikan maklumat asas mengenai kajian pertama sarang *in-situ* di Pulau Pinang, dan cadangan yang diberikan mungkin akan memberikan kebaikan untuk tujuan pengurusan Pusat Konservasi Penyu Pantai Kerachut.

Kata kunci: Keberjayaan Anak Penyu Sampai ke Permukaan Sarang, Penyu Agar, Tahap Kepantasan Renang Anak Penyu, Suhu

Abstract: The study was performed in Penang Island, Malaysia from October 2013 to March 2015. This is the first study performed by using the *in-situ* incubation method, and the eggs were incubated naturally at Kerachut. As the *in-situ* procedure is not advisable to be performed due to risk from eggs poachers and predator disturbance, only nine nests were incubated. Three objectives were formulated: First, to determine the influence of sand temperature on the hatching success and emergence success. Second, to evaluate the effects of sand temperature on hatchlings swimming speed, and third, to observe the pattern of hatchling emergence between day-time and night-time. The result shows that there is significant correlation between hatching success and emergence success on the sand temperature, and also a significant correlation between sand temperature and swimming speed. Furthermore, the study identified that the hatchling emerges during night-time rather than day-time, and the percentage was 94.18%, 80.67% and 78.05%, based on observation from Day 1 until Day 3. The overall mean hatchlings straight carapace length was 40.80 mm ± 5.08, mean hatchlings straight carapace width was 31.78 mm ± 4.55, and mean hatchlings weight was 20.5 g ± 1.22. For future conservation, first, the sample size of *in-situ* nests is suggested to be increased because the result from the study provides a new knowledge, and widens the scope of the research by including the study on hatchlings locomotors performance (crawling performance and self-righting performance). Second, additional study on the relationship between surroundings water temperatures on the swimming speed performance is also suggested. This paper provides basic knowledge for the first research of *in-situ* nesting in Penang Island, and the recommendation may benefit the management of the Kerachut Turtle Conservation Centre.

Keywords: Emergence Success, Green Turtle, Swimming Speed, Temperature

INTRODUCTION

Nest environment has a fundamental influence on sea turtle reproduction because the adult female turtles leave their eggs on the beach naturally without any other parental care (Kamel & Mrosovsky 2005), which means that the turtle eggs depend on environmental condition and rookeries properties for survival. Environmental factor such as nest temperature during incubation is able to influence the incubation period (Deeming & Ferguson 1991), hatching and emergence success (Matsuzawa *et al*. 2002; Glen *et al*. 2005), hatchling morphological characteristics (Glen *et al*. 2003; Özdemir *et al*. 2007), and hatchling locomotors performance (Booth *et al*. 2004). Furthermore, warmer sea surface temperature near the breeding sites is associated with an earlier date of first female emergence than usual (Mazaris *et al.* 2008). In addition, warmer temperature able to increases the rate of hatching success (Mazaris *et al.* 2008). As the gender of sea turtles is determined by the temperature of incubation (Mrosovsky *et al.* 2002), increase of air and sand temperatures may affect sex ratios of populations (Katselidis *et al.* 2012; Laloë *et al.* 2016). Rookery properties such as shade (i.e., vegetation characteristics), beach orientation (i.e., north or south facing, near or far from tide line), and colour of sand ranges influence the thermal environment of the nest, and accordingly change the emergence success in any particular area (Wibbels 2003) as well as the eggs' survivorship.

Upon hatching from the nests, the hatchlings find their way to the sea by themselves. This movement is known as "seafinding", influenced by two essential cues, which are light intensity and horizon elevation (Tuxbury & Salmon 2005). During light intensity, light reflected from the ocean is absorbed by vegetation behind the beach (Tuxbury & Salmon 2005). The hatchlings then crawl away from the dimmer landward horizon, and crawl by using both flippers toward the brighter seaward horizon (Van Rhijn & Van Gorkom 1983). The second cue is horizon elevation, where turtles tend to crawl away from a higher dune, which is related to shrubbery, and toward the lower, and oceanic horizon (Van Rhijn & Van Gorkom 1983). The hatchlings typically enter a "swimming frenzy" once reaching the water that quickly moves them into offshore oceanic currents (Hays *et al.* 2010). During the swimming frenzy condition, the hatchlings are able to swim continuously for approximately 24 h (Salmon *et al.* 2009), depending solely on their yolk reserves in the body (Wyneken 1997). At this point, the swimming speed of hatchlings is measured to investigate the influence of sand temperature to the swimming speed rate as hatchlings are produced from the *in-situ* nests.

