Individual nest site preference of green turtle, *Chelonia mydas*, on Mak Kepit beach and its relation with hatching emergence success

AHMAD ALI, KU KASSIM KU YAACOB, SOLAHUDDIN A. RAZAK, ZULKIFLI TALIB
Marine Fishery Resources Development and Management Department
Southeast Asian Fisheries Development Center
21080 Kuala Terengganu, MALAYSIA
Corresponding author: aaseafdec@mfrdmd.org.my

ABSTRACT
We investigated 160 individual *in-situ* nests of 24 female green turtles which laid their eggs in 4 to 10 clutches during the 2002 nesting season in order to determine whether and how nest site preferences vary among individuals. We also quantified the impact of this maternal behavior on reproductive success by evaluating offspring hatching and emergence success. We found that there was a significant repeatability of female preferences to nest under canopies at 70.6% (113 nests) compared to grasses/creeper areas at 22.5% (36 nests) and bare sand areas at 6.9% or 11 nests. The density of nests under canopies was the highest at one nest per 4.2 m^2 (1/4.2 m^2) followed by grasses and bare sand areas at 1/12.8 m^2 and 1/36.6 m^2 respectively. Most turtles preferred to lay their eggs on the right side of their first clutch and closer to the forest line. Hatching success was higher for nests situated within bare sand areas at 92.1% compared to 88.7% within grasses areas and 88.2% under canopies. The emergence success was also higher for nests situated within bare sand at 88.2% compared to 81.2% under canopies and 79.9% within grasses areas. However the percentages of undeveloped eggs were higher for nests situated within grasses areas compared to under canopies and bare sand areas at 4.7%, 2.6% and 1.5% respectively.

KEYWORDS: Nesting behaviors, green turtle, hatchlings emergence successes

INTRODUCTION

Nest site selection, which is a non-random placement of eggs within a particular area is important in species lacking parental care, because the consequences of poor nest site choice cannot be compensated for by behavior of the parents. Sea turtles are ideal animals for studying nest site preference because they lay multiple clutches within a nesting season at 10-20 day intervals, so the behaviour can be measured repeatedly within a reasonable time frame. The lack of parental care makes nest site preference particularly important for the survival of their offspring (Kamel and Mrosovsky, 2005). This behavior is also significant in sea turtles, because they show temperature-dependent sex determination. High consistency of maternal nest site choice may allow for control over offspring sex ratios and this maternal trait is a parameter included in theoretical treatments of the evolution of temperature-dependent sex determination (Bumer and Bull, 1982).

Females lay their eggs high up the beach usually adjacent to or within the vegetated strand. A warm nest during mid incubation results in all (or mostly) female hatchlings while males come from cool nests. Selection of totally unsuitable nesting substrates may result in unsuccessful incubation of eggs. The loss of nesting sites is the most serious threat to sea turtles as adult females are prevented from laying eggs at naturally selected areas. Several essential environmental conditions are required to achieve the most successful incubation of sea turtle eggs under natural conditions (Limpus, 1985; Limpus et al., 1985; Miller, 1985 and Maloney et al., 1990).

Female sea turtles usually lay their eggs at naturally selected areas in order to produce a balanced sex ratio for the population. Each female will lay her eggs at different locations on the same beach such as under a tree, under a shrub, in bare sand as well as within the grasses. Sea turtle eggs require well ventilated, low salinity, high humidity sand/soil surrounding the nest.

The objective of this study is to determine the nest site preference among individuals and to quantify the impacts of this maternal behavior on reproductive success, by evaluating hatching and emergence success of individual nests.

MATERIALS AND METHODS

In order to evaluate this particular *in situ* nesting beach, nest monitoring, beach mapping, excavation and examination of egg chambers were carried out.

Site description

Despite its relatively small length (150 m), the Mak Kepit beach on Redang Island is one of the most important green turtle *in situ* nesting beaches in Terengganu, Malaysia. Redang Island is located about 50 km off the coast of Terengganu.

