

**Relation of beach slope and vegetation on the nest site selection of the green sea turtle
(*Chelonia mydas*) on the Osa Peninsula, Costa Rica**

Dindi Hiddink Verberne (s3723909)

MSc Marine Biology, University of Groningen

COPROT, Tortugas de Osa

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Supervised by Lucia Heredero Saura & Dr. Karin de Boer

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Abstract

The green sea turtle (*Chelonia mydas*) is a keystone species inhabiting tropical and subtropical regions, playing important roles in marine and coastal ecosystems. Despite legal protections, green sea turtle populations face many threats during their life cycle. Green sea turtle parental care is limited to the nesting process and therefore, nest site selection can directly influence reproductive success. Yet uncertainties remain regarding the factors influencing nest site selection. Therefore, the aim of this study was to investigate the relation of beach slope and vegetation on nest site selection of green sea turtles on Rio Oro and Pejeperro beaches of the Osa Peninsula in Costa Rica. Our findings indicate that beach slope does not significantly influence nest site selection, while vegetation type significantly impacts nest site selection and temperature. Green sea turtles preferred to nest at locations where bare sand and low-lying vegetation coverage below 75% and high vegetation above 75% was present. The mean nest temperatures ranged from 29.90°C to 33.81°C, which was found to lie within the thermal tolerance range of 25°C - 35°C for successful incubation of green sea turtles. Besides the importance for successful incubation, temperature plays a crucial role in sexual development, since sea turtles have temperature-dependent sex determination (TSD). We observed a highly female-biased sex ratio of hatchlings, highlighting the threat of unsustainable levels of feminization in the green sea turtle populations at the Osa Peninsula. Even though no significant correlation was found between hatching success and temperature or vegetation, preserving natural vegetation along nesting beaches emerges as critical to mitigate high nest temperatures and reduce the proportion of female hatchlings. Moreover, intensive beach management, together with adequate nest reburial is a necessary conservation strategy to cope with the current threats. Lastly, offshore conservation efforts should complement coastal strategies to improve green sea turtle living environment at all stages of their life cycle.

Introduction

The green sea turtle (*Chelonia mydas*; hereafter: green sea turtle/s) is a marine reptile found in tropical and subtropical regions throughout the world's oceans ([Fig. 1](#)). They play crucial ecological roles in maintaining marine and coastal ecosystems, such as sustaining the health and productivity of seagrass beds and coral reefs, and enhancing nutrient cycling through their feeding and excretion (Thayer et al., 1984). Additionally, green sea turtles are integral to the marine food web, serving both as prey for killer whales and sharks and as predators for sponges and invertebrates (Moran & Bjorndal, 2005, 2007). Furthermore, they hold significant cultural value for indigenous human societies (Johannes & MacFarlane, 1991) and have economic importance, both through illegal trade of sea turtle eggs and the tourism industry (Wilson & Tisdell, 2001).

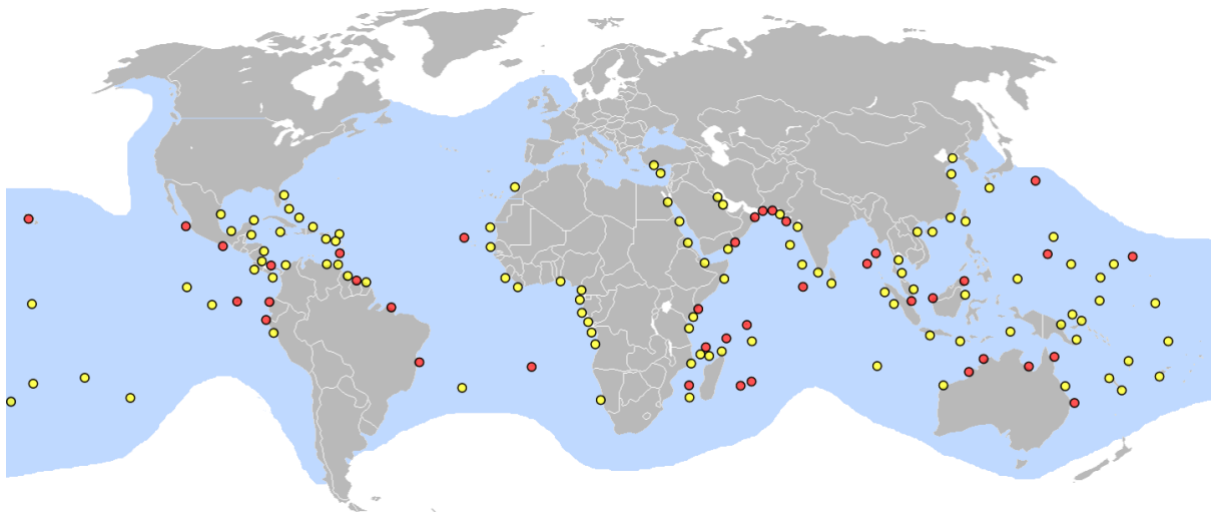


Fig. 1. Worldwide distribution of the green sea turtle. Red dots represent major nesting locations and yellow dots represent minor nesting locations (NOAA Fisheries, 2023).

Green sea turtles begin their lives as eggs, laid by adult females on nesting beaches (Carr & Ogren, 1960). After an incubation period of 54-63 days, the hatchlings emerge and quickly swim offshore (Segura & Cajade, 2010; Tiwari et al., 2006). Out at sea, these young green sea turtles enter a pelagic phase, where they remain for about 3 to 5 years (Carr & Ogren, 1997). As they grow into juveniles, they transition to coastal habitats, gradually maturing into adults (Bjorndal, 1997). Green sea turtles reach sexual maturity between 20 to 50 years of

age. As adults, they undertake long migrations of 1000-2000 km between feeding grounds and nesting beaches (Spotila, 2004). Females return to the same beaches where they were born to lay their eggs, expressing strong nest site philopatry (Bowen et al., 1992). The green sea turtle's life cycle highlights its complex and migratory nature, as well as the critical habitats required at different life stages. This complexity underscores the importance of conserving diverse marine environments for their survival.

