The effect of sand temperature on the size of hatchery relocated green sea turtle (*Chelonia mydas*) hatchlings in Pulau Perhentian Besar, Malaysia

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Abstract

Marine biodiversity is in global decline, at a rate that is alarming. This is concerning as marine habitats host a variety of keystone species such as sea turtles. According to the IUCN Red list of Threatened Species, three out of the seven species of sea turtles are currently endangered. Therefore, it is important to study the biological processes that affect the persistence of sea turtles along with what ensues from the current conservation efforts. I examined the relationship between sand temperature during incubation and the carapace length (SCL) and width (SCW) of green sea turtle hatchlings from relocated nests on a beach on Pulau Perhentian Besar, Malaysia. Additionally, I examined the relationships of other factors (such as clutch size) and the SCL and SCW of hatchlings. I found that hatchlings incubated in nests that had warmer maximum temperatures had a wider carapace. I found that hatchlings from larger clutches had a shorter carapace. Both maximum temperature and clutch size during incubation were statistically significant, but I concluded that the biological significance was small based on other effect sizes reported in the scientific literature. Thus, it is important to monitor sand temperature to prevent things such as hatchling mortality or to monitor the sex ratio, but maybe not for the size of hatchlings.

Keywords: Conservation, hatchery relocation, endangered species, hatchling, incubation temperatures

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Introduction

Marine biodiversity is in global decline, at a rate that is alarming (Ward & al., 2022). According to the World Register of Marine Species (WoRMS), there are a little more than 247 000 species that have been discovered in the marine biome in 2025. It is concerning that this biodiversity is declining as it provides many important ecosystem services such as involvement in the carbon cycle, and the production of food and energy (Ward & al., 2022). There are various factors that are contributing to the decline in marine biodiversity. Most of those factors are of anthropogenic nature such as pollution, overfishing and oceanic warming as a result of global warming (Ward & al., 2022). Climate change is a major challenge in the marine biosphere by causing shifts in species distribution and abundance, which fundamentally change the ability to function of those ecosystems (Brown & al., 2022). These anthropogenic pressures are threatening many fragile habitats in the marine biome.

One of the most threatened habitats on Earth are coral reefs (Devlin, 2022). Climate change has been especially critical to the decline in coral reefs as it causes coral bleaching. This phenomenon refers to when corals lose their symbiotic intracellular microalgae due to thermal stress, leading to their loss of colour and loss in productivity (Woesik & al., 2022). Coral reefs are a vital part of the marine ecosystem as they are a very biodiverse habitat and provide many ecosystem services such as coastal protection and food provisioning (Yuan & al., 2024). The corals in this habitat provide refuge to many small and large fish and provide food for many marine species (Beese & al., 2023; Yuan & al., 2024). This threatened state of coral reefs has also led to the vulnerability of many keystone species (Putnam & al., 2017).

Sea turtles are keystone species of coral reefs. In coral reefs, sea turtles manage vegetation density by grazing seagrass and control the population of jellyfish and sponges through their predation (Hendrix & Pérez-Espona, 2024). The grazing seagrass behaviour both provides sea turtles with food, but also protection against predation from tiger sharks during the day (Smulders & al., 2022). By preying on jellyfish, sea turtles can control their populations and prevent jellyfish blooms, in which the rapid growth in population results in a trophic cascade in the environment (Goldstein & Steiner, 2019). Coral reefs could undergo significant changes due to the loss of their presence, as they are vital for the health of the habitat.

Sea turtle populations are generally in decline. According to the International Union for Conservation of Nature (IUCN) Red list, three out of the seven species of sea turtles are currently endangered. The three endangered species are: the Kemp's ridley (*Lepidochelys kempii*), the hawksbill (*Eretmochelys imbricata*), and the green turtle (*Chelonia mydas*). Many of the anthropogenic factors that are impacting the marine biome are also directly impacting sea turtles, such as consumption of plastic waste leading to indigestion, and accidental bycatch in fishing nets (Wilcox & al., 2018; Hendrix & Pérez-Espona, 2024). The combination of the threatened state of their habitat as well as the direct impact of anthropogenic factors has led to declines in the sea turtle population.

Climate change is especially a concern for sea turtles as the increasing thermal changes affects the sex-ratio by producing more female, reduces the size of hatchlings, increases morphological abnormalities and decreases hatching success (Blechschmidt & al., 2020; Wood & al., 2014; Fisher & al., 2014). Indeed, the temperature during incubation determines the sex of the sea turtle hatchlings, with cooler temperature producing more males and warmer temperature, starting at around 29.3℃, producing more females (Blechschmidt & al., 2020). When the temperature of incubation reaches about 34℃, there is an increase in sea turtle embryonic mortality (Bladow & Milton, 2019). The importance of proper temperature during embryonic development is highlighted in studies and conservation efforts due to the fact that the National Oceanic and Atmospheric Administration (NOAA, 2015) estimates that only one out of every thousand or even every ten thousand hatchlings will reach adulthood.

Due to their endangered status and their role as keystone species in the marine ecosystem, many efforts have been made to improve conservation strategies for the sea turtle population from reducing plastic usage to relocation of clutches. Relocating clutches to a hatchery on the nesting beach is one of the many conservation management strategies. This strategy allows to protect nests from predators and tidal inundation, reduces the travel that may damage the embryo, and to monitor the temperatures of the nest. Many studies have found that this relocation strategy has the similar hatching success as natural nests and artificial incubation (Wyneken & al., 1988; Chan, 1989; Hart & al., 2016; Garcí & al., 2003; Chacon-Chaverri & Eckert, 2007). Relocation to a hatchery additionally allows for shades to be set up to help reduce the nest temperatures (Wood & al., 2014).