Besides Mak Kepit beach, there are two nesting beaches on this island namely Chagar Hutang and Mak Simpan. The Mak Kepit beach, which is situated between these two beaches, is backed by hills covered with virgin tropical green forest. The shores to the east and west of the beach are rocky. A stream discharges into the sea through the eastern part of the beach and splits this beach into two sections. Two species of turtles i.e., green and hawksbill annually nest at Mak Kepit beach. However hawksbill is very rare and in 2002 only one nest was recorded. The Redang Island is part of the Malaysia Marine Park, so all nesting beaches are totally protected and human activities around the area are regulated. The widest part of the Mak Kepit beach measured from the beginning of the sand dune to the forest line is 27 m and the narrowest is 3 m. The total nesting area is 2,153 m^2 of which bare sand area covers 916 m^2, canopies 624 m^2 and grass/creepers area 613 m^2. The sand is mixed with coral and shell fragments. The Department of Fisheries Malaysia undertook the *in situ* pioneer project at Mak Kepit Beach in 1992 in order to produce healthy hatchlings at high hatching success as well as to avoid producing hatchlings with biased sex ratio and incorrectly imprinted hatchlings. All clutches were left undisturbed and hatchlings crawled freely into the sea when they emerged. The nesting season begins in March until October each year with a peak in July.

Beach Mapping

In order to know the exact location of each egg chamber, permanent markers were placed along the beach. The 150 m beach was divided to 15 grids with 10 m interval. Fifteen posts were placed to mark the positions. A straight line rope was used as a reference for the beginning of sand dune. The rope was tied to every pole beginning from the western part of the beach which was marked as 0 m to the eastern part which was marked as 150 m. The forest line refers to the end of sand dune where nesters rarely laid beyond this area. The area of canopies was measured as the area covered by the canopy of each tree. The grasses area refers to all beach areas covered by any kind of grasses and creepers species. Bare sand area is all areas of beach which are free from any vegetation and shade.

Nesting Monitoring

Turtles were identified by Inconel flipper tags located on the first, most proximal scale at the trailing edge of both front flippers. The beach was patrolled daily and nightly for 24 hours from March to October until all hatchlings emerged and all egg chambers were excavated and examined. The nesters were measured for curve carapace length (CCL) and curve carapace width (CCW). Other information such as scale count was also recorded.

Measurements

For each egg chamber, we recorded 3 measurements. The first measurement was the position of the egg chamber along the beach (between 0 m to 150 m). The second was the distance from the egg chamber to the beginning of sand dune (a straight line rope was used as a reference for the beginning of sand dune), and the third was the distance from the egg chamber to the forest line. A pole was used to mark each egg chamber. The first egg chamber of each female was marked as a reference point for that particular turtle.

Excavation and Examination of Egg Chambers

About a week after the hatchlings had emerged, we excavated and examined all egg chambers. In order to gather information on repeatability of nesting behavior, we only investigated turtles which nested with between 4-10 nests during the 2002 season. All 160 egg chambers from 24 females were completely excavated and examined. Eggs were categorized as hatched (only the eggshell remained), pipped (at least the head protruding out of the shell), or unhatched. The unhatched eggs were opened and categorized as undeveloped, or unhatched (embryonic death). The number of live and dead hatchlings remaining in the nest was also counted. Hatching success was defined as the number of hatched eggs divided by the total number of eggs (clutch size). Pipped eggs were considered to be hatched but not emerged. Emergence success was defined as the number of hatched eggs minus the number of piped eggs and hatchlings remaining in the nest (live and dead) divided by clutch size.

Quantify Eggs and Hatchlings Destroyed by Predators

Monitoring of ants and ghost crabs on the eggs was made at all 160 nests. Identification of crab species was based on the field keys (Lovett, 1981), the characteristics of burrow were based on John (1998) and ants were based on Morita et al. (2005). The presence of characteristic snip marks on egg shells was attributed to the attack by ghost crabs and easily distinguishable to marks left by other predators such as ants.