Despite worldwide legal protection under the Endangered Species Act, green sea turtles are classified as endangered as per the IUCN's red list of threatened species (IUCN, 2023). Populations of green sea turtles in many regions have declined in abundance in recent decades due to threats at all stages of their life cycle. Offshore, they are mainly threatened by incidental capture in fishing gear (bycatch), marine pollution, habitat degradation, and boat strikes (Wallace et al., 2013). Onshore, threats include coastal development, harvest of eggs for consumption and human disturbances (de Faria et al., 2022; Fuentes et al., 2011a; Restrepo et al., 2023). Next to these direct anthropogenic impacts green sea turtle nesting grounds are being affected simultaneously by multiple climatic processes, such as increased temperature, sea-level rise, and cyclonic activity at different temporal and geographical scales (Fuentes et al., 2011).

Just like in all sea turtle species, green sea turtle parental care is limited to only the nesting process (Santos et al., 2017; Serafini et al., 2009; Zavaleta-Lizárraga & Morales-Mávil, 2013). Therefore, females that place nests in sites with favourable characteristics for embryo development can directly influence fitness (Mohd Salleh et al., 2021). Hence, nest site selection represents a crucial aspect in their reproductive biology. The selection of the nesting site is defined as the laying of eggs in zones that are not randomly chosen within a certain

area (Wilson, 1998). Many uncertainties remain about why female green sea turtles decide to emerge at a particular location along their nesting beach (Wood & Bjorndal, 2000).

Historically this was thought to be random (Mrosovsky, 1983), but more recent literature suggests that green sea turtles use several morphological cues for nest site selection. Beach slope is found to be one of the main morphological cues for nest site selection (Natih et al., 2021; Zavaleta-Lizárraga & Morales-Mávil, 2013). Green sea turtles are found to have a preference for elevated beach slopes within a slope range of 3° - 30° (Santos et al., 2017; Turkozan et al., 2011). A beach slope below 3° is found to experience high risk of seawater intrusion, affecting the humidity and temperature of the green sea turtle nests (Natih et al., 2021), whereas a slope bigger than 30° was found to make it a too strenuous exercise for green sea turtles to get to the supratidal zone to reach their nesting area (Natih et al., 2021). This preference for elevated slopes might be attributed to the association with increased nest elevation, resulting in reduced risk of inundation (Fish et al. 2005; Wood & Bjorndal, 2000). Moreover, the beach profile is dynamic, with erosion being accentuated by sea-level rise and cyclonic activity leading to loss and/or alteration of nesting beaches (Butt et al., 2016; Fuentes et al., 2011b). When the beach slope increases to a certain threshold, it will decrease beach stability, which can lead to a lower nest quality and increase the chance of abandonment, which in turn will increase the energetic demand of nesting (Spanier, 2010). Hence, changes in beach morphology may therefore deter or reduce green sea turtle nesting success.

In addition to selecting for beach slope, green sea turtles are found to select for nests in vegetated areas (Santos et al., 2017; Turkozan et al., 2011). Therefore, it is likely that green sea turtles use vegetation as a cue to select the nesting site. Nesting in vegetation was found to have clear positive implications for the nest environment, such as lower erosion and

inundation compared to nests in open beach areas (Fish et al., 2005). Furthermore, the shade of the vegetation keeps the temperature more stable and, on average, lower. Therefore, vegetation can prevent embryo mortality due to high or fluctuating temperatures (Hays et al., 2017; Kamel, 2013). These factors can explain the preference of nesting within the vegetation, even though it is found to be more energetically demanding (Hamann et al., 2022). Moreover, within vegetation, green sea turtles are found to nest more under trees, compared to grass areas (Heredero Saura et al., 2022). Green sea turtle nests were found to have a nest more and have higher hatching success when nests were located underneath trees compared to those placed in exposed area (Heredero Saura et al., 2022; Santidrián Tomillo et al., 2015; Zárate et al., 2013). Trade-offs seem to be occurring between vegetation density and the subsequent impact of dense root systems (Heredero Saura et al., 2022; Mohd Salleh et al., 2021). Despite the roots ability to stabilize the beach and increase firmness of the sand, it can lead to nest abandonment when the root system becomes too dense (Wang & Cheng, 1999).

The temperature within the nests can vary widely both within and between nesting beaches (Kamel & Mrosovsky, 2006). This variation in thermal profiles is one of the most important factors influencing embryonic development (Fuentes et al., 2023; Kamel, 2013). At higher temperatures, tissue synthesis and tissue maintenance are greater, leading to a higher rate of development and shorter development duration (Booth, 1998). However, extremely high incubation temperatures can cause mortality in developing nests (Hays et al., 2017). Green sea turtle eggs only successfully incubate within a narrow thermal range (25° and ~35°C), with incubation above the thermal threshold of 35°C resulting in hatchlings with higher morphological abnormalities and lowering hatching success (Howard et al., 2014). For

hatching success rates above 60% in green sea turtle nests, a more optimal range of 27° to 32°C is required (Bustard & Greenham, 1968).

Not only is the nest temperature critical for hatching success, but it also determines the primary sex ratio of the nest (PSR) (Standora & Spotila, 1985). This phenomenon has been described as temperature-dependent sex determination (TSD). The sex is determined during a specific period of the incubation, called the thermosensitive period (TSP), which is during the second third of the incubation period (Standora & Spotila, 1985). The temperature that produces 1:1 males and females is called the pivotal temperature (PT). In general, for green sea turtles, the PT lies between 28.9-30.3°C (Mrosovsky, 1980). However, this can differ between populations. The range of temperatures over which both sexes emerge is known as the transitional range of temperature (TRT) (Morreale et al., 1982). With warming global temperatures (Magrin et al., 2007) and most green sea turtle populations naturally producing offspring above the PT (Hays et al., 2017), it is clear that climate change poses a serious threat to the persistence of these populations in embryo development and/or feminization of populations. As mentioned above, vegetation has been found to be a significant predictor in nest temperatures, with temperatures being cooler in denser vegetation cover (Kamel, 2013; Kamel & Mrosovsky, 2006). However, other factors such as rainfall, air temperature, nest depth, grain size, sand moisture, and gas exchange can all affect the nest temperature (Broderick et al., 2000; Bustard & Greenham, 1968; van de Merwe et al., 2006). Hence, the selection of a nesting site by a female turtle holds significant implications for the temperature of the nest, consequently impacting the successful development and survival of her offspring, as well as her own fitness (Broderick et al., 2001). Therefore, examining the nest temperatures of green sea turtles becomes a crucial aspect of conservation strategies.

