

Effect of Sand Types on the Early Swimming Performance of Green Turtle Hatchlings

Nadhirah Syafiqah Suhaimi

Universiti Malaysia Terengganu

MOHD UZAIR RUSLI (✉ uzair@umt.edu.my)

Universiti Malaysia Terengganu <https://orcid.org/0000-0002-2221-1541>

David Booth

The University of Queensland

Juanita Joseph

Universiti Malaysia Sabah

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Abstract

The selection of the nest site is a critical factor whereby female turtles may choose to nest at the site that minimizes energy expenditure of hatchlings and time spent to construct the nest. Nest substrate such as grain size is hypothesized to influence hatchling fitness by affecting the amount of energy reserves used by the hatchlings during emergence. Throughout Malaysia more than half a million green turtle hatchlings are released from hatcheries annually, however the question of whether this practice is producing high-quality hatchlings remains unanswered. It is crucial to hatchery management to identify which types of sand are most suitable for egg incubation and beneficial for hatchlings during nest emergence. We measured the influence of sand types on hatchlings fitness in terms of self-righting ability and swimming performance which emerged from the coarse and fine grained sand. Ten nests which had reach 45 days of incubation duration were selected as the developing embryos would be robust enough to be transported from the nesting to the laboratory for further incubation. Eggs from each clutch were split and allowed to continuing incubating in two different experimental conditions, fine and coarse grain. After hatched, the morphological characteristics and self-righting ability of hatchlings were measured then placed inside the glass aquarium to test the swimming performance for 18 hours. Results revealed that sand types only influenced the straight carapace width (SCW) of hatchlings but had no effect on their swimming. Cooler nest temperatures and longer incubation periods in nests constructed in fine sand caused the hatchlings to have larger straight carapace width than hatchlings from coarse sand. We suggest that the relocation of eggs in the late incubation period within Chagar Hutang beach is fine. Hence, we concluded that green turtle hatchlings emerging from nests in either fine or coarse grain sands of Chagar Hutang beach did not have a significant impact on their swimming performance.

Introduction

Sea turtles are oviparous animals, with adult females emerging from the sea onto beaches in order to lay their eggs in sandy substrates. Before oviposition, the selection of a nest site is a critical factor that influences the reproductive success (Nel et al., 2013) and fitness of the resulting hatchlings (Nilsson 1984; Martin & Roper 1988; Fuentes et al., 2011). Female turtles may choose to nest at a site which minimizes their energy expenditures and time spent towards constructing a nest. Hendrickson and Balasingham (1966) state that sand characteristics, particularly coarse and fineness of sand are important in nest-site selection. Besides nest inundation and predation, key factors of the incubation environment, such as sand types, likely to influence embryonic development, emergence success, sex ratios, morphology and locomotion performance of the sea turtle hatchlings (Booth, 2017). This is supported by Wada et al. (2017) who found that female turtles had increased nesting success in finer sands, in addition to higher emergence success of hatchlings, in finer sand nests, then those incubated in coarse grain sand.

Female green sea turtles may nest in a wide range of sand types from fine sand to coarse sand due to changes in the physical characteristics of coastal nesting beaches (Kelly et al., 2017). Due to the energetic cost hatchlings face when digging out of the nest chamber, sand grain type may influence

hatchlings locomotion performance during post emergence, dispersal crawling and swimming activities (Rusli et al., 2016; Saito et al., 2019). Nest substrate has been attributed to influence hatchling fitness considering how much hatchlings may struggle to escape the nest chamber, influences the amount of energy reserves used by the hatchlings during emergence. Hatchlings have a finite amount of energy reserves stored in their residual yolk after embryonic development, and once hatched, rely on this energy reserve to power their digging, crawling and swimming activities until food can be found in the open ocean. After nest emergence, sea turtle hatchlings crawl down the beach and enter the ocean. Right after entering the ocean, sea turtle hatchling swims vigorously and continuously for approximately 24 hours (Salmon et al., 2009), depending on their yolk reserves (Wyneken and Salmon, 1992; Wyneken, 1997). The more energy they use while escaping the nest, the less energy will be available for crawling and swimming activities. Therefore, it is important to determine whether emerging through different types of sand, either fine or coarse grain, affects hatchling's fitness, in terms of its swimming performance.

Previous studies highlighted female turtles nest site preferences correlated with various environmental factors such as distance from the high-tide line (Lopez-Castro et al., 2004; Zavaleta-Lizarraga & Morales-Mavil, 2013), beach elevation (Zare et al., 2012), vegetation (Wang and Cheng, 1999; Turkozan et al., 2011), beach slope (Horrocks and Scott, 1991; Wood and Bjorndal, 2000; Cuevas et al., 2010), moisture (Bustard and Greenham, 1968; Yalcin-Ozdilek et al., 2008), and sand particle size (Mortimer, 1990; Grain et al., 1995). Saito et al. (2019) discusses how coarse and fine sand can influence hatch, emergence success, and locomotion performance of loggerhead hatchlings. The first attempt in assessing the energetic cost to turtle hatchlings during nest escape was conducted by Rusli (2016). He found that for freshwater turtle (*Emydura macquarii signata*) hatchlings, digging through a sand substrate was less energetically costly, than digging through heavy clay substrate due to differences in sand compaction and in the differences in energy spent while digging through different substrates. This may provide evidence that hatchlings incubated in less compact substrates will have larger energy reserves after emergence, than hatchlings incubated in the more compact substrates.

Differences in energy reserves after nest escape will affect hatchlings locomotion performance, influencing a hatchling's chances of surviving between nest emergence and reaching the open ocean (Gyuris, 1993, 2000; Pilcher et al., 2000). If hatchlings use more energy during nest escape, they may become fatigued, resulting in weaker locomotor performance, increasing the probability of predation during the early phase of their life. It is advantageous for hatchlings to be fast swimmers with large energy reserves, allowing them to quickly swim through predatory rich near-shore waters. In this study, we focus on early swimming effort, which has been termed as 'swimming frenzy' (Salmon and Wyneken, 1987). As most predation of sea turtle hatchlings occurs during this period, it is crucial to understand how variations in reserved energy in a hatchlings body might influence their early survivability. This study investigates the effect of sand type on hatchling's swimming performance by measuring the power-stroke rate of swimming hatchlings, and proportion of time spent power-stroking at 18-hour period.

