Monograph

The influence of incubation duration and clutch relocation on hatchling morphology and locomotor performances of green turtle (*Chelonia mydas*)

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ABSTRACT

The carapacial scute pattern of sea turtles is a conserved trait that provides taxonomic information. But non-modal scute patterns (NMSPs), *i.e.* intraspecific individual variabilities, are observed for almost all species and occur relatively often, particularly in hatchlings. We surveyed 67 nests from 61 nesting green turtle females (*Chelonia mydas*) over an eight-week study duration on Redang Island, Malaysia. During this study, eight NMSP females laid two different clutches. For all newly emerged hatchlings from each nest ($n = 4386$), we analyzed their scute pattern (*i.e.* the number of vertebral and costal scutes). For a subsample of 1144 hatchlings, we compared their carapace size, weight, and their locomotor performances (self-righting ability, running and swimming speeds). The proportion of hatchlings with NMSP varied from 0 to 70.4% (mean $\pm$ SD = 16.4% $\pm$ 16.2) per nest. We compared the scute pattern of nesting females to that of their hatchlings and found that the scute pattern of the mother does not predestine that of her descendants. However, our results revealed that the main driver of scute pattern abnormalities and hatching success was the shorter incubation duration (*i.e.* warmer incubation temperatures). Eggs hatched after a short incubation period have lowered hatching success. Our data showed also that relocated nests have a greater proportion of NMSP hatchlings compared to *in situ* nest. Conversely, the scute pattern and clutch relocation do not appear to be linked to lower hatching success or slower locomotor speeds. Our work highlights the effects of incubation duration and clutch relocation techniques on the morphology and survival of green turtle and contributes to better informed management strategies as part of global research efforts to preserve an endangered species. Based on our findings, we suggest strictly following guidelines for relocating a sea turtle nest and applying clutch relocation to populations where hatching success is very low or only as a last resort for doomed nests.

1. Introduction

Turtles, or chelonians, are one of the most ancient reptile groups. They have endured for over 220 million years and have persisted through several major environmental perturbations (Cherepanov, 2014). Much of the evolutionary success of turtles is attributed to their shell, a key morphological innovation of the order Testudines, that triggered their diversification (Zimm et al., 2017). The turtle shell, or carapace, is covered by large keratinous scales known as scutes, and the number and arrangement of these scutes form a species-specific mosaic (Kobayashi et al., 2017). The scute mosaic has two important contradictory features (Cherepanov, 2014): the scute arrangement is a

conserved trait that provides taxonomic information, and at the same time shows a wide range of individual variability, particularly in hatchlings (Zangerl and Johnson, 1957; Mast and Carr, 1989; Maffucci et al., 2019). These abnormalities consist of differences in the number, shape, or arrangement of scutes, with the most common one being supernumerary scutes (Zangerl and Johnson, 1957; Mast and Carr, 1989).

Several hypotheses have been proposed to explain these abnormalities. Gadow (1899) and Newman (1906) suggested that additional scutes are ancestral genetic traits that reappears after having been lost during evolution (*i.e.* evolutionary throwbacks or atavism), a hypothesis rapidly rejected (Parker, 1901; Coker, 1910; Grant, 1937; Hildebrend, 1930; Lynn and Ullrich, 1950; Zangerl and Johnson, 1957). Parker

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(1901) stated that the abnormality appears early in development, so early as to affect both ectodermal and mesodermal derivatives (*i.e.* the outermost and the middle of the three primary germ layers of an embryo). Coker (1910) suggested that non-modal scute patterns are caused by embryonic mutations and proposed that differences in the number of scutes between young and adults are due to differential survival between normal and anomalous individuals. More recently, evidence has increasingly shown that scute abnormalities result mostly from mechanical disorders occurring during embryonic development (Lynn and Ullrich, 1950; Moustakas-Verho et al., 2014; Zimm et al., 2017), but genetic factors have not been ruled out (Glen et al., 2003; Velo-Antón et al., 2011; Kobayashi et al., 2017).

It has been demonstrated that scute abnormalities can be caused by nest microclimate conditions (Sim et al., 2014b; Kobayashi et al., 2017; Zimm et al., 2017; Staines et al., 2019). Recently, Türkozan and Yilmaz (2007) and Sönmez et al. (2011) suggested that scute abnormalities could be linked to egg relocation. Egg relocation is a common hatchery management technique applied in marine turtle conservation programs consisting of relocating eggs just after laying, from a threatened site to a protected area, to increase hatching successes (*i.e.* the proportion of eggs that hatch) (Dutton et al., 2005). But by modifying the natural incubation environment, notably if eggs are subject to suboptimal microclimates, or by the handling in itself, relocation may affect some morphological traits of hatchlings produced such as their scute pattern, or their locomotor performance, and subsequently their long-term viability (Mast and Carr, 1989; Mrosovsky, 2006; Türkozan and Yilmaz, 2007; Pintus et al., 2009; Sönmez et al., 2011; Tanabe et al., 2021).