Overall, understanding nesting preferences and characteristics of green sea turtles is a valuable contribution for identifying specific threats and making informed conservation decisions to conserve these endangered species and their nesting beaches. Therefore, this study aimed to enhance our knowledge of green sea turtle nest site selection at Rio Oro and Pejeperro beaches on the Osa Peninsula, Costa Rica. Specifically, we sought to determine a) the importance of Rio Oro and Pejeperro beaches for green sea turtle nesting, b) the effect of beach slope on nest site selection, c) the effect of vegetation on nest site selection, temperature, and hatching success, and d) the effect of temperature on hatching success and the resulting sex ratio of hatchlings. By comprehending nest site selection of green sea turtles and their preference, researchers can better guide green sea turtle conservation efforts, which is specifically important regarding the direct and indirect anthropogenic threats green sea turtles are facing.

Method

Study site

The Osa Peninsula is located on the South Pacific coast of Costa Rica (Fig. 2a), with one of the highest conservation priorities in Central America due to its high levels of biodiversity and endemism (Ankersen et al., 2006). Many of the westerly positioned beaches on the Osa Peninsula are regarded as vital habitat for nesting female sea turtles. These include Carate, Río Oro, Pejeperro and Piro. The primary focus of this study was centred around Río Oro ($8^{\circ}32'08.95''\text{N}$, $83^{\circ}18'14.09''\text{W}$) (3km) and Pejeperro ($8^{\circ}26'12.46''\text{N}$, $83^{\circ}23'59.90''\text{W}$) (3.5km) (Fig. 2b), where green sea turtles are the second most abundant sea turtle species nesting (Chacón et al., 2007; Santidrián Tomillo et al., 2015; Venegas-Li et al., 2015).

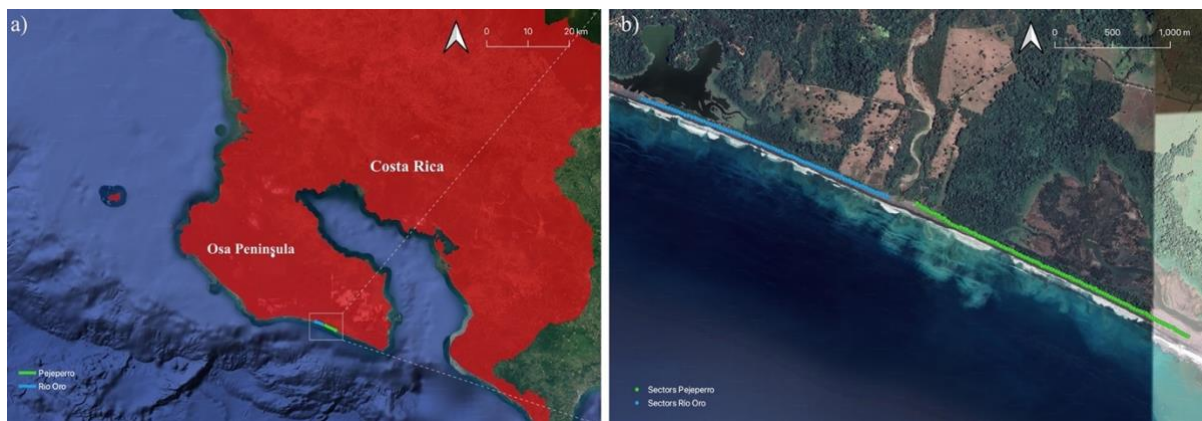


Fig. 2. Map of the study region. Left: (a) White box shows the location of Río Oro and Pejeperro beaches on the Osa Peninsula, Costa Rica. Right: (b) Río Oro (blue, sector 53.25-75.75) and Pejeperro (green, sector 77.50-103).

Nesting activity peaks are from November till March, with occasional green sea turtle nests from the beginning of June until September (Exley et al., 2022). The study area belongs to Los Refugios de Vida Silvestre Pejeperro and Río Oro which is managed by the Área Conservación Osa (ACOSA) (Quesada-Alpízar & Cortés, 2006). The climate at the Osa Peninsula is hot and humid. Average daily temperature ranges between $24.0\text{--}26.5^{\circ}\text{C}$ (Brumberg et al., 2021). It is characterized by having intense rainfall, especially from August to early December. Rainfall decreases from late December to April in which temperatures and light hours are higher compared to the other months (Brumberg et al., 2021; Quesada-

Alpízar & Cortés, 2006). Annual precipitation ranges between 3000 and 7000 mm (Brumberg et al., 2021). The surrounding terrestrial habitats consist mainly of lowland rainforest, cloud forest, palm forest, mangroves and agricultural land (Friedlander et al., 2022).

Data collection

The beach was patrolled from June to February three times a week in nesting season 2020-2021 and six times a week in 2021-2022. In nesting season 2022-2023 and 2023-2024, the beach was patrolled daily from June till March. The patrols occurred between 8:00 PM and 4:00 AM, lasting anywhere from 3 to 8 hours, based on the nesting activity level of the green sea turtle. White light can disorient nesting female turtles and therefore only red light was used when necessary. Markers in the vegetation were placed along the vegetation line to divide the length of beach into sectors separated by 25 m (numbers 53.25 to 103). Sectors 75.75 till 77.50 were present, but the river Rio Oro flows in front of these sectors and, therefore, not taken into account as nesting area for the green sea turtle. Sector 53.25 till 75.50 represent Rio Oro beach and sector 77.75 – 103 represent Pejeperro beach. GPS coordinates of each marker were registered with a handheld GPS (GARMIN eTrex® 32x). When a female was encountered, the date, time, and sector was recorded. Furthermore, for each green sea turtle nest laid in the vegetation, it was classified whether the 1m radius around the nest consisted of low-lying or high vegetation. The exact position of the nest was marked by triangulation. Each nest received a unique nest code, which consisted of the name of the beach (PP for Pejeperro or RO for Rio Oro), the species (V for green sea turtle), and a unique number (e.g., PPV10). During morning patrols, which started at 5:00am, all non-monitored green sea turtle activities of the previous night were gathered. Furthermore, depredated, and poached nests were marked. Nests that marked day 55 were checked for hatching emergence daily for the 10 following days. Nests were excavated two days after