The sea turtles are characterised by the fact that they have few enemies and high fecundity (Heppell *et al*. 2003) from the beginning of the incubation stage. The sea turtles typically suffer high levels of mortality from predators of aquatic, array of terrestrial, and aerial from eggs, hatchlings, and juvenile stages (Wyneken 2000). Two factors are identified in determining the hatchlings survivorship, which are: (1) the timing and (2) pattern of emergence of hatchlings from the nest (Glen *et al*. 2005). The critical time is during the journey of the sea turtles crawling towards the sea as they tend to attract predators. The hatchlings are vulnerable to predators within minutes after they begin swimming, because they are detected in shallow waters, have virtually no defence against an attack, and incapable of swimming faster than their predators (Whelan & Wyneken 2007). Many researchers agree that most hatchlings prefer to emerge from nest at night (Gyuris 1993) to avoid daytime lethal temperature and potential diurnal predator (Glen *et al*. 2005; Salmon *et al*. 2009). Yet, some daytime emergence has also been reported (Drake & Spotila 2001). Likewise, the pattern of emergence, which is the movement of the hatchlings to the sea in groups, is believed to reduce the probability being attacked through the predator dilution (Dehn 1990). Therefore, group formation is a common behaviour among prey species to reduce predation risk (Santos *et al.* 2016). Despite the various factors that promote intra-clutch variation leading to asynchronous hatching and emergence from nests, synchronous hatching and emergence occurs in many taxa, in eggs-laying animals (Santos *et al.* 2016).

In this paper, three objectives are formulated: (1) to determine the influence of temperature on hatching success and emergence success, (2) to evaluate the effects of sand temperature on hatchlings swimming speed, and (3) to observe the pattern of hatchling emergence between day-time and night-time. This is the pioneer study concerning the *in-situ* nest in Penang Island, as the Kerachut Turtle Conservation Centre does not prefer to authorize the *in-situ* eggs incubation due to predator attack [i.e. smooth coated otter, and common palm civet (Sarahaizad *et al*. 2012), and lack of staffs to control the poaching of eggs. Therefore, only nine nests were allowed to naturally incubate as *in-situ* without relocating it to the hatchery. The *in-situ* nests were observed for the eggs' success (hatching success and emergence success) and the correlation with the sand temperature was studied. The biological factor (incubation period), and ecological factors (nest distance, depth and moisture content) were also recorded. The pattern of hatchlings emergence was also observed between day-time and night-time. Hatchlings morphological characteristics, hatchlings swimming speed, and eggs survivorship were also studied.

MATERIALS AND METHODS

In-situ Nests Incubation Procedure

The study was conducted from October 2013 to March 2015 at Kerachut, Penang Island (Fig. 1). During the study, the procedures outlined by the Malaysia Department of Fisheries were followed whereby only limited numbers of nests (nine nests) were allowed to incubate as *in-situ* due to the unfavourable nesting ground factors (i.e., predators disturbance). The procedure of beach surveying during the nocturnal surveys in Penang Island is based on the official standard steps by the Department of Fisheries Malaysia (2016) with the guidance by staffs from Kerachut Turtle Conservation Centre. Once the turtle lands and lay nest by itself on the beach, the nest is identified for labelling purpose. Nest temperature and sand sample were collected after the green turtle lay nest and returned to the sea as the turtle is sensitive to human foot step and little nosiness may disturb the nesting process. The nest was labelled with a piece of bamboo plank, and important information such as nesting date, expected hatching date, and adult tag number were recorded. The nests were covered with squared green netlon mesh that was placed around the nests (Department of Fisheries Malaysia 2016) to avoid hatchlings from crawling themselves towards the sea, or to prevent monitor lizard and ghost crab from digging the nest. The nests (9 nests) were allowed to incubate without disturbance until hatchlings emerge from nests. According to Sukarno *et al.* (2007), the estimate days for hatchling emergence for green turtle in Malaysia was between 54–76 days. However, early emergence might occur depending on temperature and environmental condition. Incubation period was calculated from the first day of incubation until the first day of the emergence of the hatchlings from nest.