After nest emergence, sea turtle hatchlings crawl down the beach and enter the ocean. Right after entering the ocean, sea turtle hatchling swims vigorously and continuously for approximately 24 hours

(Salmon et al., 2009), depending on their yolk reserves (Wyneken and Salmon, 1992; Wyneken, 1997). This study hypothesized that hatchlings emerging from fine sand will be disadvantaged because they will spend more time and energy escaping the nest substrate and thus have less energy to spend towards crawling and swimming activity, compared to hatchlings emerging from coarse sand. It is assumed that an increase in early locomotor activity, for example, swimming ability, is advantageous because it may allow hatchlings to better avoid predators (Janzen, 1993). The purpose of this study is to discover if and how sand characteristics influence hatchlings morphology, and fitness in terms of self-righting ability and early swimming performance after nest emergence, information that will be useful for sea turtle rookery managers in making decisions about the best types of sand should be used in hatcheries.

Methods/experimental

Study site

Chagar Hutang is a crescent shaped beach 350 m in length, located at the northernmost part of Redang Island (5°44′-5°50′N, 102°59′-103°5′E), (Figure 1). The beach is divided into 35 sectors, each 10 meters wide along its length. Chagar Hutang beach consists of three distinct regions composed by sand of different particle sizes. Fine sand grain particles (<0.5 mm), characterize the substrate for the most eastern 120 m of beach length, the middle 180 m of beach is composed of medium sand grain particles (0.5 mm – 1 mm) and most western 50 m of beach length (around a small stream bed) is composed of coarse sand grain particles (1 mm – 2 mm) (Stewart et al., 2019).

Nest and egg collection

Ten nests were collected from Chagar Hutang beach. Nests which had reach 45 days incubation duration were selected as the developing embryos would be robust enough to be transported from the nesting to the laboratory for further incubation. At Chagar Hutang, researchers record the date of every nesting activity, and based on records, we can calculate which nest are in 45 days old. Due to limited electricity this experiment could not take place at SEATRU's research camp at the Chagar Hutang. Hence, nests were excavated and eggs were placed in the icebox (no cooling used) to be transported along a 15-minute boat ride from Chagar Hutang beach to SEATRU's Turtle Lab at The Taaras Beach and Spa Resort.

When collecting the eggs from the nest, a mark with a permanent marker pen was placed on top of each egg to maintain the egg orientation during transportation. Relocated clutches were expected to begin hatching within 24 to 72 hours after arriving at the laboratory. Eggs from each clutch were split and allowed to continuing incubating in two different experimental conditions, fine (< 0.5mm) and coarse grain (1-2 mm). Pipped eggs (small tear in the egg shell prior to hatching, by the hatchling with their egg tooth) and eggs which had not yet pipped were buried, in groups of varying clutches size, (15-40 eggs) in respirometry chambers filled with fine and coarse sands.

Sand grain size

One hundred grams of sand was collected at a depth of 70 cm from three different sections along the length of Chagar Hutang and left to dry under direct sunlight for three days before particle size analysis. A series of metal sieves (2000 μm , 1000 μm , 710 μm , 500 μm , 250 μm , 125 μm , 90 μm , 63 μm) were stacked in order from largest mesh size to smallest mesh size, with the sand sample placed in the top sieve (Folk, 1974). The sieve stack was then placed into an electronic shaker for 15 min which sorted the sand samples out into different sized particles. The sand remaining in each sieve was then weighed using an electronic balance, so that a sand grain size frequency distribution graph could be generated. This analysis was repeated 3 times for sample. In order to describe the particle size of Chagar Hutang beach sands, particle grain size was analysed using the Udden-Wentworth grade scale (Udden, 1941; Wentworth, 1922). The sand's sorting value reflects its compactness (Kenny and Sotheran, 2013), with poorly sorted soil are more compact than well-sorted sand (Tucker, 1996). The results analysing particle grain size (Table 1) showed that coarse sand has a mean phi value of 0.507, fine sand has a mean phi value of 2.028 represents. In terms of sorting classification, fine sand was classified as moderately well sorted and coarse sand classified as well sorted (Ali et al., 2016).

Hatchling measurements

After one week of relocating eggs from Chagar Hutang into the incubation chambers at the Turtle Lab, hatchlings began to emerge on top of the sand surface. Four hatchlings were collected at random every emergence event, and hatchling size was measured thereafter. The straight carapace length (SCL), straight carapace width (SCW), right front flipper total length (RFFTL) and body thickness (BT) of 60 hatchlings were measured (using a digital calliper (Sontax digital calliper). Carapace length and width measurements were multiplied together to calculate each hatchling's carapace size index (SI) in mm^2 . After hatchlings size measurements were completed, the self-righting time of the hatchling was measured.

Self-righting trials

Measurements of self-righting were carried out by following the method described by Booth et al. (2013), where self-righting refers to the ability of hatchlings to turn themselves over after being placed upside down on their carapace. Each of the hatchlings was placed upside down on its carapace on a flat sand surface, and the time (s) taken for the hatchling to self-right themselves was measured with a stopwatch. If a hatchling failed to self-right itself within 10 seconds, it was returned to its plastron to rest for 10 seconds before the next trial. This procedure was repeated three times for each hatchling to obtain mean self-righting times.

Swimming performance

Immediately after self-righting measurements, hatchling's power-stroke rate was measured over the course of 18 hours of swimming. Hatchlings were placed in a glass aquarium (41 cm x 25 cm x 26 cm) filled with seawater maintained at 28 °C. The walls of the aquarium were fully covered with black plastic except from one side where a dull light was placed in order to encourage unidirectional swimming activity. Hatchlings were attached to a monofilament tether using harnesses constructed from medical tape (Ischer et al. 2009) and was tied on a retort stand. The tether's length was adjusted so that hatchlings could swim freely in any direction but could not touch the bottom or sides of the aquarium in order to simulate an open-water oceanic environment (Burgess et al. 2006) (Figure 2). In order to identify the effect of sand types on the swimming performance of the hatchlings, their power-stroke rate and proportion of time hatchlings spent power-stroking, was measured for 18 hours, between hatchlings which emerged from the fine and coarse sand. Booth (2009) showed that between 12 to 18 hours after entering the ocean, the swimming efforts of green turtle hatchlings plateau at an almost constant level; hence, this is the reason why the swimming performance was measured until 18 hours. Hatchlings' swimming behaviour, over the course of the 18 hours swimming trial, was recorded using a Webcam and Bandicam software. After 18 hours swimming trial was over, each hatchling was released back to the ocean during the night. Sixty hatchlings' swimming ability was quantified by calculating the power-stroke rate per min throughout the 18 hours swimming trials.