Several studies have found that temperature affects the size of sea turtle hatchlings, with higher incubation temperatures leading to smaller carapace size of hatchlings (Glen & al., 2003; Wood & al., 2014; Sim & al., 2015). The incubation temperature impacts the phenotypic size of hatchlings because cooler temperatures prolong the incubation period, which results in a longer period during which the yolk sac is converted to tissue, thus producing larger hatchlings (Booth & al., 2004). The size of hatchlings may be important for their survival as hatchlings with longer carapaces were found to have longer flippers and be faster swimmers (Le Gouvello & al., 2020). For the future of their conservation, it is important to better understand what affects their size and what could increase their survival especially in a controlled environment like a hatchery.

In this study, I examined the relationship between sand temperature during incubation and the carapace length and width of green sea turtle hatchlings from relocated nests in a beach located on Pulau Perhentian Besar, Malaysia. I hypothesized that the increase in sand temperature decreases the size of hatchlings. I additionally examined the relationship between other factors (such as clutch size and hatching success) and the size of hatchlings.

Materials & Methods

Study site

Bubbles Turtle Conservation (BTC) is located on the Bubbles Dive Resort on the southeastern part of Pulau Perhentian Besar in the state of Terengganu, part of the east coast of Peninsular Malaysia (Fig. 1). The resort established in 2004 had noticed the nesting of green sea turtles on its beach, with approximately 85 to 230 nests per season, and decided to create BTC in response to carry out sea turtle conservation efforts in the bay area of the resort. In 2018, BTC obtained its permit from the Department of Fishery which allowed them to relocate eggs to the hatchery that is maintained on the beach and perform other sea turtle related conservation efforts. BTC is active on-site during the nesting season of sea turtles, from March to October.

The hatchery was constructed in early March of 2024 by interns and maintained every day by interns and volunteers. The hatchery was constructed using net fencing which was reinforced by plastic tubes with a dimension of approximately 11 x 6 m (Fig. 2). Tall wooden posts were installed outside the hatchery allowing for shading material to be strung across the hatchery to recreate the natural shading that is provided by trees for in-situ nests. A total of 8 temperature probes were installed in the sand at approximately the same depth that the average nests were dug (80 cm), using an auger, in the hatchery at equal distances from each other.

Data collection

All interns were trained by the project facilitator for the first 2 weeks upon arrival. This training included night patrols, rangering, hatchery relocation, hatchery maintenance, and post-hatching excavation procedures.

Night beach patrols were conducted every night and separated in two shifts, night shifts lasting from 19h00 to 02h00 and morning shifts lasting from 03h00 to 07h00. During patrols, the intern and volunteers would look for turtle crawling tracks (Fig. 3) or for a nest boil, which is when many hatchlings emerge from their nest. Low intensity red lights were employed during night patrols because red lights have a lower impact on sea turtles compared to other colors of light (Silva & al, 2017). If crawling tracks were found, the intern would then go up alone to inspect if a turtle is nesting or if there are any indications of a nest having been laid. If there is an indication of nesting, the intern would call their backup and begin the hatchery relocation process. All crawling tracks were dashed out using sticks or feet to prevent any confusion during upcoming patrols.

When oviposition began, the intern would begin to move eggs while counting quietly for the scriber. In the case where not all eggs are retrieved, a fishing line was placed in the nest to help locate the nest to allow for excavation and relocation of eggs after the mother turtle's departure.

After all the eggs were collected, they were transported in a bucket with sand at the bottom to the hatchery to begin the relocation process. A new nest was dug using an auger at a depth between 80 to 100 cm, depending on the number of eggs in the clutch to mimic a natural nest (Fig. 3). The nest was rounded by hand at the bottom to mimic a natural nest (Mortimer, 1999). The number of eggs was double checked when placing them into the relocated nest in the hatchery. Once all eggs were relocated, the nest was filled using soft hand-sifted sand to prevent damage from large coral pieces in sand. A small circle was dug around the nest to allow for net fencing to be placed and prevent hatchling from escaping during boiling over process (Fig. 3). An identification tag was then attached to the net fencing.

The post-hatching excavation procedure would begin when a nest in the hatchery was identified as boiling over. Buckets were brought based on the number of eggs per clutch, with one bucket holding a maximum of 50 hatchlings to prevent harm for hatchlings (Fig. 4). The bottom of the buckets was filled with soft sand to cushion the hatchlings. The intern would begin counting the hatchling and at every 5th hatchling, would measure its straight carapace length and width using calipers (Fig. 4). This was done with the first 50 hatchlings that boiled over, leading to a sample of 10 hatchlings per nest. If the nest had not hatched within 70 days, the nest was excavated by hand, and any living hatchlings were released. No measurements were taken in this situation to represent a natural surviving hatchling measurement. Once all hatchlings were collected, the buckets were then tipped at the top of the beach where they originally nested, and interns would stay until all hatchlings had returned to the sea (Fig. 4).

Hatchery sand temperatures were taken from the 8 temperature probes by interns every morning at 07h00 and every afternoon at 15h00.

Statistical analyses

All data were tested for normality (Shapiro-Wilk test), homoscedasticity (Breusch-Pagan test), independence (Durbin-Watson test), and multicollinearity (scatterplot matrix and VIF, or variance inflation factor). I used a multiple linear regression model to analyse the relationship between mean measurements of hatchling (straight carapace length and width) and sand temperature during incubation period (mean, maximum and minimum). Covariates were also included such as the clutch size and the hatchling success per clutch. A simple linear regression was used to analyse the relationship between mean measurements of hatchling (straight carapace length and width) and mean measurement of mother (curved carapace length and width) which is another covariant. All analyses were performed using the R statistical package (version 4.4.1; http://www.R-project.org/).