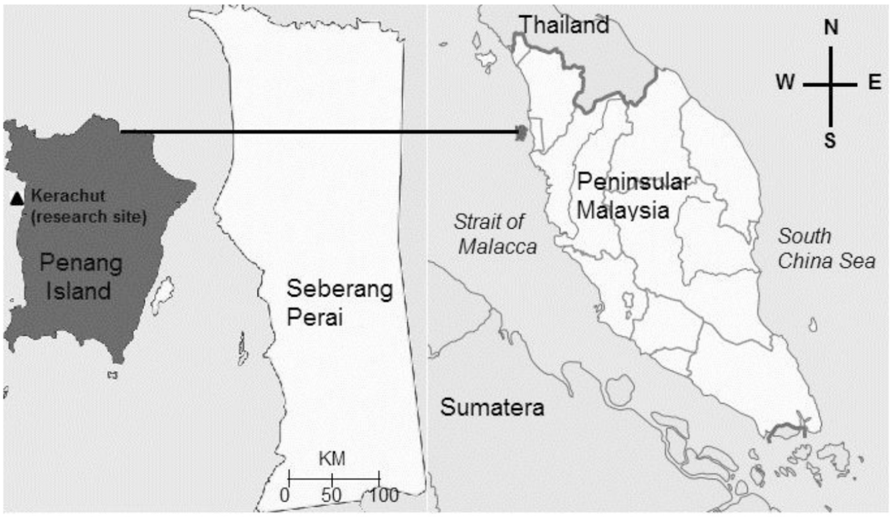

Figure 1: Location of surveyed beach, Kerachut, located in Penang Island, Malaysia.

Environmental Factors Measurements

Sand temperature was measured using the soil thermometer, ±0.1°C at 5 m depth, 1 m from nest, and at the same distance from high tide line (López-Castro *et al.* 2004). In addition, nest temperature during incubation were taken once a week consistently between 12:00 to 13:00 hrs (three readings were taken at one time and the average were calculated). Nest distance was measured using 30 m measuring tape, ±0.1 cm (the distance is from water's edge to nest). This procedure is based on the method prescribed by López-Castro *et al*. (2004); Zare *et al.* (2012).

Hatchlings Morphological Characteristics

After 45 days of incubation, the *in-situ* nests were monitored for hatchlings emergence. Hatchling that emerged were let to crawl themselves upwards without assistance, and were collected and moved into the styrofoam boxes. The time of hatchlings' emergence and temperature were recorded. Haphazard samples of 30 hatchlings from each *in-situ* nest were separated and the morphological characteristics and swimming speed were measured. Each hatchling was weighed using the electronic balance (±0.1 g) and the straight carapace length and straight carapace width was measured using the electronic calliper (±0.1 mm) (Pereira *et al*. 2012; Wood *et al*. 2014).

Swimming Speed

About 2.0 m length of monofilament fishing line was tied at both the flippers of each hatchling. Then, tethered hatchlings were brought into the open ocean of Kerachut beach and were released into the ocean. To avoid shocking before the swimming speed test begins, the hatchlings were released slowly to swim for the initial one minute around the area to stabilise with the temperature. Then, the tethered hatchlings were released and allowed to swim for 2.0 m away from the starting point or until the fishing line becomes tense (Chung *et al*. 2009). A stop watch was employed to measure the time of ±0.1 s allocated for each hatchling to complete the 2.0 m swimming speed test. After completing the first trial, the flippers were removed from the hatchlings pulled back slowly to the starting point and allowed the hatchlings to swim in the area before being released for a second trial. This test was repeated for three trials of straight-line swimming. This procedure was conducted based on the method by Chung *et al*. (2009) and Salmon *et al.* (2009).

Emergence Success, Hatching Success and Eggs Survivorship

All hatchlings were left to emerge by itself without assistance (Glen *et al*. 2005) as the exact number of hatchlings that were able to crawl upwards to the surface without any guidance was later recorded. Two type of observation were made, namely day-time and night-time. Hatchling emergence was observed between 08:00 to 18:00 hrs or known as day-time emergence, and between 20:00 to 05:00 hrs for night-time emergence. This observation was recorded for three conservative days. Nests were excavated on the fourth day to allow the hatchlings enough time to crawl to the surface. Hatched eggs fragments, unhatched eggs, and dead hatchlings were enumerated separately. Remaining survival hatchlings inside the nest were quantified for eggs survivorship calculations. After the nest was completely excavated, the *in-situ* nesting depth was measured using a 2 m measuring tape (±0.1 cm). Emergence and hatchling success were calculated.