hatching emergence was seen or on day 65 of incubation. During the excavation, the following data was recorded: alive and dead hatchlings, alive and dead pipped hatchlings, eggshells and non-hatched eggs. ‘Pipped’ refers to eggs that the hatchling has begun to open but has not completely hatched. The hatching success (HS) was determined using the following equations:

$$HS = \frac{\text{Eggshells}}{\text{Total number of eggs}} \times 100$$

Beach profile

In nesting season 2023-2024, beach profile and slope were measured using the ‘Emery method’ (Emery, 1961; Fish, 2011). The backshore slope, the area on the beach lying between the high tide mark and the dune vegetation, as well as beach width, were measured from sector 53.25 to 103, at every whole sector number. Measurements were done four times on the following dates: 11/11/2023, 7/12/2023, 4/1/2024 and 3/2/2024. A pole (1) was placed vertically next to the sector. A second pole (2) was placed to the first change in slope or to the high tide line if no slope was present. A measuring tape was run between the two poles and was placed over pole 2, the measuring tape was lowered until it was taut and level. The distance between the two poles was measured to represent the actual beach width (x). Furthermore, the distance from the top of pole 1 to the point where the measuring tape intersects pole 1 was measured to represent the change in elevation (y) ([Fig. 3](#)). If there was a second change in slope before the high tide line was reached, pole 1 was placed on the location of pole 2 from the previous measurement and pole 2 was placed to the second change in slope. This process was repeated if more changes in slope were present until the high tide line was marked. The following equation was used to calculate beach slope (α) in degrees (Hernández-Cortés et al., 2018):

$$\text{tg}\alpha = \frac{y}{x} \rightarrow \alpha = \text{arctg}\frac{y}{x}$$

Where x is level beach width and y is the change in elevation. The Pythagorean theorem was used to calculate the actual beach width (z):

$$z = \sqrt{x^2 + y^2}$$

For analysis, the total mean beach slope and total mean actual beach width was calculated over the four measured timepoints for every whole sector number.

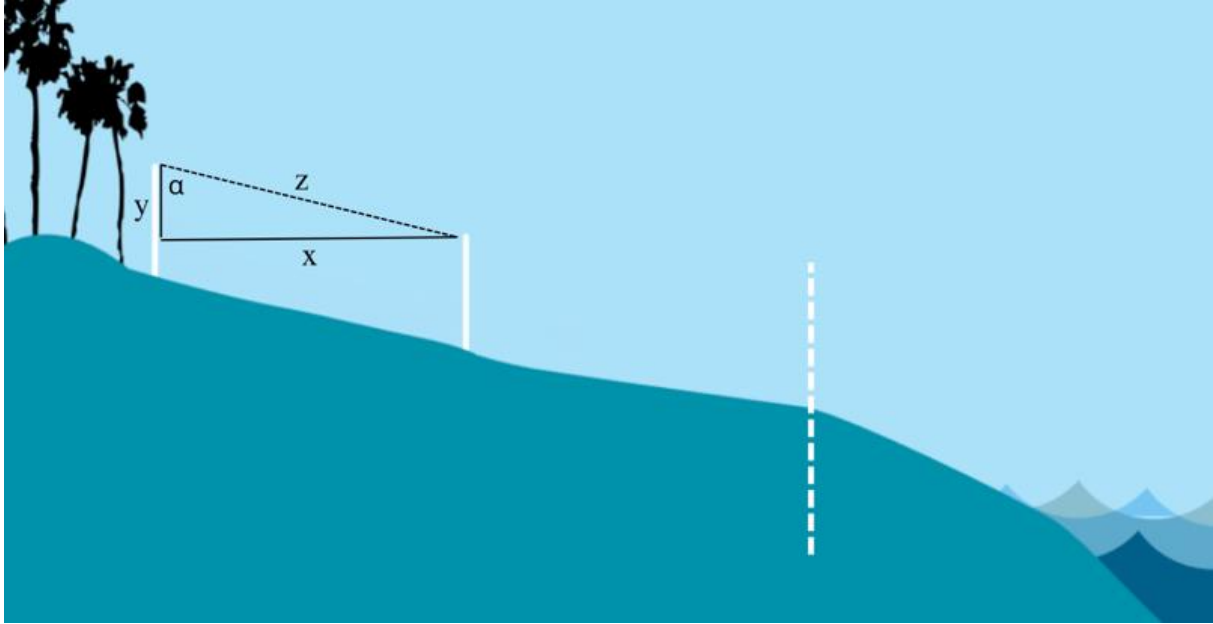


Fig. 3. Beach profile showing the division into segments based on change in slope. The first segment is shown with level beach width (x), change in elevation (y) and the actual beach width (z). α marks the beach slope. The white non-dashed line represents the measuring poles and the non-dashed line indicates a change in slope.

Vegetation

In season 2023-2024, the different vegetation types along the beach were classified using the Line Intercept method. A line transect was placed in between all sectors. The vegetation along each transect was categorized in four different vegetation types: (1) bare sand area with no vegetation, (2) low-lying vegetation (<1m), (3) medium height vegetation (1-2.5m), or (4) high vegetation (>2.5m). If two categories overlapped, the category with the highest height was counted only. If the gap between one category was less than 10cm, the measurement was continuous (Hynes, 2015). For each transect, the percentage of each vegetation type was

determined. Coverages between 0-25%, 25-50% 50-75% and 75-100% were grouped together for each vegetation category.