RESULTS

Nesting Patterns

We recorded the location of 219 nests from 49 green turtles during the study period. A total of 21,172 eggs were recorded with an average of 96.7 eggs/clutch. Of these, 24 greens (measured between 91.6-119.3 cm in CCL and 83-104.3 cm in CCW) were observed to nest 4-10 times for a total of 160 nests. The remaining 25 greens nested less than 4 times on this beach and some nesters were reported to lay their eggs elsewhere such as at Mak Simpan and Chagar Hutang beaches. The density of nests under canopies was the highest at one nest per 4.2 m^2 (1/4.2 m^2) followed by the grasses and bare sand areas at 1/12.8 m^2 and 1/36.6 m^2 respectively. In overall the average density for 219 nests within 2,153 m^2 area is one nest per 9.8 m^2 (1/9.8 m^2).

Individual Preferences

In order to analyze the individual nest site preferences, we analyzed data from 24 greens which laid their eggs between 4-10 times for a total of 160 nests. These 24 females showed a significant preference for one of the 3 mentioned nesting habitats. Most of them preferred to lay their eggs under canopies rather than within grasses/creepers or bare sand areas.

Out of 160 nests, 113 or 70.6% were situated under canopies, compared to 36 nests (22.5%) within grasses/creepers and only 11 nests (6.9%) within bare sand areas. 17 turtles laid their eggs under canopies for more than 50% of their total nests, compared to only one turtle within grasses/creepers area. None of 24 females laid their eggs for more than 50% of their total nests within bare sand areas.

Individual turtle also preferred to nest closer to the forest line rather than the seaward area as compared to their first nest. 15 females preferred to lay their eggs with 50% of their total nests close to forest line compared to 9 females close to seaward direction. 12 turtles preferred to lay their eggs on the right side compared to 10 on the left side compared to their first nest. Two turtles laid their eggs in similar proportion on the left and right side.

Clutch Size and Nest Depth

The mean clutch size and nest depth were not similar for all nesting habitats. The highest mean clutch size was recorded within grass/creepers area at 103.2 eggs (range: 61-156; SD:$\pm$24.1), followed by within bare sand areas at 101.4 eggs (78-136;$\pm$17.7) and canopies at 97.6 eggs (31-190;$\pm$30.2). In terms of the mean nest depth, the deepest nest was recorded for the nests under canopies at 77.7 cm (47-113;$\pm$12.4), followed by the nests within grasses/creepers at 76.1 cm (60-109;$\pm$11) and bare sand areas at 69 cm (58-86;$\pm$8.7). The overall mean clutch size and nest depth for 160 nests were 99.1$\pm$28.2 eggs and 76.8$\pm$12 cm respectively.

Incubation Period

The mean incubation period was also not similar for all nesting habitats. The longest incubation period was recorded for the nest under canopies at 54.6 days (range: 43-67;SD:$\pm$5.2), followed by grasses/creepers at 52.3 days (47-64 days;$\pm$4.3) and bare sand at 52.2 days (46-58;$\pm$4.2). Two nests situated under canopies were destroyed by other nesters during their excavation of egg chambers. The overall mean incubation period for the remaining 158 nests was 53.9$\pm$5.0 days. The summary is as shown in Table 1.

Table 1: The Range of Incubation Period of Nests from Different Habitats

	Number of nest			
Incubation period (days)	Bare-sand	Grass/ creepers	Canopies	Total
41-45	0	0	3	3
46-50	6	18	23	47
51-55	1	11	40	52
56-60	4	4	28	36
61-65	0	3	14	17
66-70	0	0	3	3
Total nests	11	36	111*	158

*Note: Two nests situated under canopies were destroyed by the other nesters during their excavation of egg chambers.

Hatching and Emergence Success

There was a variation in the percentage of hatching success among the nesting habitats. The highest mean hatching success was recorded for the nests situated within the bare sand areas at 92.1% (range: 67-100; SD:$\pm$9.6), followed by nests within grasses/creepers areas at 88.7% (36.9-100;$\pm$13.8) and under canopies at 88.2% (0-100;$\pm$19.2). The zero hatching success of 2 nests situated under canopies was due to the nests destroyed by other females during excavating of the egg chambers. The overall mean of hatching success for 160 nests was 88.6$\pm$17.6%.