Results

I found no relationship between the mean hatchling measurement per clutch and the mean or minimum temperature during incubation. However, I found that hatchlings incubated in nests that had warmer maximum temperatures had a wider carapace (p = 0.033, adjusted R^2 = 0.097; Fig. 5).

There was an increase in the mean straight carapace width (SCW) of 0.063 cm for every additional Celsius (Table 2).

I found that hatchlings from larger clutches had shorter carapaces ($p = 0.010$, adjusted $R^2 = 0.078$; Fig. 6). There was a decrease in the mean straight carapace length (SCL) of 0.0032 cm for every additional egg per clutch (Table 1). I found no relationship between the mean hatchling measurement per clutch and hatching success (Fig. 7). I also found no relationship between the mean hatchling measurements and mother size (Fig. 8; Table 3; Table 4).

Discussion

The effect of temperature and its impact on the embryonic development of sea turtles has been extensively studied, especially due to the rising concerns of the effect of climate change on natural nest temperatures. I focused on the effect of sand temperature during incubation on offspring size. I found no significant effect of the minimum or mean sand temperature during incubation on the size of hatchlings. I also found no significant effect of the hatching success of the clutch or the mother size on the size of hatchlings. I found that a higher maximum sand temperature during incubation resulted in a wider carapace for hatchlings, but the effect was small. I found that a larger clutch size resulted in a shorter carapace for hatchlings, but again the effect was small. Based on these findings, I have concluded that my hypothesis that increased sand temperature decreases hatchling size is not supported.

Temperature during incubation

Many studies have found that higher temperatures produce smaller sea turtle hatchlings (Glen & al., 2003; Wood & al., 2014; Sim & al., 2015). I did not find any influences of mean or minimum temperature during incubation on the size of hatchlings. However, higher maximum temperatures during incubation yielded wider hatchlings. Furthermore, the effect of my finding was small in comparison to those reported in the scientific literature (Wood & al., 2014; Sim & al., 2015). Although my findings conflict with the current literature, this may be a sign of adaptation in the population of green sea turtles. Heat shock protein expression can enhance heat tolerance of reptile embryos (Gao & al., 2014). It is possible that there is an increased expression of this protein in the sea turtle population allowing for better assimilation of yolk sack in higher maximum temperature which would explain the wider size of carapace in hatchlings. My findings allow for a better understanding of the effect of sand temperature, which may continue to rise with global warming, and other factors on the size of hatchlings which came from relocated nests. This is important for future hatchery management as priorities should not be focused on reducing temperature to produce larger hatchlings but instead keeping temperature below the thermal limit for embryonic development as well as to promote more balanced sex ratios in the sea turtle population.

Clutch size

The optimal egg-size theory proposes that there is a trade-off between the size of the clutch and the size of hatchlings to allow for the optimal maternal investment in progeniture (LeBlanc & al., 2014). I indeed found that hatchlings were shorter when they came from larger clutches, but the size difference was small.

Limitations

The possibility of confounding variables being excluded cannot be fully determined due to the observational nature of my study. Sand temperature was used as a proxy to measure the temperature during incubation. However, this may not be as accurate as nest temperature itself due to the metabolic heat emitted by the embryo development during the incubation process (Gammon & al., 2020). The metabolic heat during final semester of development raises the temperature of the nest above that of the surrounding sand (Gammon & al., 2020). Sand temperature during incubation was calculated using the nearest temperature probe, but not all nests were at the same distance from the temperature probe. It is possible that nests closer to probes had a more accurate temperature during incubation than those that were further from probes. Due to budget constraints, we could not afford to have nest probes and thus settled with using temperature probes placed into the sand at approximately the same depth as the nest chambers.

Further research

The addition of a control group as well as increased funding could help reduce variance or errors caused by the limitations mentioned previously. Thus, a future study could focus on the effect of nest temperature on the size of hatchlings while comparing those left in-situ to those relocated to a hatchery. The comparison of natural in-situ nests to those relocated to a hatchery allows for a control group to eliminate possible confounding variables. The use of nest temperature probes will allow for more accurate nest temperature analysis to reduce variance from distance between nest and the nearest temperature probe.

Another next step could be to study the impact of hatchery relocation on the sex ratio of sea turtles. Climate change has driven the current adult population into a female biased sex ratio, with 80% of the current adult population of green sea turtles being female and 99% of non-adult sea turtle population being female (Jensen & al., 2018). Relatively few studies have worked on determining sea turtle hatchlings relocated to hatcheries because sexual dimorphism only appears in sea turtles after reaching sexual maturity which may take up to 15 years (Hays & al., 2022). In the past, this meant that accurate determination of the sex was commonly performed through dissection of sea turtles such as in the study by Chan & Liew (1995). However, recent studies, such as the one published by Tezak & al. (2020), have established a way to be able to determine the sex of sea turtle hatchlings via a small blood sample. This non-invasive procedure would allow for the monitoring of sex ratio of hatchlings relocated to a hatchery and to better improve this conservation strategy with the rising temperatures due to climate change. Future studies on hatchery relocation and their impact on the sea turtle population allows for more informed practices and better conservation outcomes as sea turtles continue to be threatened by climate change and other anthropogenic stressors.

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Table 1. Coefficients of the multiple regression to explain variation in the mean straight carapace length (SCL) of green sea turtle hatchlings. The mean SCL was calculated using the width data collected of hatchery relocated hatchlings from the southeastern beach on Pulau Perhentian Besar (Malaysia) in 2024. The variables include mean temperature (T_a), maximum temperature (T_{max}), minimum temperature (T_{min}), clutch size (EN) and hatching success (NS). The statistically significant p values are in bold, along with their corresponding effect size. The adjusted R-squared value is 0.078 and the degrees of freedom is a value of 47.