Webcams were mounted on the retort stand, in order to capture the swimming behaviour of hatchlings and recorded at a rate of 30 frames per second. Webcams recorded four hatchlings at a time, allowing the two hatchlings from each sand type to be tested simultaneously per trial. QuickTime Player software was used to manipulate video speed. The power-stroke rate and length of time spent power-stroking were measured using a stopwatch. Ten power-strokes were measured 10 times every hour during the swimming trials, starting from 60 seconds to 4 hours because during this time all the hatchlings were power-stroking. However, after 4 hours time, most of the hatchlings took 10 to 40 minutes to rest before continuing to power-stroke. During this period, the hatchlings' power-stroke rate was measured immediately upon starting to power-stroke after the resting period. If no power-stroking bouts were observed in a particular hour, a power-stroke rate of zero (0) was assigned to that individual for that hour. The proportion of time spent power-stroking was measured for 2 minutes in every hour when hatchlings power-stroked continuously during the hour measurement period.

This study was approved by the Universiti Malaysia Terengganu Research Ethics Committee (UMT/ JKEPHT/ 2018/ 23), and green turtle eggs were collected under permit no: Prk. Tr. 2213 Jld 3 granted by the Terengganu State Fisheries Office.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20 software. Data of morphology, self-righting and swimming were tested for normality. A Mann-Whitney test was used to test for any differences in the morphological characteristics (SCL, SCW, BT and RRFTL)

between hatchlings which emerged from different sand types while a T-test was used for SI. A mixed model ANOVA was used to compare between self-righting time, clutch, and sand types with self-righting time as dependent variables, clutch as a random factor and sand types as a fixed factor. To test the effect of time and sand types on power-strokes rate, repeated-measures ANOVA were performed separately and repeated measures ANOVA after an arcsin transformation used to compare the proportion of time spent power-stroking (Pereira 2014; Pereira et al. 2011; Booth 2009). For power-strokes rate and proportion of time spent power-stroking, a Tukey *post hoc* test for unequal sample sizes was used for cross-comparisons at 23 different times: at 1min, 5min, 10min, 20min, 30min, 1h, 2h, 3h, 4h, 5h, 6h, 7h, 8h, 9h, 10h, 11h, 12h, 13h, 14h, 15h, 16h, 17h, and 18h of swimming trials. Statistical significance was assumed if $P < 0.05$.

Results

Hatchling morphology

The morphological characteristics of 60 hatchlings were measured (Table 2). There were no significant differences between hatchling that emerged from fine and coarse sand [SCL ($U = 444.5$, $P = 0.937$), BT ($U = 338.0$, $P = 0.344$), RRFTL ($U = 392.0$, $P = 0.391$) and SI ($t_{52.208} = -1.168$, $P > 0.160$)] across the sand types. However, 30 hatchlings from the fine sand had significantly larger SCW (Mean = 35.05) than 30 hatchlings from the coarse sand (Mean = 25.95), $U = 313.5$, $P = 0.039$.

Self-righting time

The self-righting time of the hatchlings was assessed as an indicator of hatchling fitness. Righting response time for hatchlings from the coarse sand ranged from 1.08 s to 7.67 s (2.985 0.272), while for hatchling from the fine sand ranged from 1.33 s to 7.14 s (3.396 0.313) (Figure 3). Mixed model ANOVA indicated neither sand type ($F_{1,42} = 0.055$, $P = 0.81946$) nor clutch ($F_{9,42} = 1.198$, $P = 0.41559$) have a significant effect on self-righting time of hatchling, however there was a significant differences between clutch vs. sand type interaction ($F_{7,42} = 2.915$, $P = 0.01406$) (Figure 4 & Figure 5). Table 3 represents the means and standard deviations of self-righting time within sand types from each clutch and Table 4 represents the summary of the statistic results.

Swimming performance

Power-stroke rate

This study investigated the influence of emerging between fine and coarse sand of hatchlings' (i) power-stroke rate and hours of swimming, (ii) the proportion of time spent power-stroking and hours of swimming with the type of sands. Hatchlings from the coarse sand produced more power-stroke (50% decline) than hatchlings from the fine sand (48% decline) during the first 16 hours of swimming (Figure 6). After 16 hours of swimming, hatchlings from the fine sand produced more power-strokes. Stroke rate during a power-stroke bout by hatchlings from the coarse sand decreased (17%) very rapidly within the

first 20 minutes, then decreased at a slower rate over the first 9 hours and continued to decrease at a slower rate until the 18 hour marks. Hatchlings from fine sand had a similar pattern as the coarse sand, the stroke rate decreased very rapidly (20%) in the first 20 minutes, then decreased at a slower rate over the first 5 hours and did not change until reaching the 18 hour marks. The power-stroke rate of hatchlings from coarse sand decline to 59%, while the power-stroke rate of hatchlings from fine sand decline to 45%.

ANOVA repeated measures were performed to compare variables among sand types. A repeated-measures ANOVA in which sand types were the dependent variables and the power-stroke rate was the repeated independent variables that was applied and showed that sand types did not influence hatchling power strokes. However, time and sand type vs. time interaction show significant differences (Table 5). This means that the stroke rate changed with time in a different way in hatchlings from coarse sand, then in hatchlings from fine sand. A Tukey *post-hoc* test indicated that the power-stroke rate of hatchlings decreased significantly with time from 1 minute to 9 hours but from 10 to 18 hours power-stroke rate remained constant for both sand types (Appendix 1). The power-stroke rate during a power stroking bout decreased as hours of swimming increased.

Proportion of time spent power-stroking

Hatchlings from coarse sand spent more time power-stroking than hatchlings from fine sand (Figure 7) between 5 to 12 hours and continued from 14 to 15 hours of swimming, however, at 17 to 18 hours, fine sand spent more time power-stroking than hatchlings from coarse sand. The proportion of time spent power-stroking by hatchlings from coarse sand appeared to increase at a slower rate within the first 3 hours then decreased at a slower rate until 13 hours and decreased very rapidly after 13 to 18 hours of swimming trials. Alternatively, the proportion of time spent power-stroking by hatchlings from fine sand begins to increase at a slower rate within the first 3 hours then decreased very rapidly until 7 hours, after which it stays consistent. The proportion of time spent power-stroking by hatchlings from coarse sand decreased by 18% within 18 hours of swimming trials and decreased by 7% for hatchlings from fine sand respectively.

ANOVA repeated measures indicated no effect of sand type on the proportion of time spent power-stroking and sand type vs. time interaction (Table 5). This means that the change in the proportion of time spent power-stroking over time was not influenced by sand type. However, only time is indicated as significant. A Tukey *post-hoc* test indicated that the power-stroke rate of sea turtle hatchlings decreased significantly with the time between 2 to 3 hours and 17 to 18 hours for both sand types (Appendix 2).