The mean emergence success was not similar to hatching success for all nesting habitats. A total of 13,015 hatchlings successfully emerged. The highest number was from the nests situated under canopies at 9,038 hatchlings followed by the nests within grass/creepers at 2,975 hatchlings and the nests from bare sand areas at 1,002 hatchlings. The highest mean emergence success was for the nests situated within bare sand areas at 88.2% (range: 67-100; SD:$\pm$9.5), followed by nests under canopies at 81.2% (0-100;$\pm$22.7) and within grasses/creepers areas at 79.9% (2.9-100;$\pm$21.6). The overall mean emergence success for 160 nests was 81.4$\pm$21.8 %.

Undeveloped Eggs

The highest mean in terms of percentage of the undeveloped eggs was recorded for the nests within grasses/creeper areas at 3.4% (range: 0-25.4%; SD:$\pm$5.6) followed by under canopies at 2.6% (0-47;$\pm$6.7) and bare sand areas at 1.5% (0-5;$\pm$1.7). The overall mean in term of percentage of undeveloped eggs was 2.7$\pm$6.2%.

Predator

In general, a total of 727 (eggs + hatchlings) were destroyed by the predators especially ghost crab (176) and red ant (551). The highest number of eggs predated by ants and ghost crabs was recorded from the nests under canopies at 389 and 124 followed by the nests situated within grasses/creepers at 144 and 52 respectively. Only 18 nests were predated by ants from the nests situated within bare sand areas.

DISCUSSION

Most scientists recommended that sea turtle eggs should be incubated in their natural nest in order to produce natural sex ratio and for the survival of the population. Limpus (1993), recommended that a hatchling sex ratio should be about 70% females. Sea turtles also posses temperature-dependent sex determination, where the nest temperature above the pivotal temperature produces mostly female hatchlings while below the pivotal temperature will produce mostly male hatchlings.

According to Mrosovsky and Yntema (1980), a change of 1-2°C can make a considerable difference to the sex ratio of the hatchlings. This is particularly important especially during the middle third of incubation or between 15-20 days of incubation where sexual differentiation occurs. There are numerous factors that can affect the temperature of the incubating turtle eggs ranging from climate, sand and beach characteristics, vegetation, water table and metabolic heating from the eggs themselves.

Liew et al. (2002) reported that the metabolic heating effects were greater in larger clutches compared to smaller clutch sizes, however this heating does not come into effect in the first 20 days of incubation. Certain activities on the beach such as the presence of crab burrows can exert some effect on the nest temperature too, causing them to fluctuate to a greater degree in tandem with air temperature. There are no reports about the effect of the density of nests to nest temperature, such as recorded on this beach, especially under canopies which was about 1 nest per 4.2 m^2.

In the case of Mak Kepit nesting beach where all nests were incubated naturally, we found that most turtles prefer to lay their eggs under canopies which was considered as low temperature as compared to open areas such as within grass/creepers and bare sand. This maternal behavior could tend to produce more male hatchlings rather than a balanced sex ratio as expected and recommended.

We found the mean incubation period of nests situated under canopies was 54.6 days which was longer by about 2 days when compared to the nests situated within grasses/creepers (52.3 days) and bare sand areas (52.2 days). The incubation period also can be used to predict the sex ratio of sea turtle hatchlings by which a short period will produce all female, an intermediate will produce 50:50 and the longer will produce all male. Normally, a short incubation period is related to a warm temperature and a longer period to a cool temperature

At present this population has no problem with the number of adult males but the low number of adult females should be studied seriously. This is significantly true because the overall mean of unfertilized eggs was only 2.7%, which indicated that all females have mated successfully with males. However the tagging data from 1993-2000 showed that only 391 females laid their eggs on this beach with an average of 48 nesters a year. In 2001 and 2002 only 62 and 49 females laid their eggs on this beach respectively. The highest number was recorded in 2004 with 80 nesters and the lowest in 2005 when only 20 females laid their eggs on this beach. This figure was considered low as compared with an average of more than 30,000 hatchlings released yearly from all beaches on this Island.