The number of scutes does not seem to influence the hydrodynamics of the carapace. However, anomalies may be phenotypic manifestations of underlying morphological or physiological instabilities that could harm an individual's ability to move or survive (Maffucci et al., 2019). Mast and Carr (1989) showed that for the Kemp's ridley turtle (*Lepidochelys kempii*), deceased embryos and hatchlings that failed to emerge from the nest had higher proportions of scutes with abnormal patterns than live hatchlings. Sim et al. (2014b) compared morphological parameters of loggerhead (*Caretta caretta*) and flatback turtle (*Natator depressus*) hatchlings and found that hatchlings with modal scute pattern were larger and heavier than those with non-modal scute pattern; moreover, they demonstrated that *N. depressus* hatchlings with normal patterns were more efficient swimmers compared to those with abnormal scute patterns. Kobayashi et al. (2017) found lower survival rates of green turtle (*Chelonia mydas)* hatchlings showing non-modal scute patterns with growth impediments and high incubation temperatures. Similarly, Maffucci et al. (2019) observed in *C. caretta* that individuals with non-modal scute pattern were lighter than individuals with modal scute pattern, and that the former have a lower survival rate.

To date, the seven extant sea turtles species are imperiled (IUCN, 2021; the flatback turtle is listed as data deficient in the IUCN Red List of Threatened Species, but as a nationally vulnerable species on the Australian Commonwealth's Endangered Species Protection Act). Turtles are under pressure from both terrestrial and marine predators. But they are also particularly at risk from anthropogenic effects including bycatch, marine pollution, consumption of eggs and meat, and climate changes (*e.g.* habitat loss, changes in reproductive periodicity and foraging success, warmer incubation temperatures causing female-biased sex ratios and higher embryo mortality) (Maurer et al., 2021; Patrício et al., 2021). To restore their declining populations, it is therefore crucial to identify the factors that may impact their survival. The present study focuses on the green turtle *Chelonia mydas*. Widely distributed in tropical and subtropical waters of the Atlantic, Pacific, and Indian oceans, *C. mydas* has nesting rookeries in >80 countries worldwide and inhabits coastal waters of over 140 countries (Seminoff, 2004). In Malaysia, Redang Island is by far the most important nesting area (Groombridge and Luxmoore, 1989). However, Malaysian turtle populations have declined by >80% since the 1950's, mainly due to a long history of egg exploitation for human consumption, nesting beach

development, and incidental captures of turtles in fishing gear (Seminoff, 2004).

The modal scute pattern of green turtles consists of five vertebral scutes along the longitudinal line of the carapace, four series of bilaterally paired costal scutes on either side, one nuchal scute flanked by 11 smaller pairs of marginals, and one pair of supracaudal scutes (Özdemir and Türkozan, 2006; Ergene et al., 2011; Kobayashi et al., 2017) (Fig. 1). But non-modal scute patterns (NMSP; Fig. 1) have been regularly observed in this species with no general conclusions about the implications of these anomalies on the fitness of individuals (Durmus et al., 2010; Kobayashi et al., 2017; Cherepanov and Malashichev, 2018).

The scute anomalies have been attributed to various and non-exclusive factors, including environmental conditions (Kazmaier and Robel, 2001; Sim et al., 2014b), epigenetic mechanisms (Caracappa et al., 2016; Zimm et al., 2017; Maffucci et al., 2019), management practices (Mast and Carr, 1989; Suganuma et al., 1994; Türkozan and Yilmaz, 2007; Tanabe et al., 2021; Unda-Díaz et al., 2022) and heritable factors (Velo-Antón et al., 2011; Kobayashi et al., 2017). But the intricate origins of these anomalies in natural populations, particularly their interplay during the decisive incubation phase, remain only partially understood.

In this study, our objectives were three-fold. *(i)* We compared scute pattern, morphological traits, and survival between hatchlings from different nest environments to test if lower incubation duration and/or relocated nests tend to have higher percentage of individuals with non-modal scute pattern. *(ii)* We also tested whether the scute pattern could be explained by an inheritable genetic component, by comparing the scute pattern of nesting females to that of their hatchlings. *(iii)* Lastly, through three complementary locomotor performance experiments, we tested whether hatchlings with non-modal scute pattern might spend more time in the predation environment compared to hatchlings with modal scute pattern, which would reduce their survival rate.

2. Material and methods

2.1. Study site and nest monitoring

This study was conducted at the Chagar Hutang Turtle Sanctuary on Redang Island, Terengganu, Malaysia, from June 22nd until August 21st, 2019. The beach is about 350 m long, accessible only by boat and surrounded by hills with undisturbed thick tropical rainforest and located about 23 km off the east coast of Peninsular Malaysia in the South China Sea. The Sea Turtle Research Unit (SEATRU) volunteer program was introduced at Chagar Hutang in 1998, and has since been conducting yearly monitoring, tagging, and *in situ* egg incubation research from April to September. In 2005, Chagar Hutang was declared as a turtle sanctuary through the Department of Fisheries, and it was closed to the public and thus protected against poaching (Tanabe et al., 2021). At the sanctuary, clutches are relocated if the threat of predation or erosion were high, or if the nest was in areas characterized by high density of rocks or tree roots. Clutches are removed within a two-hour window, and relocated to an area of the beach that replicates the original shaded and sunny conditions. Note that volunteers and rangers responsible for monitoring nests are trained, but despite this formation, the precise method of relocation may slightly vary.