1. Emergence success (%): [Clutch size – (unhatched eggs + dead hatchling + survival hatchling in clutch)/ clutch size ×100] (Formula by Wood *et al*. 2014).

2. Hatching success (%): [(Clutch size – number of unhatched eggs)/total clutch size × 100] (Hitchins *et al*. 2004; Zare *et al*. 2012).

Eggs survivorship was divided as survival hatchlings, dead hatchlings, and unhatched eggs. Survival hatchlings were calculated as: (number of hatched eggs fragment – number of dead hatchlings)/ clutch size × 100 (Chan 2013), and dead hatchlings were calculated as: [(number of hatched eggs fragment – number of survival hatchlings)/clutch size × 100. Unhatched eggs were calculated as: Clutch size – [(number of hatched eggs fragment + dead hatchlings)/clutch size] × 100.

Statistical Analysis

SPSS 17.0 version and Microsoft Excel were used to analyse the data. Spearman's rank correlation coefficient (ρ) was employed as the numbers of *in-situ* nests investigated were small. Spearman's rank correlation coefficient (ρ) was used to find a significant correlation between eggs success and hatchlings morphological characteristics. Microsoft Excel was utilised to calculate mean and standard deviation for biology and ecological factors of *in-situ* nests. An independent sample *t*-test was used to compare the significant difference between hatchling emergence of day-time and night-time, as the test were used to compare mean between two groups (Pallant 2002).

RESULTS

Sand Temperature, Nesting Depth, Moisture Content, and Incubation Period

Mean nest distance for nine *in-situ* nests were 32.14 m ± 7.36, ranged between 19.9 to 45.70 m, follow by mean nesting depth was 68.56 cm ± 7.21, ranged = 56.0 to 83.0 cm. The lowest mean sand temperature was 26.1°C and the highest mean sand temperature was 32.4°C (Table 1). Overall, mean sand temperature was 28.81°C ± 1.96. The sand moisture content was measured, and the mean was 0.42% ± 0.17, ranged from 0.1% to 0.7%. Meanwhile, the mean incubation period for nine *in-situ* nests was 51.0 days, or between 44 to 57 days for eggs to hatch. No significant correlation was found between sand temperature and incubation period with Spearman's rank correlation coefficient indicating (ρ) = –0.152, $p > 0.05$.

Table 1: Breeding biology and ecology factors of *in-situ* nests in Malaysia.

Nest	Date incubation	Date hatching	Nest distance (m)	Nest depth (m)	Mean temp. (°C)	Moisture content (%)	Incubation period (days)
1	19 October 2013	10 December 2013	36.3	75	27.3	0.3	52
2	25 October 2013	15 December 2013	27.5	65	30.5	0.7	51
3	5 November 2013	1 January 2014	26.1	70	26.1	0.1	57
4	15 November 2013	7 January 2014	31.1	62	27.8	0.4	53
5	5 December 2013	23 January 2014	32.0	70	27.4	0.5	49
6	18 March 2014	4 May 2014	30.0	83	27.4	0.4	44
7	28 April 2014	19 June 2014	19.9	68	29.7	0.6	52
8	8 May 2014	30 June 2014	45.7	68	32.4	0.5	53
9	4 March 2015	24 April 2015	40.7	56	30.7	0.3	48

Hatching Success, Emergence Success and Eggs Survivorship

A total of 1,129 eggs were collected from nine *in-situ* nests, and clutch size ranged between 104 to 146 eggs. All eggs were calculated for eggs survivorship after the incubation process, and result shows a total of 934 eggs, or 82.73% successfully hatched (survival hatchlings) and 144 eggs or 12.75% considered as unhatched eggs (Table 2). Furthermore, 47 hatchlings were identified dead, which contributed to 4.16%, and another unexpected four hatchlings (0.35%) were considered as abnormal hatchlings as these hatchlings were examined. The abnormalities identified were hatchlings with blind eyes, abnormal flippers, and unsymmetrical carapace. The mean hatching success for *in-situ* nest was found at 86.54% ± 11.14 (ranged = 59.62 to 97.60%, Table 2), where the percentage is considered as high.

Table 2: Eggs survivorship, hatching success, emergence success and preferable sand's surface temperature for hatchlings emergence.