Discussion

Hatchling morphology

Sand types were expected to influence hatchling's morphology. However, green turtle hatchlings morphology such as SCL, BT, RFFTL, and SI were not different between hatchlings emerging from either

fine or coarse sand in this study (Table 2). The maternal effect could be one of the reasons in which the hatchlings retained similar fitness characteristics, as they resulted from a single clutch that was split and incubated into different sand substrate types. In this study, eggs clutches were split in half and incubated in separate chambers filled with fine sands and coarse sands to investigate the effects on hatchlings locomotion not on the morphology of the sea turtle hatchlings. For future studies, collecting eggs from different mothers and incubated the eggs in different types of sand should be considered in order to eliminate the maternal effects. The previous study has reported the same result on the green turtle hatchling carapace size index where the sand types do not influence the hatchling carapace size index (Stewart et al., 2019).

Although SCL, BT, RFFTL, and SI were similar across sand types, the SCW of hatchlings from fine sand were slightly larger in size than hatchlings from coarse sand (Table 2). This might be due to cooler nest temperatures and longer incubation periods in nests constructed in fine sand (Saito et al., 2019) which provide more time for hatchlings to convert yolk into the tissue before hatching to develop into larger hatchlings (Booth and Astill, 2001; Booth, 2006, 2017). To study this relation is important to consider that nest temperatures might influence the hatchlings' body sizes (Gyuris, 2000) since nests with cooler temperature appear to produce larger hatchlings (Ischer et al., 2009). Hatchling sizes may affect hatchling survivor rates as larger body sized have an advantage when they are dispersing across the predatory-rich reefs environments, as they are more capable of escaping the gauntlet of predators (Congdon et al., 1999; Gyuris, 2000; Janzen et al., 2000; Booth, 2006).

Self-righting time

Self-righting ability has been applied in previous studies as an indicator of hatchlings' fitness (Hosier et al. 1981; Burgess et al. 2006; Maulany et al. 2012a,b; Read et al. 2013; Sim et al. 2014; Wood et al. 2014). In this study, neither clutch nor sand type was shown to influence the self-righting time of hatchlings (Table 3). However, in the previous study by Saito et al. (2019) and Stewart et al. (2019), hatchlings incubated in coarse sand are poor self-righters with lower self-righting propensity than hatchlings incubated in the fine sand. From both studies, it is proved that the loggerhead and green turtle hatchlings that emerging from coarse sand are poor self-righters. Self-righting has been identified as an important aspect of sea turtle hatchling fitness (Delmas et al., 2007). Occasionally, sea turtle hatchlings encounter objects such as rocks, coral rubbles, and tree branches on the beach, which cause them to fall upside down onto their carapace. The longer the time they take to turn themselves upright, the more vulnerable they are to predation and death by exposure to increased temperature (Booth et al., 2013). According to Booth et al. (2013), neck length is an important parameter for self-righting ability since sea turtle hatchlings self-right by flexing their head against the substrate, but has not been measured in this study. It is important to measure the neck length of the hatchlings for future study. There is an interaction between clutch vs. sand types of fine and coarse sand (Figure 4). This means that in some clutches, hatchlings from the coarse sand had faster self-righting times, but in other clutches, hatchlings from the fine sand had faster self-righting times. This might be a result of differences in the genetic makeup and/or the proportion of nutrients transfer from different females to their eggs.

Swimming performance

Power-stroke bouts are produced by the up and down movement of the hatchlings front flippers to generate forward thrust, and lasted for 2 to 20 seconds (Carr and Ogren 1960; Davenport et al. 1984; Salmon and Wyneken 1987; Wyneken 1997; Burgess et al. 2006; Pereira et al. 2011). During hatchling dispersal, power-stroke bouts are inter-dispersed with a dog-paddling style of swimming for 1 to 5 seconds during which hatchlings heads break the surface to breath air (Salmon and Wyneken 1987; Wyneken 1997; Burgess et al. 2006). The swimming efforts of sea turtle hatchlings normally decreases with time spent swimming, with a rapid decline occurring within the first two hours of entering the water (Pilcher & Enderby 2001; Booth et al. 2004; Booth 2009; Ischer et al. 2009; Booth & Evans 2011; Pereira et al. 2011; Sim 2014). Additionally, Booth (2009) reported hatchlings' swimming effort, in terms of power stroke rate, to sharply decrease during the first 20 minutes of swimming, termed the rapid fatigue phase. Between 60 seconds until 16 hours during the swimming performance trials, hatchling from coarse sand showed a higher power-stroking rate than hatchlings from fine sand (Figure 6). However, after 16 hours, the rate of power-stroking is higher in the fine sand type than in the coarse sand type.

The graph (Figure 6) shows fluctuating trends when relative long resting periods occur after 5 hours of swimming. This means that the hatchlings from fine sand tend to stop and rest temporarily, in order to gain their energy back before power-stroking commences again. Eventually, muscle fatigue is the cause of decreased swimming effort during this time (Burgess et al. 2006). As hatchlings time swimming in the ocean continues, the power-stroke rate lessens, dog-paddling bouts grow longer, and periods of rest may occur where there are no flipper movement.

Compared with hatchlings from coarse sand, the stroke rate of hatchlings from fine sand was much lower and power-stroking bouts were much shorter (Figure 6 and Figure 7). It is assumed that larger body size of sea turtle hatchlings are better locomotors (Ischer et al., 2009). However, green turtle hatchling morphology were similar between hatchling emerging from either fine or coarse sand. The better locomotion of hatchlings in the coarse sand might be attributed to a lighter body in a similar sized frame needing to do less work overcoming gravity (Stewart et al., 2019). Although the graphs show differences in power-stroking rate and time spent power-stroking, statistical analysis concluded that differences in sand type did not influence the early swimming performance of green turtle hatchlings. This indicates that further studies to compares the energetic cost of swimming for 18 hours between hatchlings from both sand types, with focus on power strokes rate produced throughout time. This may provide insight into why swimming efficiency changed over time and provide a real-time comparison of the energetic cost of swimming between treatment types.