The beach was patrolled daily for 8 weeks between dusk and dawn. For each nesting female, flipper tag number and scute pattern were recorded, as well as curved carapace length (CCL) and curved carapace width (CCW) by using a measuring tape. For each nest, we recorded the distance from vegetation (m), the incubation duration (*i.e.* the time from the egg-laying until hatchling emergence), whether it was primarily in shade or sun, and if the clutch was relocated or left *in situ*. Nests that spent more than half the day under vegetation or even partially under a branch or tree were considered as "shaded nests" ($n = 29$), and conversely, nests that spent more time partially in the sun or in the open sunlight were classified as "sunny nests" ($n = 38$).
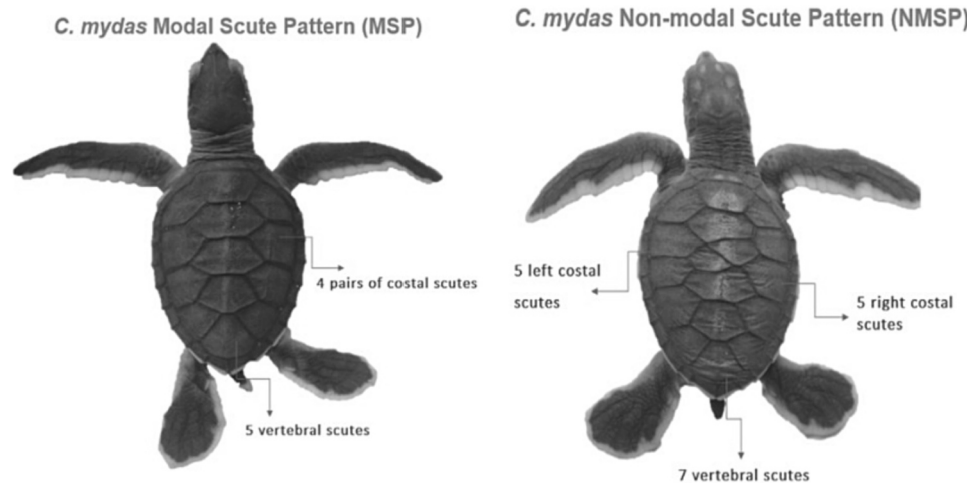
Fig. 1. Green turtle hatchlings (*Chelonia mydas*) with modal scute pattern (MSP) (left) and non-modal scute pattern (NMSP) (right). Modal scute pattern consists of 4 pairs of costal scutes and 5 vertebral scutes. Both hatchlings are from Redang Island, Malaysia (personal pictures) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

After 45 days of incubation, an enclosure made of plastic mesh was placed at the center of the nest at dusk to prevent hatchlings from emerging and escaping to the ocean. These nests were checked every hour between dusk and dawn to ensure that hatchlings were not on the surface too long before being evaluated for locomotor performance tests. Two days after hatchling emergence, nests were carefully excavated by hand. The percentage of unhatched eggs, dead embryos and alive hatchlings stuck in the nest chamber were counted. The hatching success (%) was calculated using the following formula: [(# of empty *eggshells* /# total number of eggs that can be counted) × 100] (Miller, 1999).The number of vertebral and costal scutes were counted for all hatchlings even for unhatched embryos by opening the eggs.

2.2. Morphology and locomotor performance of hatchlings

A random sample of ten MSP and ten NMSP hatchlings were selected from each nest and labelled with a unique number on their plastron. If there were <10 hatchlings of one type, we completed to 20 with hatchlings of the other type. The 20 hatchlings were weighed using an electronic balance (Electronic Compact Scale, SF-400C, 500 ± 0.01 g). Their straight carapace length (SCL) and straight carapace width (SCW) were measured from the widest point using a digital caliper (Hardened Stainless digital caliper, 0–150 ± 0.01 mm) and these measurements were used to calculate the carapace size index (SCL x SCW) (Read et al., 2012). Three proxies were used to evaluate locomotor performances: self-righting (*i.e.* the capacity of individuals to recover from a carapace upside down position), running speed (*i.e.* the capacity to crawl down the beach from the nest to the ocean) and swimming speed (*i.e.* the capacity to quickly swim through the near-shore environment before reaching the open ocean) (Booth et al., 2013). Within two hours of hatchling emergence, the locomotor performance tests were carried out at night on the sub-sample of 20 hatchlings. Self-righting ability was quantified by placing the hatchlings upside-down in a flat-bottomed plastic bucket filled with sand and using a stopwatch to time how long they took to self-right (Sim et al., 2014b). Between each trial, the hatchling had 10 s of rest, for a total of three self-righting tests. Self-righting attempts were deemed a failed attempt if hatchlings took longer than 30 s.

Immediately following the self-righting experiment, the running and swimming tests were conducted. The running and swimming speeds were measured using a PVC guttering (2.9 m × 0.18 m × 0.12 m) sealed at each end and filled with fresh sand or seawater each night, perpendicular to the sea. A flashlight was placed at the seaward end of the raceway to encourage unidirectional running or swimming. The time taken to run and swim (in 2 min max. For one trial each) from the landward to the seaward end of the track was measured and converted into running or swimming speeds (cm/s). Once locomotor tests were recorded, hatchlings were released on the beach, 1 m from the water's edge to crawl freely to the ocean (Sim et al., 2014b).