Variables	Estimate	Standard error	t value	p value	Effect size
Intercept	2.960	1.205	2.457	**0.018**	-
T_a	0.003	0.021	0.128	0.898	0.0027
T_{min}	-0.001	0.021	-0.043	0.966	-0.0018
T_{max}	0.057	0.033	1.713	0.093	0.0430
EN	-0.004	0.001	-2.689	**0.010**	**-0.0032**
NS	0.086	0.174	0.494	0.624	0.0830

Table 2. Coefficients of the multiple regression to explain variation in the mean straight carapace width (SCW) of green sea turtle hatchlings. The mean SCW was calculated using the width data collected of hatchery relocated hatchlings from the southeastern beach on Pulau Perhentian Besar (Malaysia) in 2024. The variables include mean temperature (T_a), maximum temperature (T_{max}), minimum temperature (T_{min}), clutch size (EN) and hatching success (NS). The statistically significant p values are in bold, along with their corresponding effect size. The adjusted R-squared value is 0.097 and the degrees of freedom is a value of 47.

Variables	Estimate	Standard error	t value	p value	Effect size
Intercept	0.396	1.155	0.343	0.733	-
T_a	0.022	0.020	1.069	0.290	0.0190
T_{min}	0.033	0.020	1.632	0.109	0.0350
T_{max}	0.070	0.032	2.195	**0.033**	**0.0630**
EN	-0.002	0.001	-1.336	0.188	-0.0011
NS	-0.167	0.167	-0.998	0.323	-0.0880

Table 3. Coefficients of the linear regression to explain variation in the mean straight carapace length (SCL) of green sea turtle hatchlings. The mean SCL was calculated using the width data collected of hatchery relocated hatchlings from the southeastern beach on Pulau Perhentian Besar (Malaysia) in 2024. The mean curved carapace length of mother turtle is represented by the variable aCCL. The statistically significant p values are in bold, along with their corresponding effect size. The adjusted R-squared value is 0.174 and the degrees of freedom is a value of 11.

Variables	Estimate	Standard error	t value	p value	Effect size
Intercept	3.447	0.501	6.886	**< 0.001**	-
aCCL	0.010	0.005	1.878	0.087	0.0099

Table 4. Coefficients of the linear regression to explain variation in the mean straight carapace width (SCW) of green sea turtle hatchlings. The mean SCW was calculated using the width data collected of hatchery relocated hatchlings from the southeastern beach on Pulau Perhentian Besar (Malaysia) in 2024. The mean curved carapace width of mother turtle is represented by the variable aCCW. The statistically significant p values are in bold, along with their corresponding effect size. The adjusted R-squared value is 0.06474 and the degrees of freedom is a value of 11.

Variables	Estimate	Standard error	t value	p value	Effect size
Intercept	4.108	0.383	10.721	**< 0.001**	-
aCCW	-0.006	0.004	-1.353	0.203	-0.006

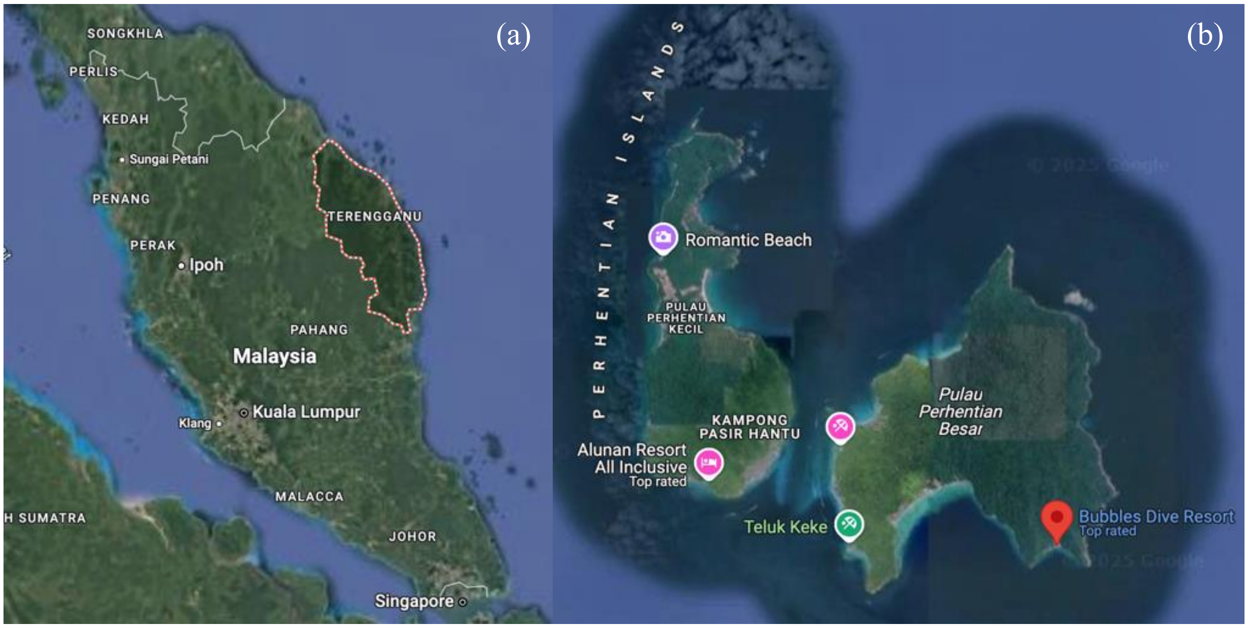

Figure 1. Google Maps image of study site in 2024 for green sea turtle population of southeastern beach on Pulau Perhentian Besar (Malaysia). (a) Malaysia Peninsular with the state of Terengganu outlined in red, along with the islands associated with the state. (b) Perhentian Islands with a red pin on the Bubbles Dive Resort, located on the southeastern part of Pulau Perhentian Besar, which was the specific study location.