This study's results are in contrast with a recent publications by Saito et al. (2019) and Stewart et al., (2019), which reported that hatchlings from fine grain sizes have stronger swimming ability and fitness than hatchlings from coarse grain sizes. This can be explained by the tendency for the walls of nest chambers constructed in sand consisting of large particles grain sizes collapse more easily, making it more difficult for hatchlings to dig out from the nest (Mortimer, 1990) and hinder group emergence (Saito

et al., 2019). It is theorised that hatchlings consume more energy escaping a nest comprised of large sand grain particles, leaving less energy reserves in the hatchlings body for post-emergence activities, like crawling and early swimming, adversely impacting the locomotor performance of the hatchlings. According to Carr and Hirth (1961), hatchlings that emerge in small groups were relatively weaker because they are more exhausted, compared to hatchlings which emerged in large groups. Additionally, reported by Rusli et al. (2016), group emergence helps to reduce the energy expenditure of individual hatchlings by distributing the workload of the digging process across multiple hatchlings. From the results, green turtle hatchlings emerging from nests in either fine or coarse grain sands of Chagar Hutang beach did not have a significant impact on their swimming performance. Therefore, the relocation of eggs in the late incubation period within Chagar Hutang beach is fine.

Better locomotor performance, for example swimming ability, is advantageous because it may allow hatchlings to better avoid predators (Janzen, 1993). Predation of hatchlings is directly linked to the time hatchlings spend crawling down the beach and swimming across coral reefs. The longer the time spent crawling on the beach and swimming in the coral reef areas, the higher the possibility of hatchlings to be consumed by predators. The highest mortality rate occurs during hatchlings' dispersal across the coral reef areas, where primary predators are relatively active (Gyuris, 1994; Pilcher et al., 2000). Malaysia is a tropical country, with extensive coral reef areas dominating the shallow water environment and host many predators. In order to avoid such predation, a hatchling needs to swim across the coral reef areas faster and, once they reach the open sea, the predation pressure decreases and swimming effort becomes lower. Therefore, optimizing nest conditions is important to produce hatchlings with strong performances and increase the survival rate.

Clearly, more studies on the effects of different types of sand on sea turtle eggs and hatchlings are needed since the current study could not disprove the null hypothesis and provide clear results. This is important in order to come to a conclusion whether sand types may or may not influence the quality of sea turtle hatchlings in terms of their fitness and locomotor performance. Better understanding how sand types may influence the quality of sea turtle hatchlings can help implement better hatchery management practices and improve our knowledge of sea turtle nesting ecology. Therefore, determining the best nest conditions for incubating sea turtle eggs is a critical step in order to produce fit hatchlings with strong swimming abilities.

Conclusions

This study has demonstrated that sand types only had an influence on the morphology, the SCW of the green turtle hatchlings but not their early swimming performance. Hatchlings which emerged through fine sand had larger straight carapace width than hatchlings from coarse sand. Although there were no significant differences in hatchlings' power-stroke and proportion of time spent power-stroking, the swimming effort of green turtle hatchlings from both sand types decreased as swimming time increased. This decrease in swimming effort over time is probably because of muscle fatigue. As the hatchlings

spend a longer time in the ocean, the power-stroke rate reduces, dog-paddling bouts grow longer and periods of rest may occur, where there is no flipper movements.

Abbreviations

SCL: Straight Carapace Length; SCW: Straight Carapace Width; BT: Body Thickness; RFFTL: Right Front Flipper Total Length; SI: Body Size Index

Declarations

Availability of data and material

Please contact the corresponding author for data requests.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

MUR and DTB has contributed in proposed the topic, conceived, designed the study and reviewing of the manuscript. DTB also has contributed in statistical analysis of data. NSS carried out the experimental study, analyzed the data and writing the manuscript. JJ collaborated with the corresponding author in the construction of manuscript and reviewing of the manuscript. All authors read and approved the final manuscript.

Authors' information

Sea Turtle Research Unit (SEATRU), Institute of Oceanography and Environment (INOS), Universiti Malaysia Terengganu, 21030, Kuala Nerus, Terengganu, Malaysia.

Nadhirah Syafiqah Suhaimi, Mohd Uzair Rusli

Department of Zoology and Entomology, School of Life Sciences, The University of Queensland, ST. Lucia, Queensland, Australia

David T. Booth

Endangered Marine Species Research Unit, Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia

Juanita Joseph

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Tables

Table 1. Range of coarse and fine sand by using the Udden-Wentworth, 1922 grade scale.

Label	Coarse	Fine
Parameter	Phi	
Mean (x)	0.507	2.028
Sorting	0.429	0.706
Skewness	0.250	-0.877
Kurtosis	7.001	4.447

Table 2. **Hatchling morphology**. Data is shown as mean SE. Mann-Whitney test was used for statistically comparing SCL, SCW, BT, and RRFTL, and the T-test was used for SI. P-values with significant differences are highlighted by asterisk.

Variables	Sand Type		P-value
	Coarse	Fine	
	Mean		
SCL (mm)	46 0.257	46 0.321	0.937
SCW (mm)	35 0.243	36 0.344	0.039*
BT (mm)	17 0.202	18 0.184	0.344
RRFTL (mm)	41 0.400	41 0.454	0.391
SI (mm)	1626 16.786	1660 23.732	0.160

Table 3. Data are means and standard deviations of self-righting time of hatchlings emerging from fine and coarse sand from each clutch.

Clutch Number	Coarse Sand		Fine Sand	
	N	Self-righting Time	N	Self-righting Time
289	2		3.80 0.87	-
293	2		6.56 0.87	1 2.56 1.23
398	2		2.61 0.87	2 3.45 0.87
421	4		2.84 0.61	4 5.58 0.61
500	7		3.21 0.46	8 4.21 0.43
519	2		2.59 0.87	2 2.84 0.87
683	6		2.14 0.50	4 1.59 0.61
758	-		-	4 1.93 0.61
1036	1		1.86 1.23	4 3.81 0.61
1120	4		2.48 0.61	1 1.48 1.23

Table 4. Mixed model ANOVA was used to test differences between self-righting time, clutch, and sand types. P-value with significant differences is highlighted by asterisk.

	Self-righting time (sc)
	Probability statistic
Clutch	$F_{9,42} = 1.198$ $P = 0.41559$
Sand types	$F_{1,42} = 0.055$ $P = 0.81946$
Clutch vs. sand types interaction	$F_{7,42} = 2.915$ $P = 0.01406^*$

Table 5. Power-stroke rate and proportion of time spent power-stroking. Data is shown as mean SE and probability statistics. ANOVA repeated-measures compare 3 main effects (sand types, swimming time and interaction between these two factors). P-value with significant differences is highlighted by asterisk.