2.3. Statistical analyses

Statistical analyses were performed with *R* software (version 3.6.2; R Development Core Team, 2019) using the *RStudio* interface (version 1.3.959; RStudio Team, 2015). We used the packages *Rmisc* (Hope, 2013), *tidyverse* (Wickham et al., 2019), *MASS* (Venables and Ripley, 2002), *car* (Fox and Weisberg, 2019), *nnet* (Venables and Ripley, 2002), *ordinal* (Christensen, 2019), *multcomp* (Hothorn et al., 2008), *lme4* (Bates et al., 2015) and *GGally* (Schloerke et al., 2020) for statistical analyses, and *ggplot2* (Wickham, 2016) and *ggpubr* (Kassambara, 2020) for data visualization. Normality and variances homogeneity of the data were tested by Shapiro-Wilk and Levene tests. For ANOVA models, the normality of residuals was tested using the Shapiro-Wilk test and by plotting the standardized residuals against a perfect normal distribution of theoretical quantiles. Non-normal data and/or data showing heteroscedasticity were transformed using the logit $[\ln(p/(1 - p))]$ and Box-Cox transformations (Box and Cox, 1964; Sokal and Rohlf, 1995). We used parametric tests when the data or the transformed data met the assumptions of normality, homogeneity of variances and independence, and non-parametric tests when they did not.

To explore effects of female scute pattern (*i.e.* females with NMSP *vs.* females with MSP), of female morphology, of nest microenvironment (*i. e.* shade *vs.* sun) and of relocation on incubation duration, clutch size and hatchlings (hatching success and hatchlings scute pattern), we used multiple regression models for controlling extraneous variables. The regressions in this study included not only the independent variables being examined for their effects on the dependent variable but also any potential confounding variables, *i.e.* additional factors that not only impact the dependent variable (outcome) but also influence the primary independent variable. Fisher's exact test was used to determine if there were nonrandom associations between two categorical variables. Ordinal logistic regression was used to test how scute pattern and relocation impact self-righting success of hatchlings; odd ratios were used to compare the self-righting success. The self-righting success represented a score from 1 to 3 (*i.e.* 1 = one success out of three attempts to self-right, 2 = two successes out of three attempts and 3 = three

successes out of three attempts). To obtain the self-righting ability, self-righting success was multiplied by the inverse of self-righting mean time (*i.e.* the average time taken to self-right across all three self-righting attempts). A higher numerical value for self-righting reflects a greater self-righting ability (Booth et al., 2013). Because all hatchlings from a given nest are pseudo replicates, we applied mixed-effects models with a random intercepts term for nests. For all statistical tests an alpha-level of <0.05 were considered statistically significant.

3. Results

We surveyed 67 nests over an eight-week study. For all encountered females ($n = 61$, eight NMSP females laid two different clutches) and for all newly emerged hatchlings from each nest ($n = 4386$), the scute pattern was recorded. In hatchlings, the percentage of non-modal scute pattern (NMSP) varied from 0 to 70.4% per nest (mean ± SD = 16.4% ± 16.2). We observed a total of 36 variants (including the MSP 5-4-4; number of vertebral - number of left costal - number of right costal). The most common NMSP was 6-4-4 (35.0%), 6-5-4 (10.6%) and 5-5-4 and 6-4-5 (both at 7.5%). In nesting females, the percentage of NMSP was 15.8%, only 8 variants (including the MSP) were observed, and the most common NMSP were 6-4-4 (40.0%), and the scute pattern 5-5-4, 7-5-4 and 8-5-5 (all 13.3%).

3.1. Effects of female scute pattern on clutches and hatchlings

The carapace length of MSP females is significantly larger (mean ± SD = 97.6 ± 5.2 cm) than the carapace length of the NMSP females (93.8 ± 6.3 cm) (Wilcoxon rank sum test, *p*-value = 0.049). Carapace width (MSP females: 85.7 ± 4.9 cm; MSP females: 82.9 ± 7.6 cm) and size index (MSP females: 8386.1 ± 869.4 cm^2; NMSP females: 7826.8 ± 1183.9 cm^2) of the nesting females did not differ between MSP and NMSP females (Wilcoxon rank sum tests, *p*-values = 0.332 and 0.090, respectively).

The clutch size is similar between MSP females (97.7 ± 28.8) and NMSP females (86.5 ± 50.2), while controlling for the size of the females (multiple regression model, $p = 0.384$). However, hatching success, while controlling for female size, incubation duration (*i.e.* a proxy of the incubation temperature), the effect of shade and whether eggs were relocated or not, decreases by, on average, 18.2% for females with a NMSP compared to females with MSP (multiple regression model, $p = 0.046$; MSP: 79.2% ± 14.3; NMSP: 66.9% ± 22.2). According to this model, 40.8% of the hatching success is explained by the female scute pattern, the female size, the incubation duration, and the effects of shade and relocation. The scute pattern of the hatchlings does not relate to the scute pattern of their mother. The percentage of NMSP hatchlings per nest, while controlling for the same four confounding variables, did not differ between modal (17.5% ± 18.7) and non-modal (25.0% ± 22.9) females (multiple regression model, $p = 0.386$).