Figure 2. Hatchery installed on the southeastern beach on Pulau Perhentian Besar (Malaysia) in 2024. (a) Hatchery before any clutch relocation was performed. (b) Plastic ant barrier, which was installed at the back of the hatchery, where vegetation was present, to reduce ant predation on relocated nests.

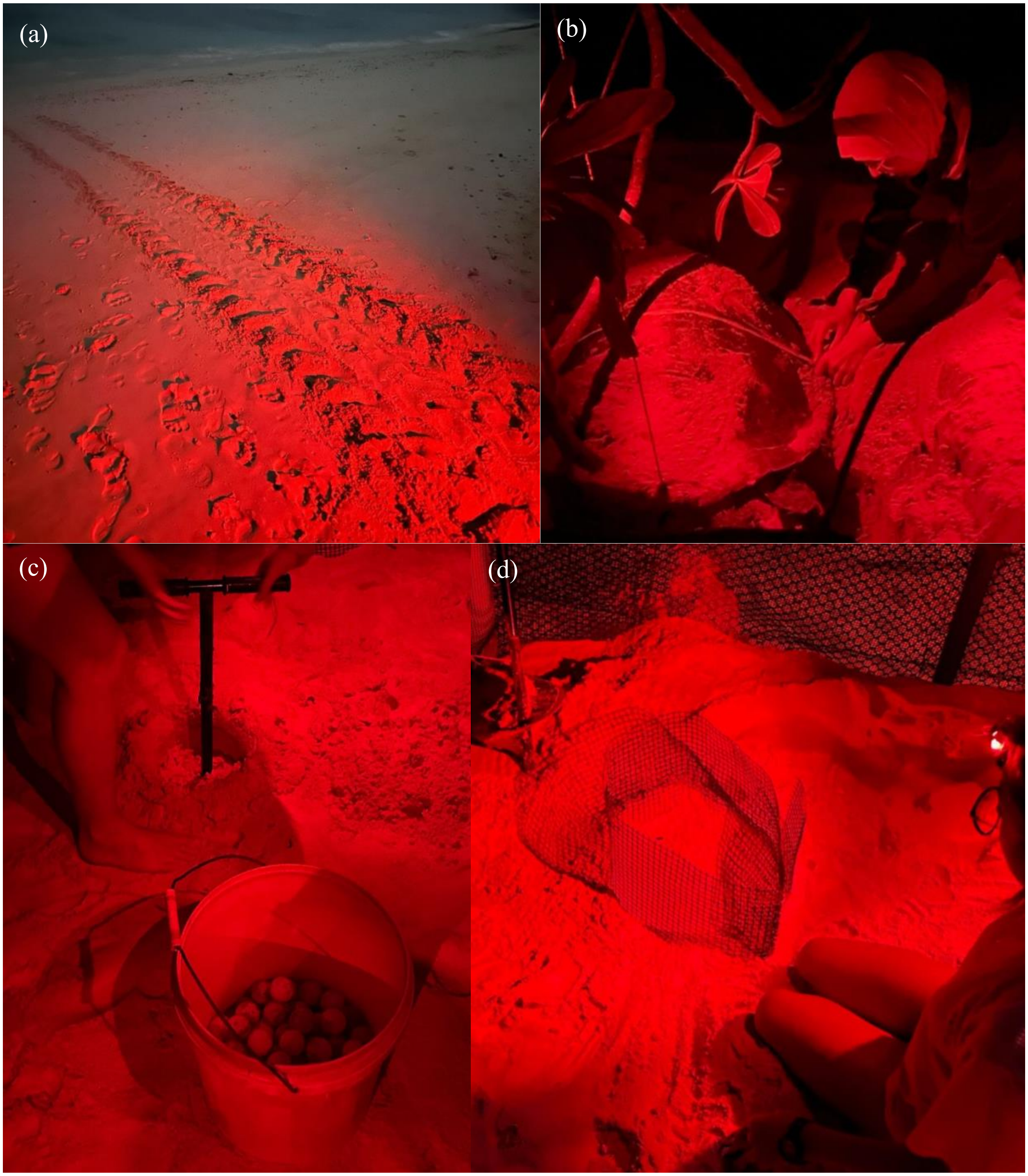

Figure 3. Photos of the ex-situ relocation process on the southeastern beach on Pulau Perhentian Besar (Malaysia) in 2024. (a) Uphill tracks of a mother turtle attempting to nest. (b) Intern taking measurements of curved carapace width of mother turtle after oviposition using measuring tape. (c) Intern digging relocated the nest in the hatchery using auger, the white bucket beside her contains sand from the original nesting location and all the eggs laid by the mother turtle of the clutch. (d) Net fencing around the nest along with a nest identification tag attached to it.

Figure 4. Photos of post-hatching excavation procedure on the southeastern beach on Pulau Perhentian Besar (Malaysia) in 2024. (a) Intern began digging to allow living hatchlings under the sand to be released with hatchlings that boiled out naturally. (b) Intern measuring the straight carapace width of hatchling using a caliper. (c) Bucket containing soft sand and 50 live hatchlings. (d) Hatchling released down the beach in the same sector where the mother turtle had nested.