	Coarse	Fine	Swimming time	Sand type	Sand types vs. Time interaction
Power-stroke rate (stroke/min)	92 1.26	91 5.13	$F_{22,1276} = 68.766,$ $P = 0.000^*$	$F_{1,58} = 1.383,$ $P = 0.244$	$F_{22,1276} = 2.357,$ $P = 0.000^*$
Proportion power-stroke (%)	75 1.68	70 1.74	$F_{22,1276} = 1.993,$ $P = 0.004^*$	$F_{1,58} = 1.865,$ $P = 0.177$	$F_{22,1276} = 0.518,$ $P = 0.968$

Figures

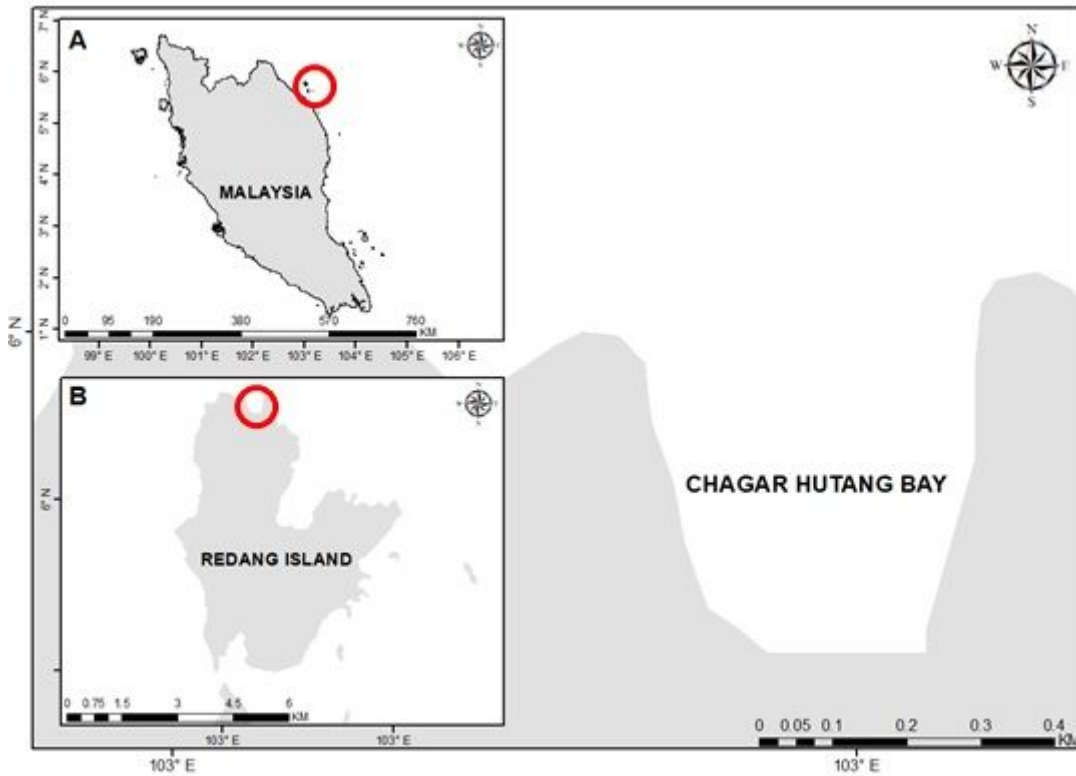


Figure 1

Map of the study area. (A) Peninsular Malaysia; (B) Redang Island; (C) Close up view of Chagar Hutang Turtle Sanctuary.

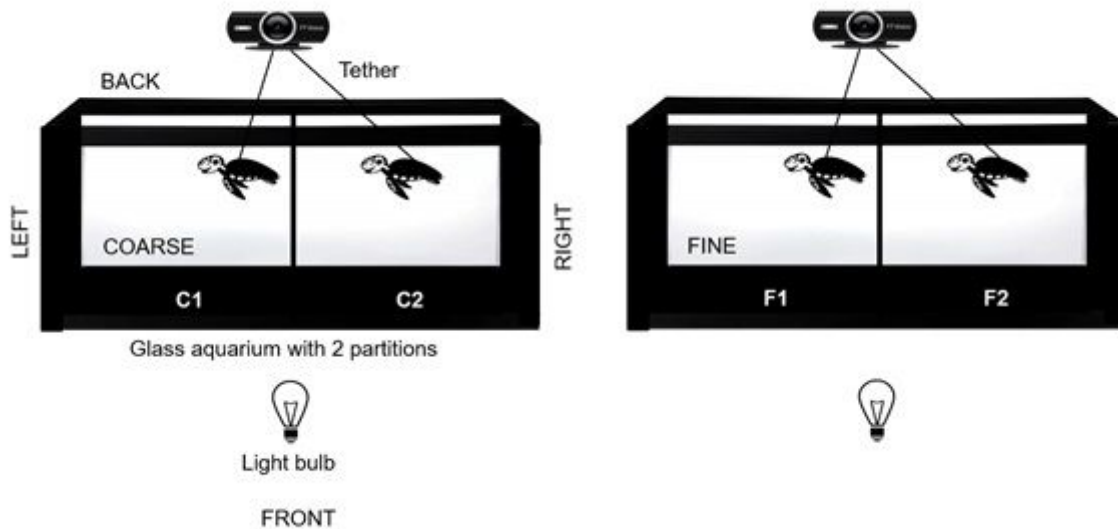


Figure 2

Experimental setup for swimming performance analysis. All sides were covered with black plastic except the front panel, where a dull light was positioned to influence unidirectional swimming. Two webcams were used to record the aerial view of swimming hatchlings between the two treatments.