3.2. Effects of nest microenvironment on hatching success and hatchling scute pattern

Whether nests were predominantly in the shade or sun did not appear to affect the incubation duration (shade: 51.9 ± 2.2 days; sun: 48.7 ± 2.2 days; multiple regression model controlling for female scute pattern, female size, and relocation, $p = 0.269$), the hatching success (shade: 84.0% ± 12.4; sun: 74.7% ± 16.7; multiple regression model controlling for female scute pattern, female size, incubation duration and relocation, $p = 0.177$) or the percentage of hatchlings with NMSP (shade: 9.1% ± 1.0; sun: 22.1% ± 18.0; multiple regression model controlling for female scute pattern, female size, incubation duration and relocation, $p = 0.500$). Interestingly, multiple regression analysis controlling for shade effects, female scute pattern and size index and relocation reveals a significant and positive relationship between the hatching success and the incubation duration, all else being equal. So,

for an increase of one day in the incubation duration, the hatching success increases, on average, by 5% ($p = 0.022$).

3.3. Effects of clutch relocation on hatching success and hatchling scute pattern

Multiple regression analysis controlling for shade and temperature effects and female scute pattern and size index reveals a significant and positive relationship between the incubation duration and the relocation, all else being equal ($p = 0.024$). Relocated nests had a significantly shorter incubation duration (49.4 ± 2.9 days) than *in situ* nests (50.9 ± 2.1 days). Conversely, we found no relationship between the hatching success and the relocation of nests ($p = 0.901$) when the scute pattern of the nesting females, their carapace size index and the effects of the temperature and the shade stay constant (mean hatching success of relocated nest ± SD = 76.6% ± 15.0; mean hatching success of *in situ* nest ± SD = 82.5% ± 15.9). However, multiple regression analysis controlling for the same four confounding variables reveals a significant and positive relationship between the percentage of hatchlings with NMSP and the relocation, all else being equal ($p = 0.040$). Relocated nests showed a greater percentage of NMSP (mean ± SD = 21.5% ± 17.8; $n = 38$) compared to *in situ* nests (9.9% ± 11.3; $n = 29$). This association between relocation technique and hatchling scute pattern abnormalities is confirmed by a Fisher's exact test for count data (*p*-value <0.001): the percentage of NMSP hatchlings was significantly higher in relocated nests (35.2%, $n = 223$) than in *in situ* nests (18.9%, $n = 97$).

3.4. Effects of clutch relocation and scute pattern on hatchling morphology

Morphological differences in carapace size index and weight of hatchlings, according to scute mutation (MSP *vs.* NMSP), relocation (*in situ vs.* relocated nests) and incubation duration, were assessed using nested ANOVA and Box-cox transformed data. The carapace size index of hatchlings was significantly associated with the incubation duration ($t = 2.215$; *p*-value = 0.031), but not with the hatchling scute pattern (*p*-value = 0.126) nor with clutch relocation (*p*-value = 0.108) (Fig. 2). However, the weight of hatchlings seems to be influenced by the incubation duration ($t = 2.030$; *p*-value = 0.047), and the hatchling scute pattern ($t = -3.245$; *p*-value = 0.001), but not with the clutch relocation (*p*-value = 0.846) (Fig. 2).

3.5. Effects of clutch relocation and scute pattern on hatchling locomotor performances

To assess if hatchling scute pattern (MSP *vs.* NMSP) and clutch relocation were associated with their success to self-right, self-righting tests were studied through an ordinal logistic regression. Success level was negatively linked to both independent variables. The regression model and odd ratios (OR) showed that hatchlings from relocated nests had a lower likelihood to success to self-right than hatchlings from *in situ* nests (*p*-value = 0.048; OR = 0.48, 95%CI = 0.28–0.80) and, to a lesser extent, NMSP hatchlings have more difficulties to self-right when they have already self-righted once, and even more difficulties when they self-righted twice (*p*-value = 0.013; OR = 0.53, 95%CI = 0.32–0.88) (Fig. 3). Controlling for confounding variables refines this first result: NMSP hatchlings have more difficulties to self-right (*p*-value = 0.009 OR = 0.33, 95%CI = 0.15–0.77), the hatchling size also interferes the self-righting success, the bigger the carapace, the more likely an individual is to get back on its feet (*p*-value = 0.021; OR = 1.00, 95%CI = 1.00–1.01); the effect of relocation is no longer significant (*p*-value = 0.087).