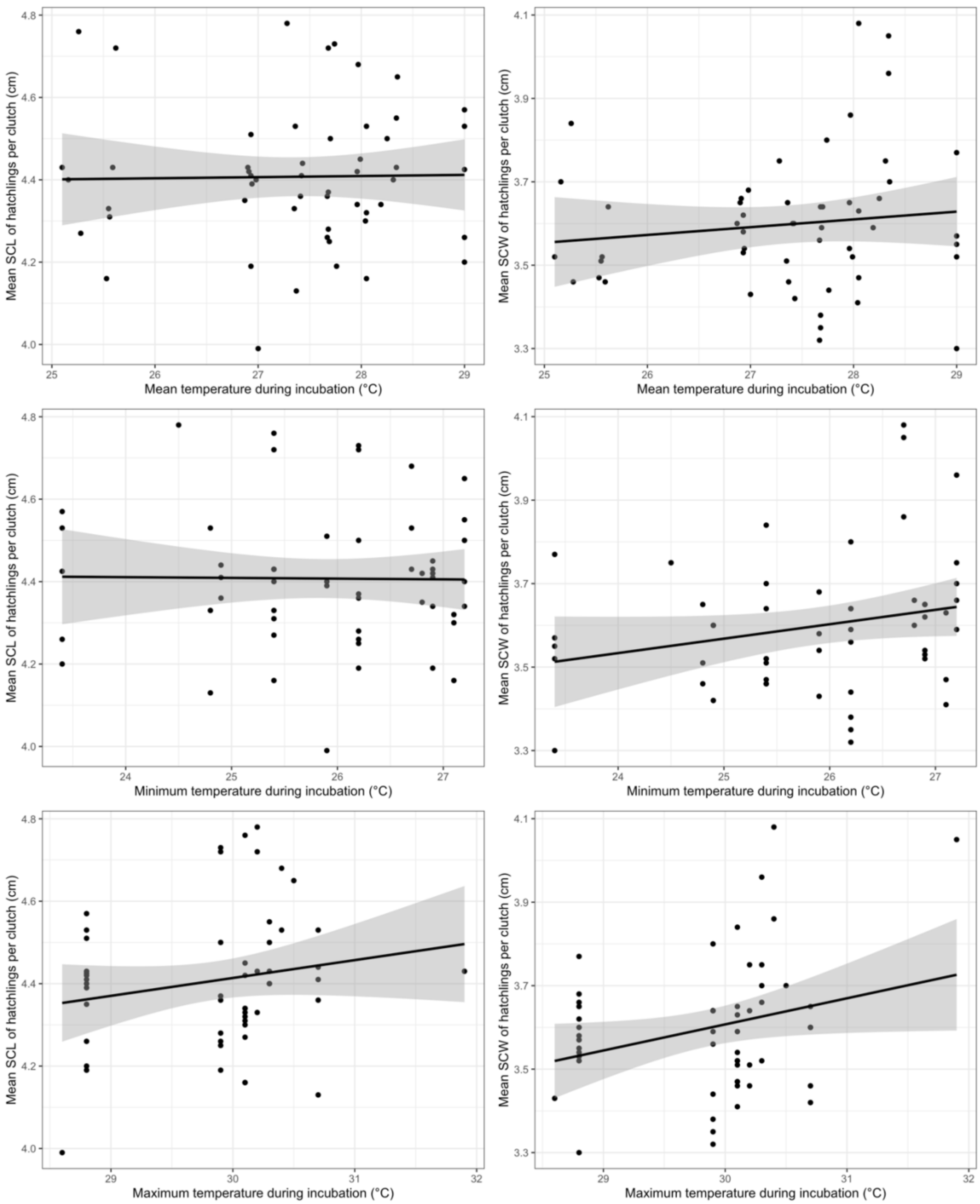

Figure 5. Linear regressions of the mean green sea turtle hatchling measurement per clutch (cm) as a function of the temperature during incubation period (°C) (n = 48). The figures on the right have a y axis representing the mean SCL (straight carapace length) of hatchlings per clutch (cm). The figures on the left a y axis representing the mean SCW (straight carapace width) of hatchlings per clutch (cm). The figures on the top are an x axis representing the mean temperature during incubation period (°C). The figures in the centre are an x axis representing the minimum temperature during incubation period (°C). The figures on the bottom are an x axis representing the maximum temperature during incubation period (°C).