The maternal behavior of most nesters in laying their eggs under canopies also attracted predators and some nests were destroyed by other nesters during excavation of egg chambers and become the major factors of low hatching and emergence success in this area. Two nests recorded zero hatching success due to excavation of egg chambers by other nesters. There is a link between ghost crabs and the other predators. The initial harm occurs when the ghost crabs make tunnels into the nest chamber and break open several eggs. This begins a sequence of events, which normally results in the entire clutch being destroyed although the crab may actually eat only a few eggs. This direct access into the nest provided by the ghost crabs, acts as a pathway for second predators such as red ants and fungi. Fungi and harmful microorganisms find a suitable medium on the broken eggs and spread slowly, encompassing eggs at different stages of embryonic development (Ahmad and Kamarruddin, 2002).

Based on this finding we suggest that random sampling should be conducted to monitor the sexes of hatchlings emerging from different nesting habitats on this nesting beach in order to get a better picture of sex ratio of hatchlings produced. This is important for the survival of this population in the future. The information of hatchling sex ratio could be used in the management of this nesting beach. Even if the turtles laid their eggs naturally on this beach it does not mean that they can produce natural sex ratio.

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REFERENCES

Ahmad, A. and Kamarruddin, I., 2002. Crab predation on green turtle (*Chelonia mydas*) eggs incubated on natural beach and in turtle hatcheries. In: Proceedings of the 3rd Workshop on SEASTAR 2000: 95-100

Bulmer, M.G. and Bull, J.J. 1982. Model of polygenic sex determination and sex ratio control. Evolution. **36**, 13-26.

John, A.H.F., 1998. Ghost crab (*Ocypode* spp) predation on green turtles (*Chelonia mydas*) nests and hatchlings in Cagar Hutang, Pulau Redang. Final year project report, Bachelor of Marine Science, Faculty of Applied Science and Technology, Universiti Putra Malaysia. 92 p.

Kamel, S.J. and Mrosovsky, N. 2005. Repeatability of nesting preferences in the hawksbill sea turtle, *Eretmochelys imbricata*, and their fitness consequences. Elsevier. 10 pp.

Liew, H.C., K.Tatsukawa., S.L. Chan., E.H.Chan, M. Charuchinda, and Kamarruddin, I. 2002. Clutch size and incubation temperatures of green turtle eggs. In: Proceedings of the 3rd workshop on SEASTAR200: 101-104

Limpus, C.J. 1985. A study of the loggerhead turtle in Queensland. Ph.D. Thesis, University of Queensland: Brisbane.

Limpus, C.J. 1993. Recommendation for conservation of Marine Turtles in Malaysia. Report to Department of Fisheries Malaysia, Ministry of Agriculture, Malaysia. 60p

Limpus, C.J., Reed, P.C. and Miller, J.D. 1985. Temperature dependent sex determination in Queensland sea turtles: Intraspecific variation in *Caretta caretta*. In 'Biology of Australasian Frogs and Reptiles. (Eds. G. Grigg, R. Shine and H. Ehmann). Royal Zoological Society, New South Wales: 343-351

Lovett, D.L., 1981. A guide to the shrimp, prawns, lobsters, and crabs in Malaysia and Singapore. Universiti Pertanian Malaysia.

Maloney, J.E.,Darian-Smith, C.,Takahashi,Y. and Limpus. C.J. 1990. The environment for development of the embryonic loggerhead turtle (*Caretta caretta* in Queenslands. Copeia 1990: 378-387

Miller, J.D. 1985. Embryology of marine turtles. In: Biology of the reptilian. Vol. 14.(Eds. C. Gans, F. Billet and P.F. A. Maderson). Wiley-Interscience, New York: 271-328

Morita, M., Chan, E.H. and Ahmad, A.H. 2005. Marauder ant predation of green turtles in Chagar Hutang, Redang Island and Measures to Protect the nests. Paper presented at the 2nd International Symposium on SEASTAR2000 and Bio-logging Science, 13-14 December 2005, Bangkok, Thailand.

Mrosovsky, N and C.L. Yntema. 1980. Temperature dependence of sex differentiation in sea turtles: Implications for conservation practices. Biological Conservation. **18**(4):271-280