Effects of relocation (*in situ vs.* relocated nests) and scute pattern (MSP *vs.* NMSP) on other hatchling locomotor performances were assessed using nested ANOVA on box-cox transformed scores, and
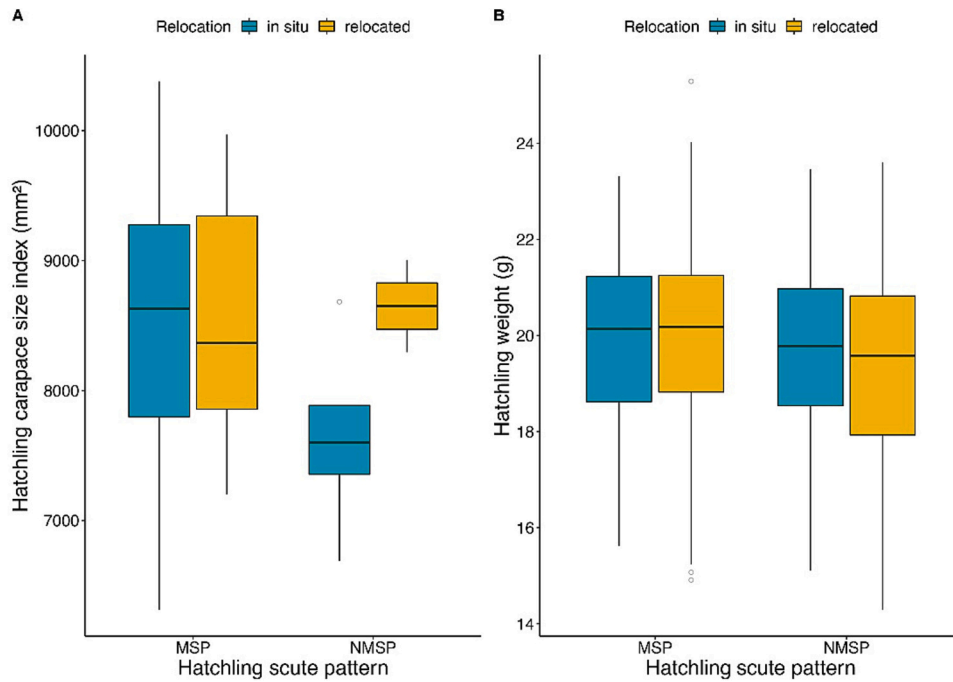
Fig. 2. Box plots of hatchling carapace size index (mm^2) (A), and of hatchling weight (g) (B) incubated *in situ* and relocated between hatchlings with modal (MSP) and non-modal scute pattern (NMSP). Box plots show median and first and third quartile; whiskers include 95% of all observations.
Carapace size indices differ between relocated and *in situ* hatchlings; other comparisons are non-significant (mixed-effects modelling with a random intercepts term for nests).
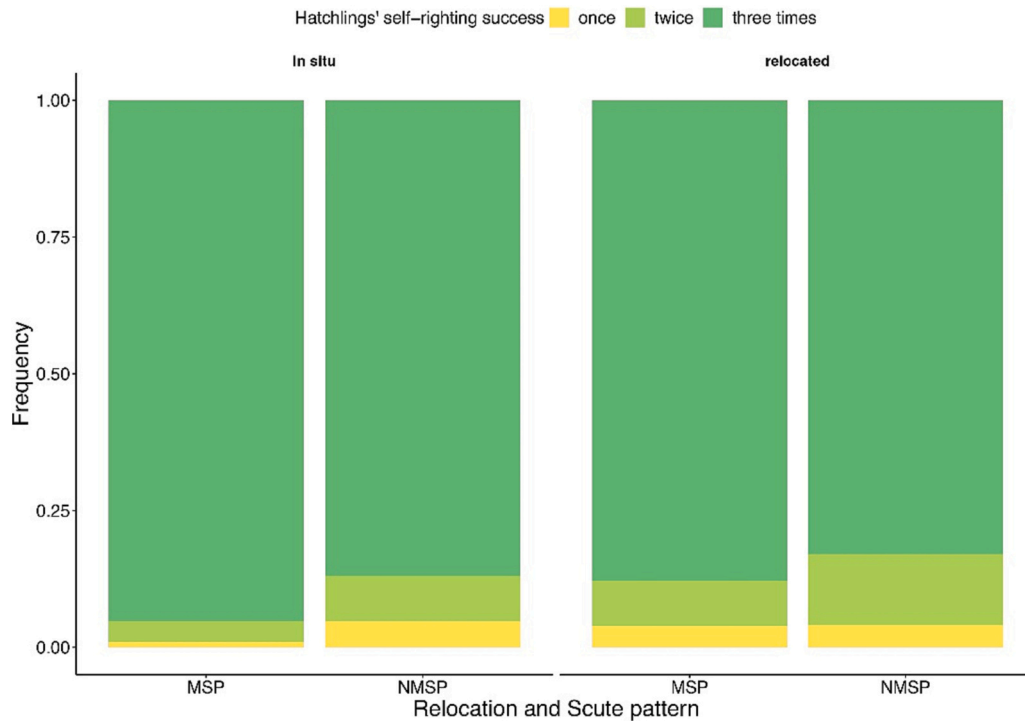

Fig. 3. Stacked bar chart showing hatchling success to self-right once, twice or three times from a carapace upside down position, for hatchlings with modal (MSP) and non-modal scute pattern (NMSP), and from relocated (right) and *in situ* (left) nests. Ordinal logistic regression model showed that hatchlings with NMSP have more difficulties to self-right when they have already done once, and even more difficulties when they have already done twice and a fortiori thrice than hatchlings with MSP. The effect of relocation (relocated nests had a lower likelihood to success compared to hatchlings from *in situ* nests) is only significant if the confounding variables are not considered.

controlling for the incubation duration, the size, and the weight of the hatchlings and whether the nests were mainly at sun or shade. For the self-righting time, our analysis revealed a significant interaction between hatchling scute pattern and clutch relocation ($t = -2.034$; *p*-value $= 0.042$): hatchings with MSP from relocated nests take longer to self-right but will succeed just as well as hatchings from *in-situ* nests or

with NMSP (Tukey's multiple comparison test, $z = 3.344$; *p*-value = 0.004). Self-righting ability of the hatchlings were not affected by their scute pattern, nor by whether their nest was relocated (all *p*-values >0.080). Similarly, still controlling for confounding variables, the running and swimming speeds of the hatchlings were not affected by their scute pattern, nor by the clutch relocation (running speed: all *p*-values >0.128; swimming speed: all *p*-values >0.213) (Fig. 4).