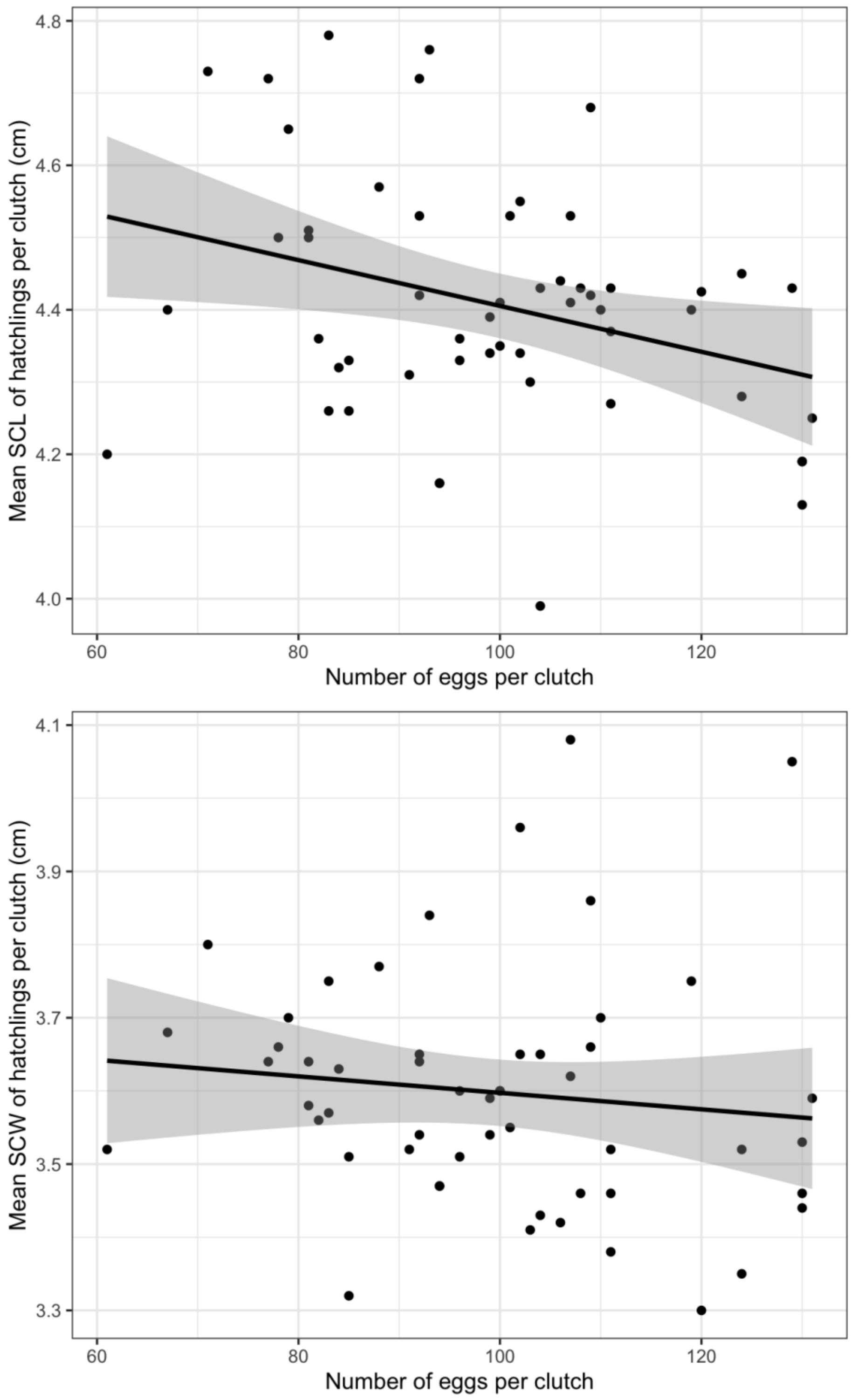

Figure 6. Linear regressions of the mean green sea turtle hatchling measurement per clutch (cm) as a function of the clutch size (n = 48). The figure on the top has a y axis representing the mean SCL (straight carapace length) of hatchlings per clutch (cm). The figure on the bottom has a y axis representing the mean SCW (straight carapace width) of hatchlings per clutch (cm).