Nest	Clutch size	Survival hatchlings	Dead hatchlings	Unhatched eggs	Abnormal hatchlings	HS (%)	ES (%)	Mean sand's surface temperature for hatchlings emergence (°C)
1	114	89	3	22	0	80.70	74.56	28.3
2	125	117	5	3	0	97.60	60.80	28.18
3	126	101	2	23	0	81.75	29.37	27.68
4	128	105	6	13	4	89.84	79.69	28.35
5	104	60	2	42	0	59.62	32.69	28.4
6	142	113	9	20	0	85.92	57.04	27.93
7	137	113	14	10	0	92.70	62.77	29.15
8	146	140	2	4	0	97.26	92.47	29.15
9	107	96	4	7	0	93.46	86.92	29.28

Correlation between hatching success of *in-situ* nests with ecological factors such as sand temperature, moisture content, and nesting depth were investigated. The result shows that hatching success significantly correlate with sand temperature, Spearman's rank correlation coefficient (ρ) = 0.862, $p > 0.001$, and with nesting depth as indicated by Spearman's rank correlation coefficient (ρ) = –0.672, $p < 0.05$. No correlation was found for hatching success with nest distance and moisture content (Table 3).

Mean emergence success was 64.03% ± 20.91 (ranged = 29.37 to 92.47%). The emergence success was found to correlate with sand temperature, as indicated by Spearman's rank correlation coefficient (ρ) = 0.728, $p < 0.05$ (Table 3). The overall mean sand surface temperature was 28.49°C ± 0.54, and the mean sand surface temperature ranged from 27.68 to 29.28°C per nest, which was considered as a preferable temperature for hatchlings to emerge from nest (Table 2). Emergence success per nest also significantly correlate with the sand's surface temperature as indicated by Spearman's rank correlation coefficient (ρ) = 0.703, $p < 0.05$.

Table 3: Spearman's rank correlation coefficient (ρ) of hatching success and emergence success with ecology factors.

	Nest distance (m)	Nest depth (m)	Mean temperature (°C)	Moisture content (%)
Eggs success				
Hatching success (%)	$p > 0.05$	$p < 0.05$ (sig.)	$p < 0.001$ (sig.)	$p > 0.05$
Emergence success (%)	$p < 0.05$ (sig.)	$p > 0.05$	$p < 0.05$ (sig.)	$p > 0.05$
Hatchlings morphological characteristics				
Straight carapace length (mm)	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Straight carapace width (mm)	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Weight (g)	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

An independent sample *t*-test was used to compare the hatchling emergence between day-time and night-time for the nine *in-situ* nests. There was a significant difference between hatchling emergence in day-time (M = 10.22 ± 9.86) and night-time (M = 70.78 ± 33.04); t (16) = –5.27, $p < 0.001$. The emergences of the hatchlings were recorded for three conservative days, and the differences of hatchling emergence between day-time and night-time are presented in Fig. 2. The frequency of hatchling emergence in day-time was 22 in Day 1, 52 in Day 2, and 18 in Day 3, which contribute to 5.82%, 19.33%, and 21.95% from total hatchling emergence per day. In comparison, there are 356, 217, and 64 emergence of hatchlings or 94.18%, 80.67%, and 78.05% percentage of hatchling emergence in Day 1, Day 2 and Day 3, respectively.

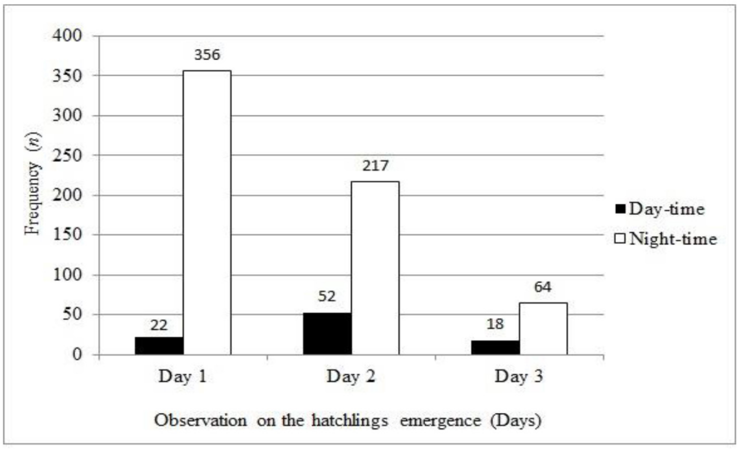

Figure 2: Comparison of hatchling emergence between day-time and night-time.