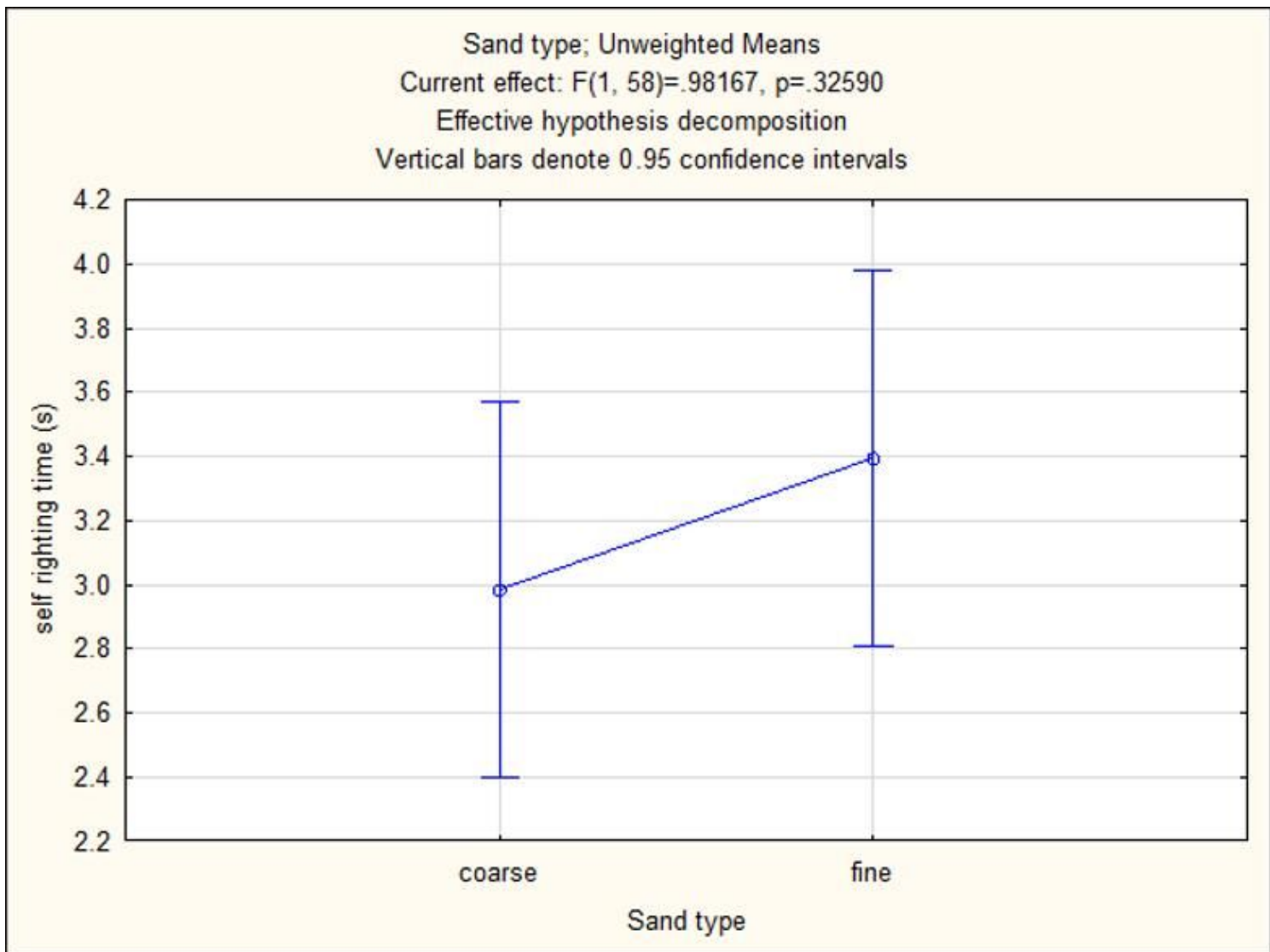


Figure 3

The self-righting time of 30 individual hatchlings from the coarse sand and 30 individual hatchlings from the fine sand.

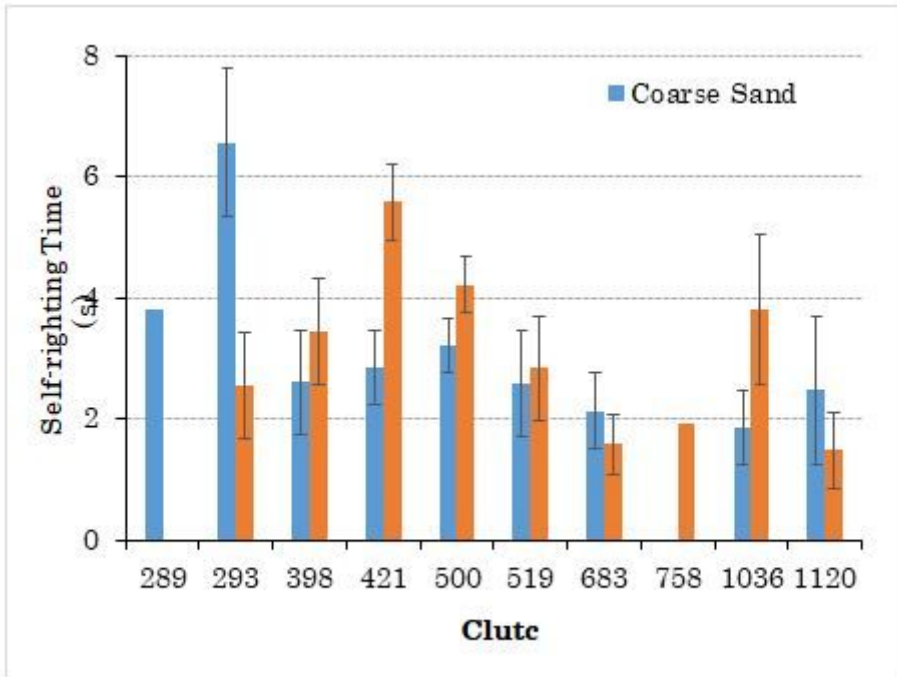


Figure 4

Mean self-righting time of hatchlings between sand types (fine and coarse) for each clutch.