Temperature

Four temperature loggers (HOBO by Onset UA-002-64, USA) (resolution of 0.01°C, accuracy range of $\pm 0.18^\circ\text{C}$) were placed above the vegetation line and at a depth of 45cm, resembling the natural depth of the centre of green sea turtle nests. Two loggers were placed in an area with high vegetation cover (in sector 69.5 & 83.75) and two were placed in bare sand with no vegetation (sector 74 & 79.75). Control temperatures were measured between October 21, 2023 till December 21, 2023 and between February 28, 2024 and March 28, 2024. In 18 green sea turtle nests a temperature logger was placed. Each logger was carefully placed in the middle of the nest after the nesting female laid a total of 30 eggs. 8 out of 18 temperature loggers were found to be predated (n=6), poached (n=1) or malfunctioning (n=1). Therefore, data from these 8 loggers was discarded from the analysis. [Table 1](#) shows an overview of the location of the functioning temperature loggers. Each datalogger was retrieved during excavation of the nest. Temperature data was used from the time the nesting female started covering the nest until the day of hatchling emergence or one day after a sudden drop in temperature was seen in the data, since this indicates hatching emergence (van de Merwe et al., 2006). For each nest with temperature data, we calculated mean temperature of the whole incubation period and of the second third of the incubation period (TSP in sea turtles). Sacrificing hatchlings to extract their gonads has ethical implication, therefore the TSD curve of a nearby population is often used (Santidrián Tomillo & Spotila, 2020). In this study, the TSD curve of the green sea turtle population on the Caribbean Coast of Costa Rica, in Tortuguero was used (Morreale et al., 1982; Standora & Spotila, 1985). The PT of this population is 28.3°C, with temperatures below 28 °C resulting in 100% male hatchlings and

temperatures above 30.2 °C producing 100% females (Morreale et al., 1982; Standora & Spotila, 1985). Within the TRT, sex ratios were estimated as the proportion of female hatchlings for every increment of 0.1°C.

Table 1. Overview of 10 green sea turtle nests with temperature loggers on Rio Oro (sector 53.25 – 75.50) and Pejeperro (sector 77.75 -103) beaches, Osa Peninsula, Costa Rica.

Nest number	Date of nest laid	Sector	Vegetation type surrounding the nest
1	27/10/2023	90.25	High vegetation
2	29/10/2023	84	Low-lying vegetation
3	28/12/2023	79.25	High vegetation
4	02/01/2024	94	High vegetation
5	05/01/2024	81.50	High vegetation
6	15/01/2024	79	High vegetation
7	17/10/2023	63.5	High vegetation
8	08/11/2023	66.6	High vegetation
9	29/01/2023	70.75	High vegetation
10	31/01/2024	69	High vegetation

Statistical analyses

Data analysis was performed using RStudio R version 1.4.1103 (R Core Team, 2021). To evaluate whether nests were randomly distributed along all sectors, chi-squared tests were performed. Nesting data of all four nesting seasons (2020-2024) was used. To evaluate the effect of beach slope on the frequency of nests and activity without actual nesting, negative binominal models were applied. Beach slope and beach width were both used as fixed factors and were log transformed to obtain homogeneity of variances. To evaluate the effect of vegetation on the number of green sea turtle nests, generalized linear models were fitted using Poisson distribution due to the count nature of the data. If overdispersion was present, a

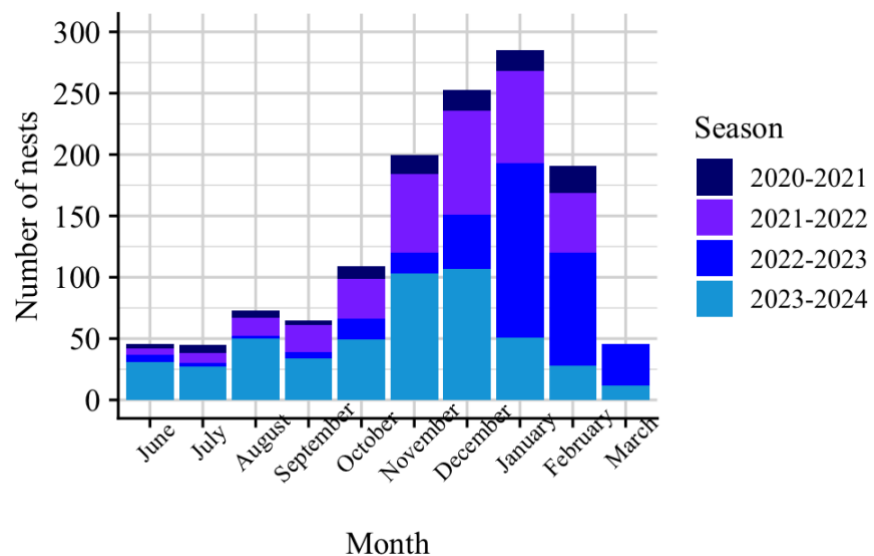
Quasi-Poisson distribution was fitted. The level of vegetation coverage was taken into account as a fixed factor. The effects of vegetation coverage on the number of nests were assessed using one-way ANOVAs. Pairwise differences between outcome variables were examined with Tukey-adjusted post hoc comparisons using the emmeans package (Searle et al., 1980). To evaluate the effect of low-lying or high vegetation on hatching success, linear models were applied. The effect of vegetation on hatching success was assessed using a one-way ANOVA. To evaluate the effect of location on temperature, linear models were fitted. The location of the temperature logger was taken into account as a fixed factor. The effect of location on the mean temperature was assessed using a one-way ANOVA. Pairwise differences between outcome variables were examined with Tukey-adjusted post hoc comparisons using the emmeans package (Searle et al., 1980). To assess the strength and direction of the relationship between mean temperature of green sea turtle nests and hatching success the Spearman's rank correlation coefficient was used. To visualize the relationship between incubation temperature and hatching success, LOESS (Locally Estimated Scatterplot Smoothing) was applied. For all analysis, effects were considered significant at $\alpha = 0.05$. For negative binominal and (generalized) linear models, normality and homogeneity of residuals were visually examined using the performance package (Lüdtke et al., 2021). For the data analysis of beach slope, vegetation coverage, and temperature, data of nesting season 2023-2024 was used. The car package was used to correct for unbalanced designs (Fox & Weisberg, 2011).

Results

Nesting numbers

A total of 1311 green sea turtle nests were recorded on Rio Oro and Pejeperro beaches, Osa Peninsula, Costa Rica, between June 2020 and March 2024. The total number of nests per season varied, ranging between 103 in 2020-2021 and 492 in 2023-2024. We observed year-round nesting with distinct peak of laying in December and January and the lowest level of nest being laid in July & March when considering all four nesting seasons together ([Fig. 4](#)).