Hatchlings Morphological Characteristics

The overall mean hatchlings straight carapace length for nine *in-situ* nests was 40.80 mm ± 5.08 (ranged = 34.53 to 47.32 mm), mean hatchlings straight carapace width was 31.78 mm ± 4.55 (ranged = 25.82 to 37.33 mm), and mean hatchlings weight was 20.5 g ± 1.22 (ranged = 18.79 to 22.3 g, Table 4). No significant correlation was found between hatching morphological characteristics with any of the ecological factors (i.e., temperature, and moisture content, Table 3), which shows that nest distance, nest depth, sand temperature and sand moisture do not influence the hatchling sizes (straight carapace length, straight carapace width and weight).

Table 4: Hatchlings morphological characteristics of *in-situ* nests.

Nest no.	Length (mm)	Width (mm)	Weight (g)	Swimming speed (m/s)
1	47.32	37.33	22.3	0.055
2	35.87	27.12	21.8	0.066
3	35.89	28.32	18.96	0.045
4	34.65	26.16	19.28	0.069
5	34.53	25.82	18.79	0.061
6	44.61	35.15	21.7	0.045
7	45.37	36.38	20.64	0.049
8	45.31	36.2	20.74	0.066
9	43.65	33.51	20.19	0.091

Swimming Speed

Overall mean swimming speed for nine *in-situ* nests was 0.061 m/s ± 0.014 (ranged = 0.045 to 0.091 m/s). A significant correlation was found between sand temperature and swimming speed as indicated by Spearman's rank correlation coefficient (ρ) = 0.696, $p < 0.05$.

DISCUSSION

Most hatchlings prefer to emerge during night-time (Gyuris 1993). In this study, the hatchling prefers to emerge during night-time; between 20:00 to 05:00 hrs rather than day-time; between 08:00 to 18:00 hrs, as the sand temperature is lower during the former. Hatchlings generally crawl from the nest to the sea after the surface sand cools down (Salmon *et al*. 2009), probably because they prefer a cooling effect during the non-daylight hours. This is supported with a significant correlation found between emergence success and sand's surface temperature. It was believed that hatchlings' crawling at night may reduce the risk of the mortality (Santos *et. al.* 2016) as hatchlings are exposed to minimum risk by land predators such as ghost crab, monitor lizard, and eagles. The nocturnal emergence will prevent death by overheating and desiccation, and decrease predation by visual and diurnal predators (Drake & Spotila 2001). At the same time, the hatchlings crawling upwards towards the surface in a group to avoid the predators attack (Glen *et al*. 2003).

The phenomenon of emergence synchrony is predicted to be favoured by natural selection (Glen *et al.* 2005), because mass departure with large groups of hatchlings should saturate the foraging ability of predators, thereby reducing the predation threat to individuals (Dehn 1990). The result can be seen in Fig. 2, where clustered emergence was higher at night-time rather than day-time. The researcher observed that the higher clustered emergence was in a group of 115 hatchlings, which occurred at night-time (28.1°C) and the lowest was in a group of two hatchlings (28.7°C) occurred in day-time (Sarahaizad, personal observation). This performance was typically believed to reduce the predation as the synchrony of sea turtle hatchlings emerged within a nest (Tucker *et al.* 2008). Santos *et al.* (2016) also supported that the increase of group size (number of hatchlings emerging together from a nest) reduces green turtle (*Chelonia mydas*) hatchling predation. Research by Peterson *et al.* (2013) using freshwater turtles as a proxy for sea turtle hatchlings shows a decrease in the per capita predation risk with increased group size of hatchlings. However, although the large groups formed by hatchlings during their emergence from nests have long been predicted to be an anti-predator strategy (Spencer & Janzen 2011), the relationship between their group size and predation risk remained unknown (Heithaus 2013), and this hypothesis remains to be tested (Heithaus 2013). Additionally, according to Santos *et al.* (2016), delay in emergence within an evening was associated with a decrease in risk, particularly for hatchlings emerging in smaller group sizes, due to fewer predators and an increase in the proportion of those already preoccupied with prey. This result suggests that delaying emergence, rather than synchrony, would be advantageous at the scale within the evening.

The result shows that temperature does influence the swimming performance of hatchlings. Result in this study proves that higher sand temperature does influence the increase of swimming speed rate, and lower sand temperature reduces the energy of hatchlings swimming speed. Hatching were incubated at sand temperature ranging between 26.1°C to 32.4°C. Higher temperature is believed to increase the energy of the hatchlings to swim and move the flippers. This finding is consistent with previous study by Burgess *et al.* (2006) that green turtle hatchlings from eggs incubated at 25.5°C and 26°C were poor swimmers than hatchlings from eggs incubated at 28°C and 30°C.