4. Discussion

We reported that the percentage of the modal scute pattern (5–4-4) is relatively similar between hatchlings and nesting females (72.1% and 85.2%, respectively), and that the percentage of emerged hatchlings with non-modal scute pattern ranges from 0 to 70.4% per nest with an average value of 16.4%. In Japan, Kobayashi et al. (2017) counted the vertebral and costal scutes of 44,537 hatchlings from 574 nests and reported that the percentage of *C. mydas* hatchlings with NMSP was 5.8%. In Cyprus, Özdemir and Türkozan (2006) investigated the number of marginal, costal, vertebral, supracaudal and nuchal scutes of 718 *C. mydas* hatchlings and concluded that 40.4% had carapacial scute variation. In Turkey, Ergene et al. (2011) examined the same carapacial scute series on 917 *C. mydas* hatchlings and found a percentage of 21.9% of scute abnormalities. Conversely, in Sri Lanka, *C. mydas* is characterized by a stable scutation as only 3% (out of 341) of hatchlings have additional abnormal scutes (Cherepanov and Malashichev, 2018). The variability observed within nests of a same population and between *C. mydas* populations suggests that an interplay between environmental and genetic factors may affect scute variability of individuals.

4.1. Effects of genetic factors on hatchling scute pattern

We did not observe that NMSP females were more likely to produce to NMSP hatchlings while we controlled for the effects of environmental nest conditions (*i.e.* female scute pattern, female size, incubation duration and relocation). Conversely, Kobayashi et al. (2017) found that the frequency of *C. mydas* hatchlings with NMSP produced by NMSP females

was significantly higher compared to the frequency of hatchlings with NMSP by MSP females; for these authors, scute abnormalities are heritable. However, they observed that the specific scute patterns were not coherent between mother and hatchlings: only 30% of hatchlings from 6 nests displayed the same scute pattern as the mother. They concluded that the low frequencies of hatchlings with NMSP may be related with high genetic diversity of this *C. mydas* Japanese population (Kobayashi et al., 2017).

It is important to note that multiple paternity is a common mating system among sea turtles (Lee et al., 2018). Joseph et al. (2017) found that 36% of *C. mydas* clutches in Redang Island are produced by females who have mated with two different males. The number of males involved in consecutive nests laid by a single female over a single season could therefore affect the hatchling scute abnormalities by the direct additive genetic influence of the female but also by those of its different mates. Based on the relationship between the mother scute pattern and those of her hatchlings, our results suggest that non-modal scute pattern is not heritable and did not influence their laying. Unfortunately, observing male turtles and investigating their scute pattern is difficult because only females have a terrestrial life cycle during nesting activities (Maffucci et al., 2019). Further studies are required to investigate how paternal genetic components could affect phenotypic traits such as scute abnormalities in sea turtles (Roberts et al., 2004; Velo-Antón et al., 2011; Booth et al., 2013).

4.2. Effects of nest microenvironment on hatchling scute pattern

Our results revealed that whether nests were incubated mainly at sun or shade does not significantly affect the incubation duration (sun: 48.1 ± 2.1 days; shade: 51.8 ± 2.2 days), the hatching success (sun: 74.6% ± 16.0; shade: 84.0% ± 12.0) nor the percentage of NMSP hatchlings (sun: 22.1% ± 17.0; shade: 9.1% ± 10.0). Yet, we found a positive correlation between incubation duration and hatching success. The longer the incubation duration, the higher the hatching success rate. As incubation duration is used as a proxy for incubation temperature, these results suggest that shorter incubation duration and/or warmer incubation
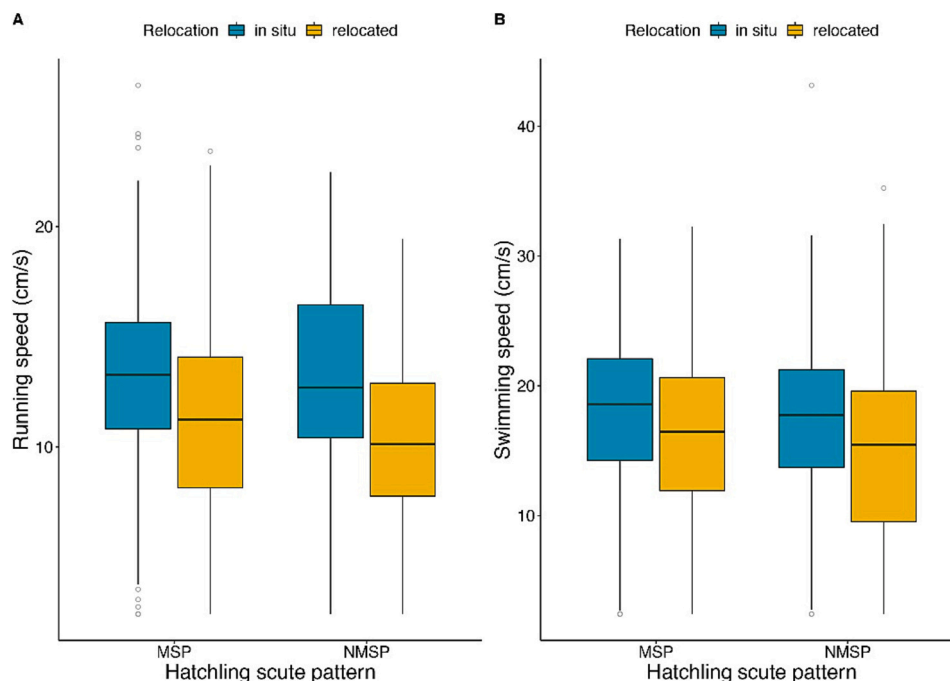
Fig. 4. Box plots of running speeds (cm/s) (A), and swimming speeds (cm/s) (B) of hatchlings incubated *in situ versus* relocated and compared between hatchlings with modal (MSP) and non-modal scute pattern (NMSP). Box plots show median and first and third quartile; whiskers include 95% of all observations. Running speeds significantly differ between relocated and *in situ* hatchlings; other comparisons are non-significant (mixed-effects modelling with a random intercepts term for nests).