Fig. 4. Barplot showing the frequency of nests laid per month, separated for the four different nesting seasons at Rio Oro and Pejeperro beaches, Osa Peninsula, Costa Rica.



High variation of frequency of nests occurred between sectors along both beaches for all four nesting seasons 2020-2024 ([Fig. 5](#)). The nests were not randomly distributed along the beach (X^2 , $p < 0.001$). Considering all four nesting seasons together, the least frequent sectors were located at both ends of both beaches. No nests were located in sectors 52.5, 56.25, 56.5, 58.5, 78, 79, 99, 100.75, 101.25, 101.75, and 103. In general, sectors between 63.25 & 74 in Rio Oro and sectors between 80.50 & 84.75 in Pejeperro had a higher number of nests. Sector 66.25 had the highest number of nests with 24 nests over the four seasons.

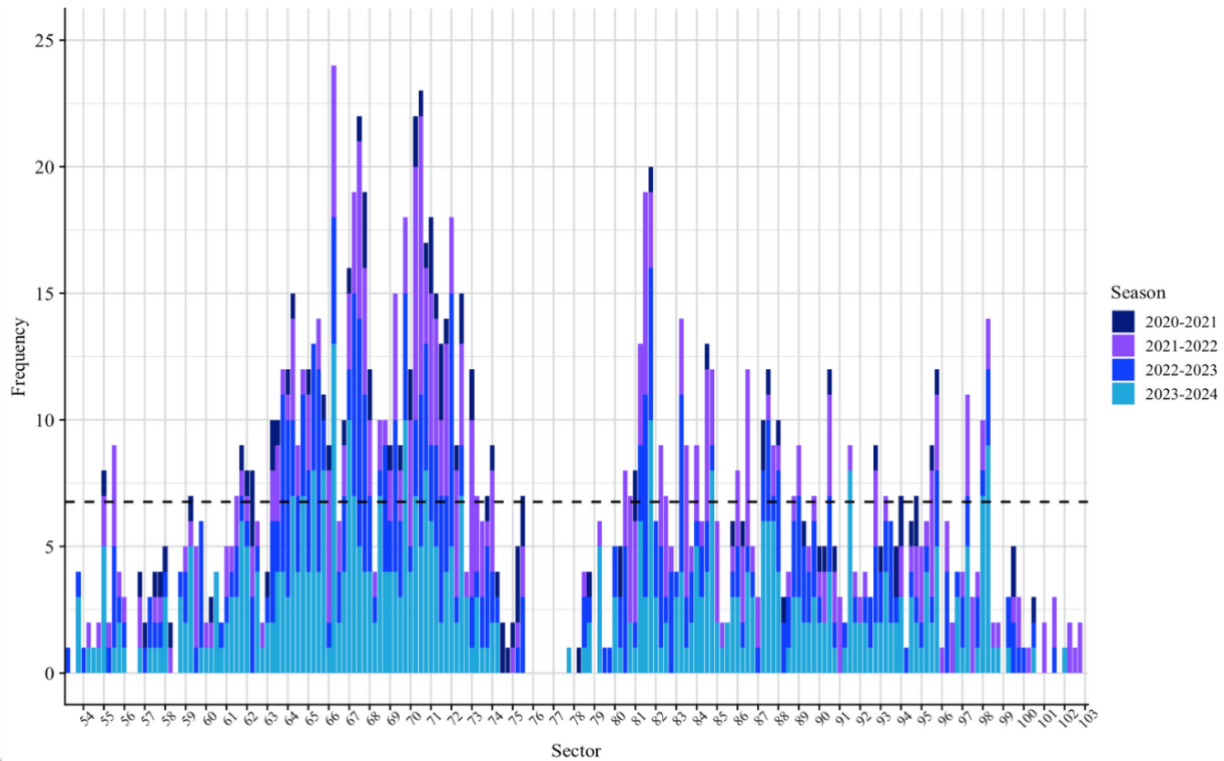


Fig. 5. Bar plot showing the frequency of nests laid within each sector separated for the four different nesting seasons (2020-2024). X-axis shows only whole sector numbers, but bars represent each individual sector (per .25). The dashed line shows the average number of nests overall sectors over the four nesting seasons at Rio Oro and Pejeperro beaches, Osa Peninsula, Costa Rica. Sector 53.25 till 75.50 represent Rio Oro beach and sector 77.75 – 103 represent Pejeperro beach.

Beach profile

The mean overall slope of the beach from the high tide line to the vegetation line was $6.97^{\circ} \pm 4.59$. It had a range of 2.71° to 26.5° along the beach (Fig. 6). The overall mean of the beach width was $1758 \text{ cm} \pm 392$ and ranged from 1147 cm to 2881 along the beach (Fig. S1). An exponential trend was observed of a higher number of nests under a lower beach slope (Fig. 7a). However, beach slope was not strong enough to yield a significant correlation on the number of nests (NBM, $p = 0.519$). In addition, we did not find any significant interactive effect of beach slope and width on the number of nests (NBM, $p=0.544$). Moreover, there was no significant correlation for beach slope (Fig. 7b) on the frequency of unsuccessful nesting attempts (false crawls, abandoning after digging or only cleaning) (NBM, $p = 0.469$,

$p=0.510$). Also, no significant interactive effect of beach slope and width was found (NBM, $p=0.471$).