As the same neuromuscular systems are used in swimming and crawling, researchers anticipate similar correction of crawling behaviours with sand temperature (Ischer *et al.* 2009). However, the result contradicts. The summary by Ischer *et al.* (2009) explained that hatchlings from high temperature nest tend to be faster swimmer, but not synchronised with crawling speed; whereby crawling speed is greater in green turtle hatchlings that emerge from cooler nests. According to Ischer *et al.* (2009), hatchlings from cool nests had larger carapace dimensions and longer limbs than hatchlings from warmer nests, suggesting that more yolk was converted to hatchling tissue during embryonic development in cool nests. The inter-individual differences in swimming motivation indicated by differences in the time spent power stroking (swimming performance) rather than physiologically constrained differences in muscle flippers performance (crawling performance) may explained for the differences of swimming and crawling behaviour (Ischer *et al.* 2009). As this study only allows incubating of only nine *in-situ* nests for this testing, there is a need to request for permission from the Malaysia Department of Fisheries for further testing. Researchers of this study suggest increasing the sample size for this testing; as larger sample size of *in-situ* nest may provide stronger interaction between temperature and the effect on hatchlings swimming speed.

Climate plays a critical role in the survival of sea turtles both on the beach and at sea and further study is required to improve the understanding of the connection between environmental features and sea turtle survival (Patel *et al*. 2016). For example, warm water temperature is also able to increase the swimming speed, with hatchling swimming in warm water have faster stroke rate than cooler sand temperature (Booth & Evans 2011). With an increase in body temperature, warmer water temperature generally increases in the muscle metabolism by the cardiovascular system (Booth & Evan 2011). Additionally, an increase in heart rate with surrounding warmer water temperature is a common phenomenon in ectothermic vertebrates, and can result in an increase in the rate of delivery of nutrients and oxygen (Hill *et al.* 2004). Under such circumstances, the faster the hatchlings swim, the greater chance of making to the relative safety of deeper off-shore water (Booth & Evan 2011) for survival.

The current study indicates that global warming may affect sea turtle hatchlings swimming ability and their survival through the effects on increasing nest temperature and sea surface temperature (Booth & Evan 2011). As sand temperature increases and precipitation declines in the future, hatchlings demographic could skew towards a more female bias (Patel *et al*. 2016). According to Patel *et al*. (2016), sea turtles are vulnerable to climate change impacts in both their terrestrial of nesting ground and oceanic habitats. Higher sand temperatures have been demonstrated to clearly reduce hatching success of sea turtles (Santidrián-Tomillo *et al.* 2014). In addition, it also impacted behaviours of adult turtles. As warm temperatures are in both air and sea, it can result in earlier shifting in nesting seasons and could substantially reduce the mean clutch sizes in future (Patel *et al*. 2016). A shift in phenology from the impact of temperature increase may have effect on the balance of the potential decline in clutch size, and may have far-reaching effects on the condition of reproductive success (Patel *et al*. 2016) in Mediterranean Sea. This may produce a net negative impact on the future population.

This study also found that hatching success of *in-situ* nest does correlate with sand temperature. As this is the first publication pertaining the result of *in-situ* nest in Penang Island, the comparison was made with the result of translocate nests in Penang Island. The result is found to be similar (Sarahaizad *et al.* 2017) as incubation temperature does influence the rate of hatching success (Wood *et al.* 2014). When eggs incubate at high temperature, the metabolic process of embryonic eggs increases (Booth & Evans 2011), and the process leads to rapid egg development and produces a higher hatching success. Moreover, when eggs incubate at lower temperature, the growing process of the foetus reduces, and decreases the rate of hatching success. At higher temperature, more tissues are synthesised (Booth & Evans 2011). However, there is a limitation and hatching success will start decreasing at extreme environment when nest temperature exceeds 34°C (Maulany *et al*. 2012). High incubation temperature leads to a shorter incubation period duration (Glen *et al.* 2005), and suggested duration of the incubation period is influenced by temperature (Hays *et al*. 2002). However, Georges *et al.* (2005) suggest that eggs incubated in *in-situ* nest are also able to survive when exposed to temperature as low as 18°C and as high as 45°C, but for a shorter period.