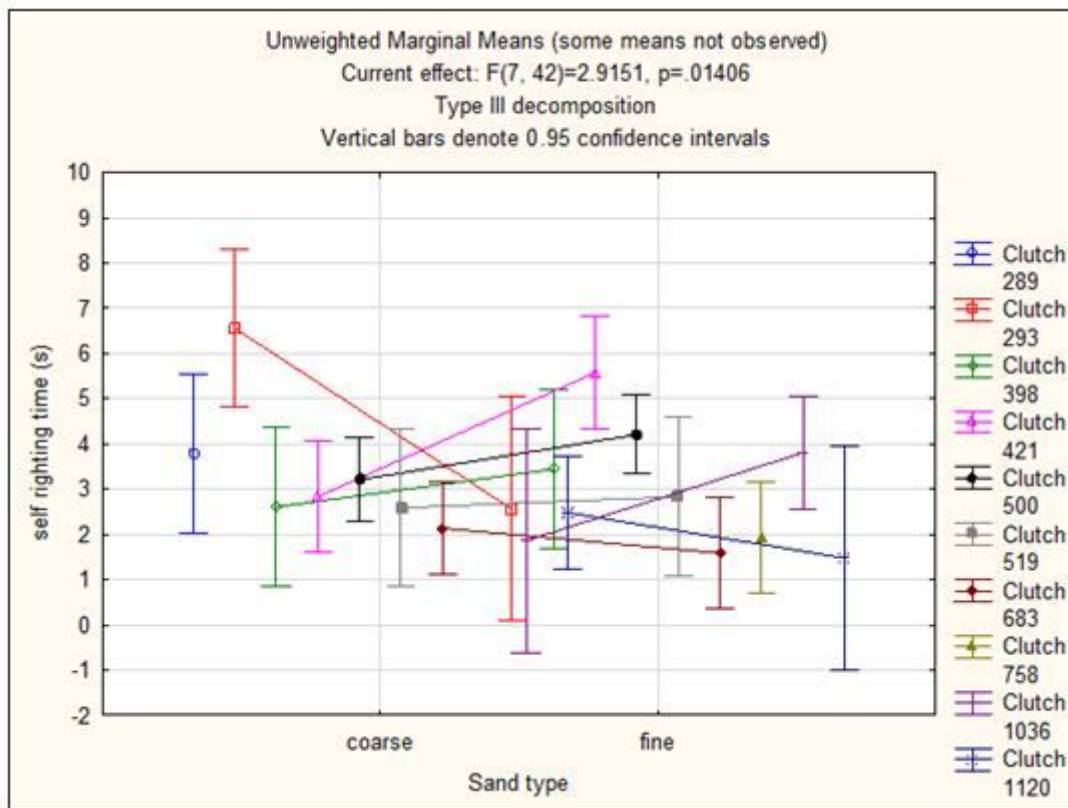


Figure 5

Plots of interaction between sand types and clutches.

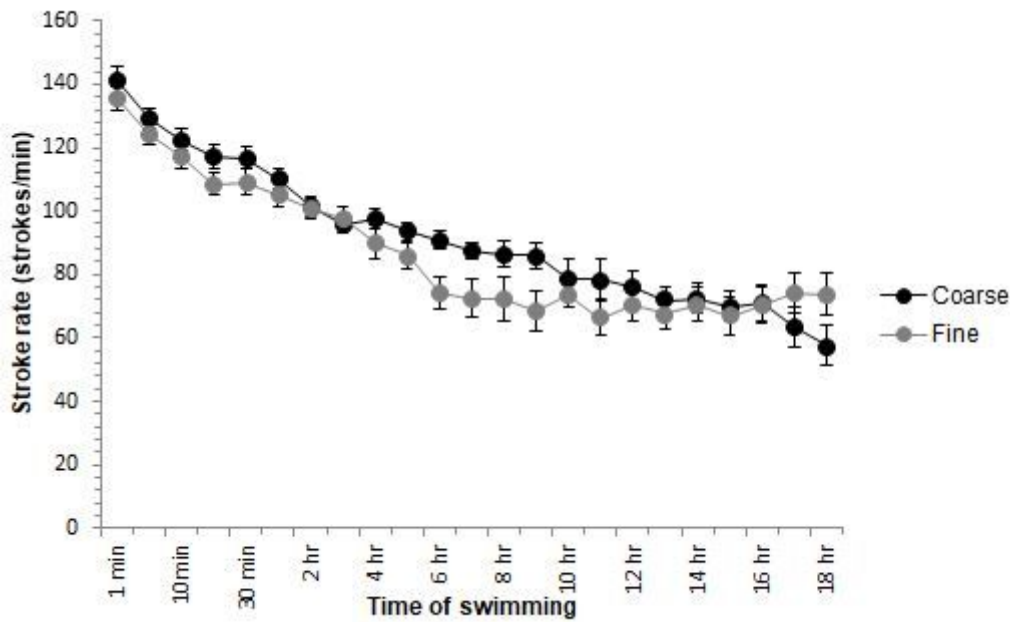


Figure 6

Rate of hatchling's power stroke. Graph of stroke rate during power-stroke bout during the first 18 hours of swimming frenzy activity in green turtle hatchlings from Chagar Hutang, Redang Island. The graph shows the mean stroke rate during a power-stroke bout for 30 individuals for each treatment, from 1 minute to 18 hours. Mean power stroke rates were calculated from 30 individuals from each treatment. Repeated measures (ANOVA) indicated power-stroke rates for both treatments decreased with time $F(22,1276) = 68.766$. $P = 0.000$.

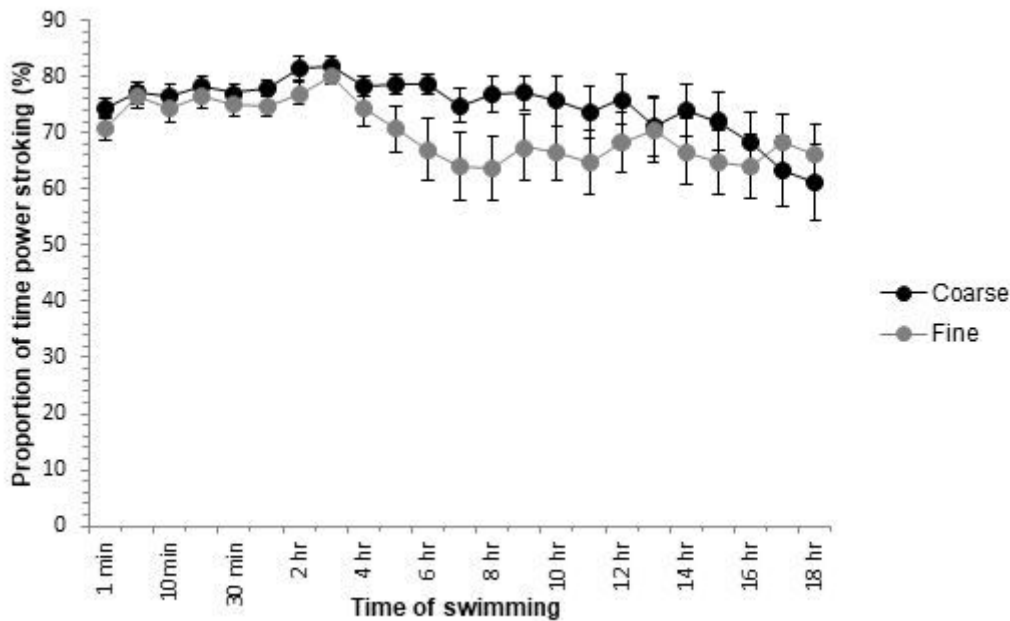


Figure 7

The proportion of time spent power-stroking. Graph of the proportion of time spent power-stroking during the first 18 hours of swimming frenzy activity in green turtle hatchlings from Chagar Hutang, Redang Island. The graph shows the mean proportion of time spent power-stroking for 30 individuals for each treatment, from 1 minute to 18 hours. Mean power stroke rates were calculated from 30 individuals from each treatment. Repeated measures (ANOVA) indicated power-stroke rates for both treatments decreased with time $F(22,1276) = 1.933$. $P = 0.004$.

Supplementary Files

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