differences between MSP and NMSP individuals. Our measurements concerned two dimensions only, whereas the sphericity of the carapace could differ between hatchlings. The height of the carapace could hinder the self-righting of the hatchling. The remaining quantity reflects the amount of fat present in hatchling and is greater when the duration of the individual's development has been shorter. The amount of fat could influence the time or ability of the hatchling to self-right (Fisher et al., 2014; Usategui-Martín et al., 2019). Geometric morphometric analyses could help to determine if the shape of the carapace influenced the survival and performances of individuals.

5. Conclusion

Our study at Chagar Hutang Turtle Sanctuary found diverse scute patterns in *C. mydas* populations, particularly in hatchlings, but the modal scute pattern was predominant. We observed that the mother's scute pattern does not determine that of her offspring. In contrast, we identified shorter incubation periods and higher temperatures as the main factors affecting scute pattern anomalies and hatching success. Eggs with shorter incubation periods had lower hatching success. Scute pattern and relocation did not affect hatching success or locomotor speeds significantly, but their impact cannot be completely ruled out.

Relocating clutches for endangered species conservation is a common practice, but previous studies (Mrosovsky, 2006; Pintus et al., 2009; Najwa-Sawawi et al., 2021; Tanabe et al., 2021) and our results indicate that it may not be favorable. Relocated nests have a greater proportion of NMSP hatchlings compared to *in situ* nests, and relocated hatchlings also faced difficulties in self-righting compared to non-manipulated hatchlings. Relocating clutches should be undertaken when hatching success is very low or, as in our population where hatching success of *in situ* nests was very high (near 90%), only as a last resort for doomed nests. It is imperative that sea turtle conservation programs be aware of the effects of such techniques upon populations and strictly apply a standard and approved egg-relocation procedure to ensure the production of performant hatchlings (Pintus et al., 2009; Tanabe et al., 2021).

Artificial man-made nests may offer suboptimal microclimatic conditions for eggs compared to natural nests. Differences in depth, shape, and width of egg chambers can lead to mechanical disorders during embryonic development, despite precautions taken during relocation (Tanabe et al., 2021). Moreover, given that green turtles deposit between 80 and 120 eggs in each nest and lay between 1 and 11 nests during the nesting season, the handling of multiple nests per nesting season poses challenges (Mast and Carr, 1989). Cooling nest microclimate conditions through shade *in situ* can help reduce the proportion of non-modal scute patterns and promote the development of hatchlings with good predispositions for their future (Kobayashi et al., 2017).

Even if sea turtles have overcome climate change in their evolutionary past, the rapid rate of climate change raises concerns about sea turtles' ability to adapt (Van De Merwe et al., 2009; Patrício et al., 2021). The combined effects of elevated temperatures on embryonic mortality, clutch sex ratios, hatchling phenotype and locomotor abilities suggest that global sea turtle populations may be at risk of collapse if rookery beach temperatures continue to rise (Roberts et al., 2004; Velo-Antón et al., 2011; Booth et al., 2013). Further conservation research is required to understand the influence of environmental effects, genetic diversity, and notably paternal genetic components on gene expression, affect phenotypic traits such as scute abnormalities in sea turtles. The decline of this keystone species may have serious implications for marine and terrestrial ecosystems (Seminoff, 2004).

Ethical approval

All experimental procedures were approved by the Universiti Malaysia Terengganu animals ethics committee (approval no. UMT/RMIC/2–2/1/23).

CRediT authorship contribution statement

Marion Steenacker: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Lyndsey K. Tanabe:** Methodology, Investigation. **Mohd Uzair Rusli:** Resources, Supervision, Funding acquisition. **Denis Fournier:** Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.
Mohd Uzair Rusli reports financial support was provided by Universiti Malaysia Terengganu. Denis Fournier reports financial support was provided by Université libre de Bruxelles- ULB.

Data availability

Data will be made available on request.

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