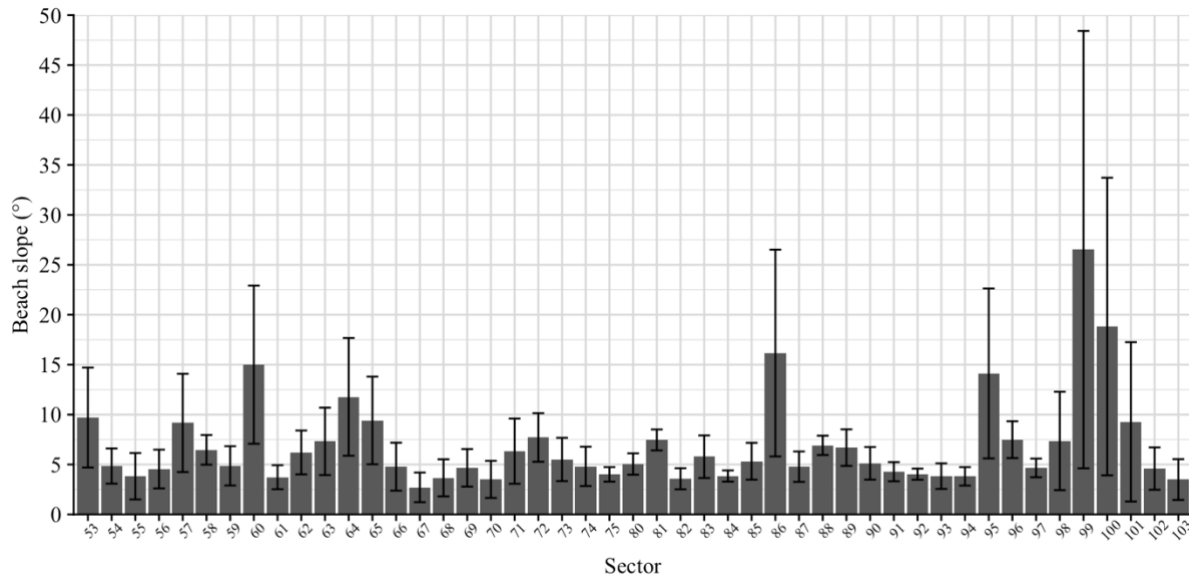


Fig. 6. Bar plot showing the mean beach slope of each whole sector over a time period of four months (November 2023 till February 2024) along Rio Oro (sector 53-75) and Pejeverro (sector 80-103) beaches, Osa Peninsula, Costa Rica. Error bars represent the standard deviation.

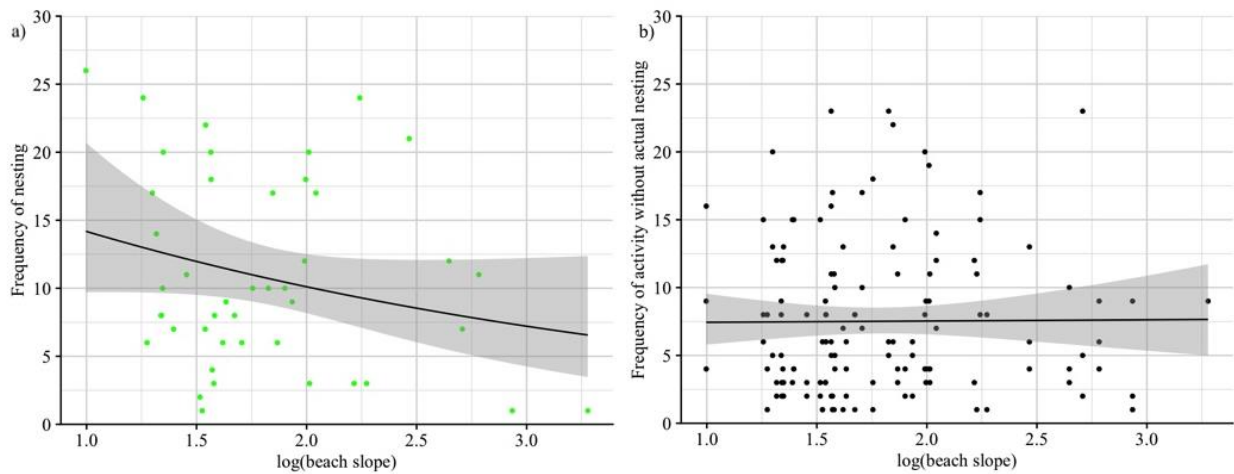


Fig. 7. Regression plots with regression line showing effect of beach slope width on the frequency of (a) nesting ($n = 492$) and (b) activity without actual nesting on Rio Oro and Pejeverro beaches, Osa Peninsula, Costa Rica in nesting season 2023-2024. Beach slope and width were log transformed to obtain homogeneity of variances. No significant correlation was found for the frequency of nesting or activity without actual nesting and beach slope (NBM, $p=0.544$, $p=0.469$, respectively).

Vegetation

In nesting season 2023-2024, the vegetation along the vegetation line of the study area mostly consisted of high vegetation, making up 45.92% of the beach. Furthermore, along the transect

line there was on average 26.50% bare sand, 19.80% low lying vegetation, and 3.3% medium height vegetation ([Fig. 8](#)). Below sector 53.25-55 and 73.5-75.75 consisted mostly of low-lying vegetation. Sector 99-102.75 consisted mostly of bare sand (>sector 99). Sectors 63-73 and sector 86-96 consisted mostly of high vegetation.