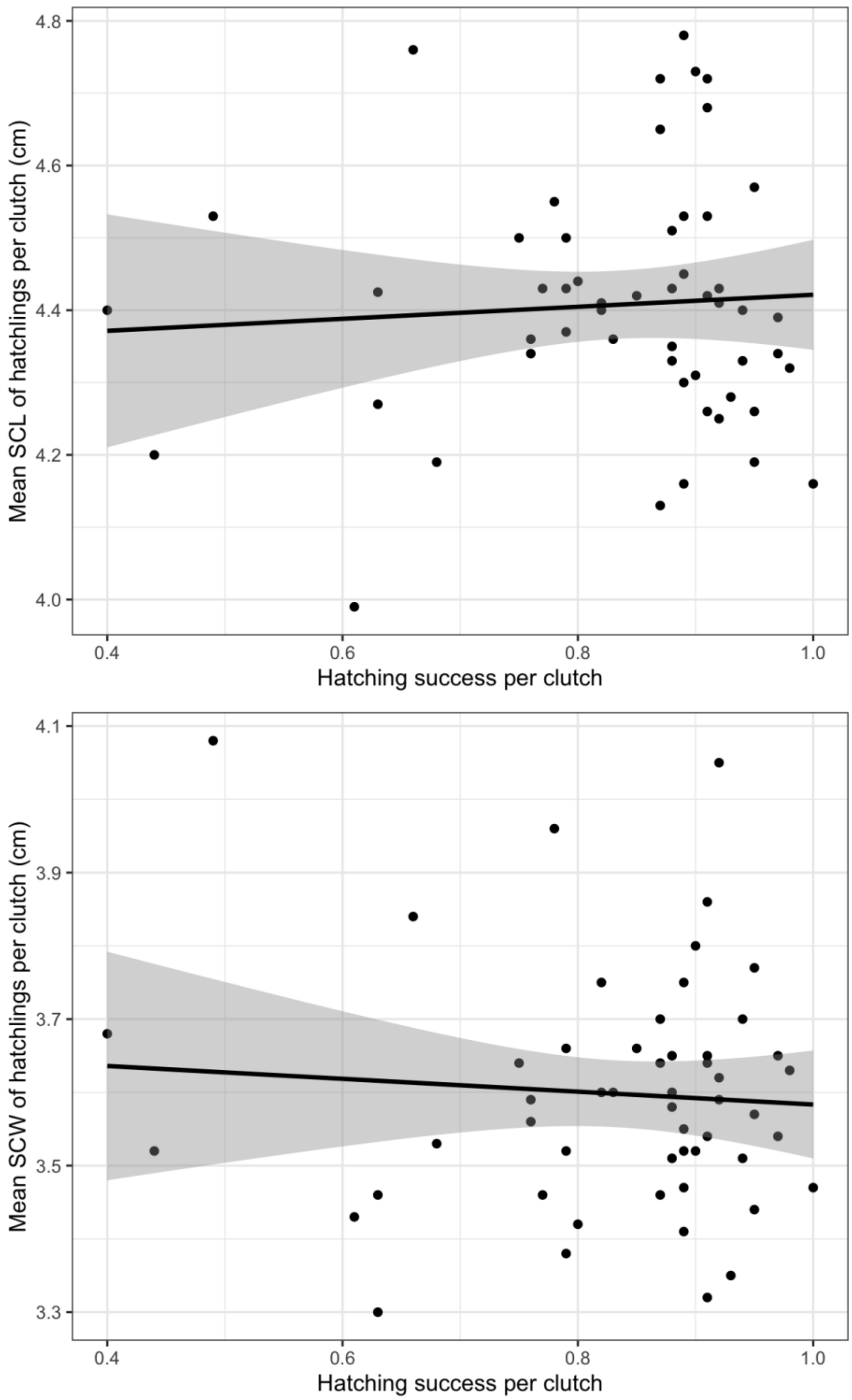

Figure 7. Linear regressions of the mean green sea turtle hatchling measurement per clutch (cm) as a function of hatching success per clutch (n = 48). The figure on the top has a y axis representing the mean SCL (straight carapace length) of hatchlings per clutch (cm). The figure on the bottom has a y axis representing the mean SCW (straight carapace width) of hatchlings per clutch (cm).

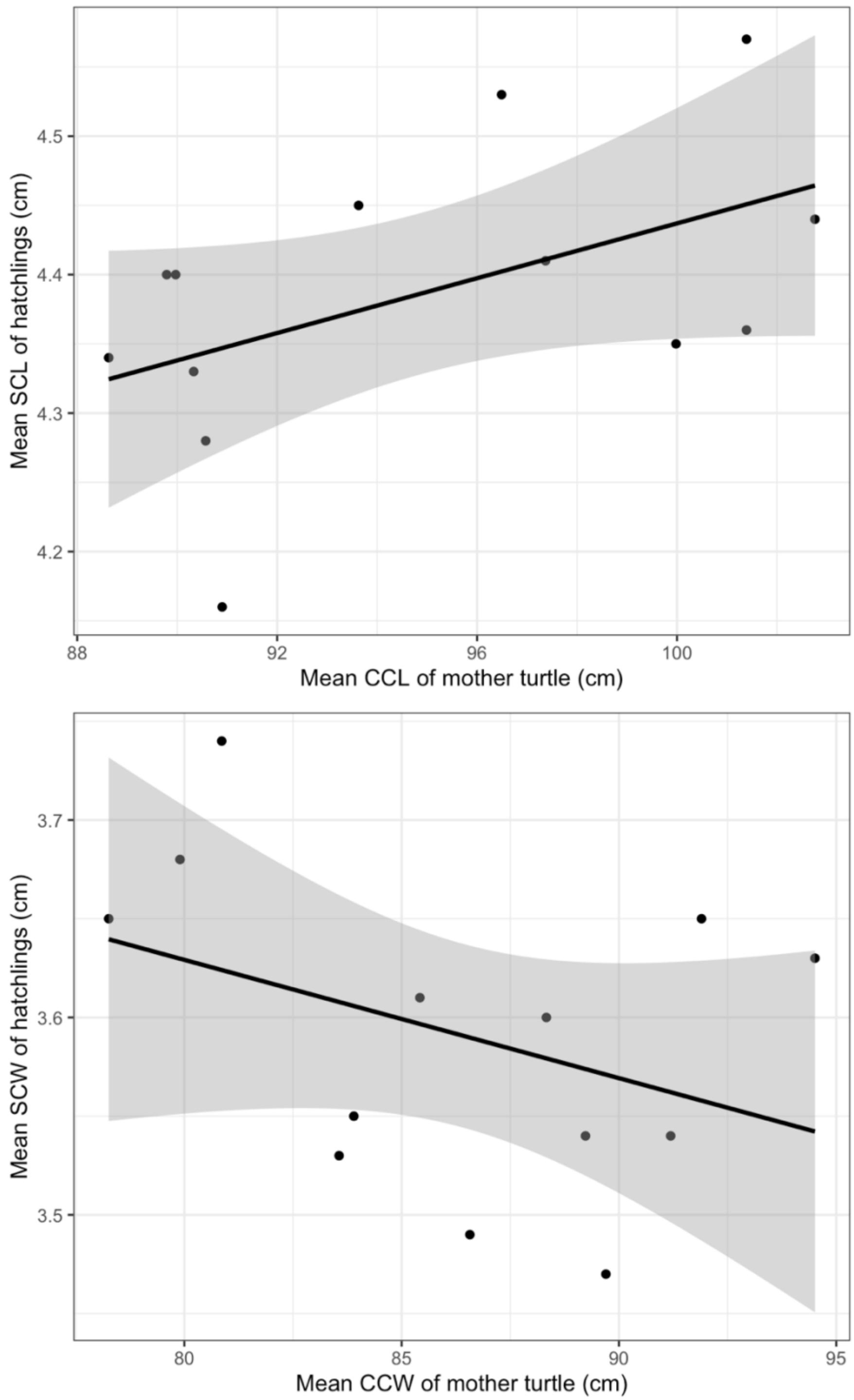

Figure 8. Linear regressions of the mean green sea turtle hatchling measurement (cm) as a function of mother size (cm) (n = 12). The figure on the top has a y axis representing the mean SCL (straight carapace length) of hatchlings per clutch (cm) and an x axis representing the mean CCL (curved carapace length) of the corresponding mother turtle (cm). The figure on the bottom has a y axis representing the mean SCW (straight carapace width) of hatchlings per clutch (cm) and an x axis representing the mean CCW (curved carapace width) of the corresponding mother turtle (cm).