There was no significant correlation between temperature and incubation period, whereas the result contradicted with the translocated result in the previous studies (Sarahaizad *et al.* 2017) in Penang Island. The reason is probably because translocate nest were constantly incubated under the constant temperature in Styrofoam nests. However, *in-situ* nests were exposed to environmental temperature that fluctuated as well as extreme and low temperature. The fluctuating temperature such as heavy rain and strong sunlight may influence the incubation period. In addition, hatching's straight carapace length, width, and weight do not correlate with sand temperature. The result was similar to the study of Ischer *et al.* (2009) in Australia. It was probably due to the natural temperature and other environmental factor such as moisture content that influenced the sizes of the hatchlings.

A significant correlation was not found between hatchlings' morphological characteristics and sand temperature. As only the sand temperature was investigated, monitoring the whole nest temperature was suggested; where eggs heating will be monitored for the whole incubation (Booth *et al*. 2004). From the reading, the exposure of the eggs to a longer or shorter period of extreme temperature was also investigated, which influences the 12.75% unhatched eggs, 4.16% dead hatchlings, and 0.35% abnormal hatchling produced from *in-situ* nests (Table 2). Moreover, it was suggested that the position of eggs during incubation, the density of the sand, and anthropogenic factors also influence the mortality of eggs and hatchlings from *in-situ* nests.

In addition, there are a few recommendations listed for future conservation in Penang Island. First, further discussion with the Malaysia Department of Fisheries for continuing the study of *in-situ* nests in Penang Island was suggested. As this experiment produces new outcomes, the sample size of *in-situ* nests is requested to be widen by including the study on hatchlings' locomotors performance (crawling performance, self-righting performance). The future result of hatchling locomotors performance (crawling performance) in *in-situ* nests might be useful for the conservation of green turtle in Penang Island, since many studies suggest that hatchlings are fast crawler and are able to escape from land predators. Moreover, increased energy enables to prolong the survival of the hatchlings while crawling towards the sea (Rusli *et al*. 2016). In addition, recent findings advice that it is 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). Currently, it was estimated that 50% of yearly nests (Sarahaizad, personal observation) collected at Penang Island would incubate as split eggs. The study recommends reducing the procedure of split eggs, as it will impact the population of hatchling as the methods are decreasing the energy of the hatchlings (Rusli *et al.* 2016). The study on locomotors performance of the hatchlings can be conducted by referring to previous method by Booth *et al*. (2004). Second, the study on the effect of water temperature and sand temperature (Booth & Evans 2011) on the ability of swimming performance of *in-situ* nests is important since hatchlings with greater energy reserves are good swimmers, and presumably are able to increase the survival rate before finding food (Rusli *et al.* 2016). Therefore, the study on the relationship between water temperature and sand temperature on the swimming speed performance is suggested. Third, the comparison of hatching success and emergence success between *in-situ* and translocate nests is also suggested to be conducted. This test can be achieved by referring to the procedure by Ischer *et al.* (2009), and Booth and Evans (2011). Fourth, the study on the hatching success and the relationship with the attack by land predators are suggested to be performed. From the test, the exact number and percentage of *in-situ* nest that are destroyed from natural land predators can be calculated. Moreover, the types of destroyed nests by different land predators are to be investigated. Therefore, the exact percentage of nests that are destroyed by predator is advised to be investigated. This study can be achieved by referring to the study by Madden *et al.* (2008) where predated nests on the beach were accurately identified. Lastly, the study should be widening on the prediction of climate change impact on nesting distribution in Penang Island. For example, the study by Patel *et al.* (2016) predicts that global warming has a negative impact on future loggerhead population. The rising of sea surface temperature and air temperature have a significant effect on the quantity, timing, and quality of loggerhead nesting in the Mediterranean Sea (Santidrián-Tomillo *et al.* 2014). Therefore, the same research could be applied in Penang Island by using a climate model projection, by referring to the method by Patel *et al.* (2016).

CONCLUSION

This study provides basic knowledge for the first research of *in-situ* nesting in Penang Island. By updating the current result of *in-situ* nest, the results might be helpful for the purpose of conservation. Therefore, the *in-situ* and translocation eggs experiment is suggested to be further investigated as it is perceived beneficial. The recommendation given in this paper may provide benefit for the reason to upgrade the management of the Kerachut Turtle Conservation Centre and the Malaysia Department of Fisheries in the future.

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