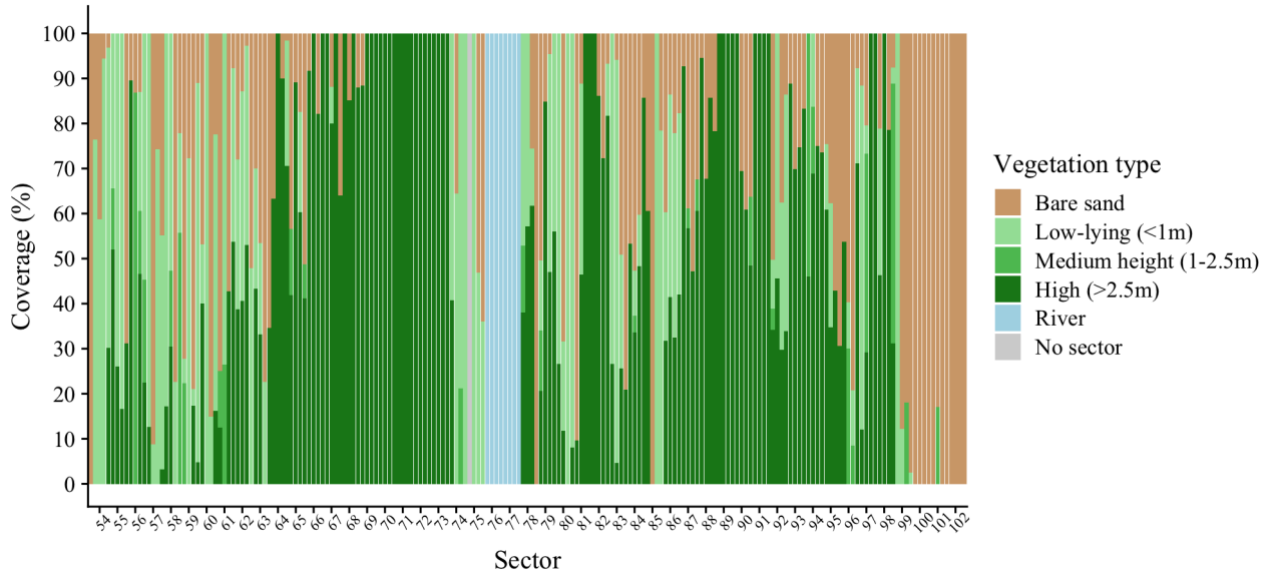


Fig. 8. Stacked bar plot showing the coverage in percentage along the horizontal transects of the four different vegetation categories along Rio Oro (sector 53.25-75) and Pejeperro (77.50-102.75), beaches, Osa Peninsula, Costa Rica in nesting season 2023-2024. X-axis only shows whole sector numbers, but bars represent each individual sector (per .25).

For three out of four vegetation categories the number of nests was significantly affected by the level of coverage ([Fig. 9](#)). The three vegetation categories with significant effects were bare sand (GLM, $p < 0.001$, low vegetation (GLM, $p < 0.00169$), and high vegetation (GLM, $p < 1.832e-14$). A significantly lower number of nests (0.70 ± 0.25) was found in 75-100% bare sand coverage compared to 0-25% (3.02 ± 0.26), 25-50% (2.62 ± 0.32), and 50-75% bare sand (2.50 ± 0.49) (Tukey test, $p = 0.0006$, $p = 0.0053$, $p = 0.0138$, [Fig. 9a](#)). Furthermore, a significantly higher number of nests (3.01 ± 0.24) was found for a coverage of 0-25% of low vegetation compared to 75-100% low vegetation coverage (1.18 ± 0.26) (Tukey test, $p = 0.0272$, [Fig. 9b](#)). Moreover, a significantly higher number of nests (4.19 ± 0.29) was found at 75-100% coverage of high vegetation compared to 0-25% (1.15 ± 0.16), 25-50% (2.88 ± 0.39) and 50-75% coverage (2.31 ± 0.39) (Tukey, $p = < .0001$, $p = 0.0468$, $p = 0.0058$, [Fig. 9d](#)).

Lastly, a significantly lower number of nests (1.15 ± 0.16) were found at 0-25% coverage of high vegetation compared to 25-50% (2.88 ± 0.34), and 50-75% (2.31 ± 0.39) (Tukey, $p = <.0001$, $p=0.0093$). No significant effects of the coverage of medium vegetation were found for medium height vegetation coverage (GLM, $p = 0.2771$, [Fig. 9c](#)).

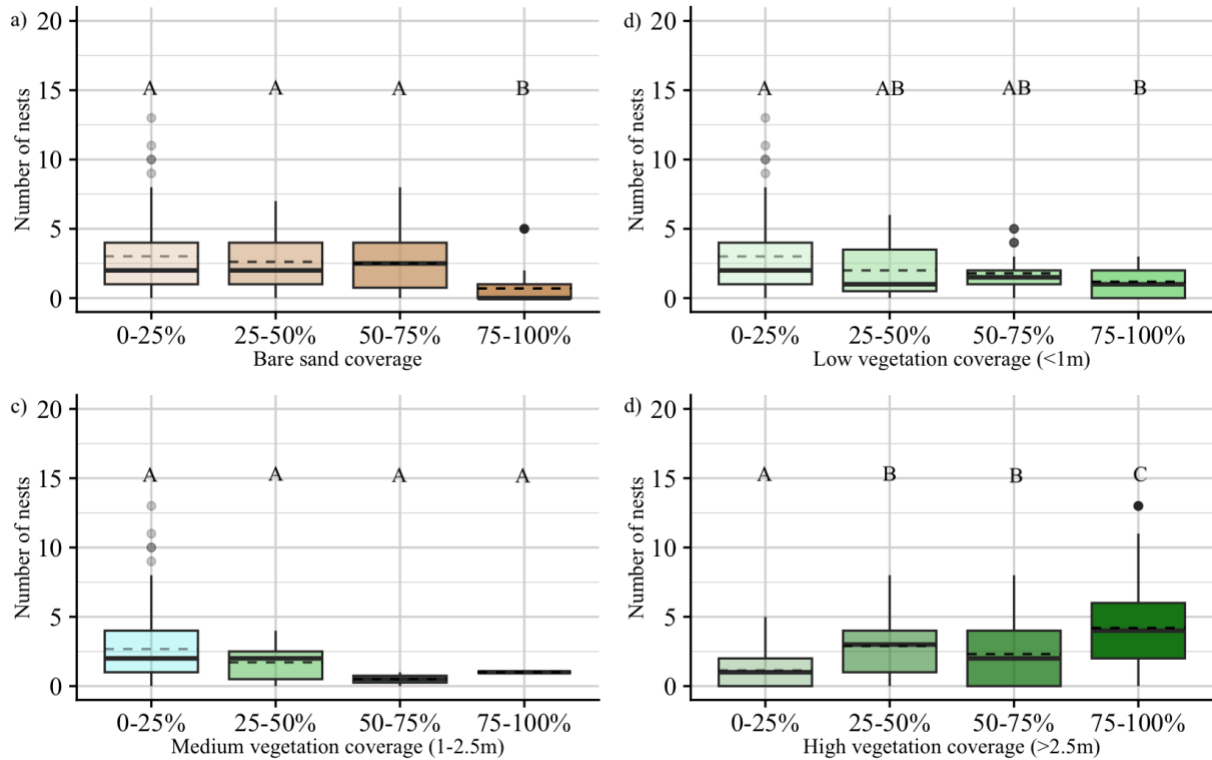


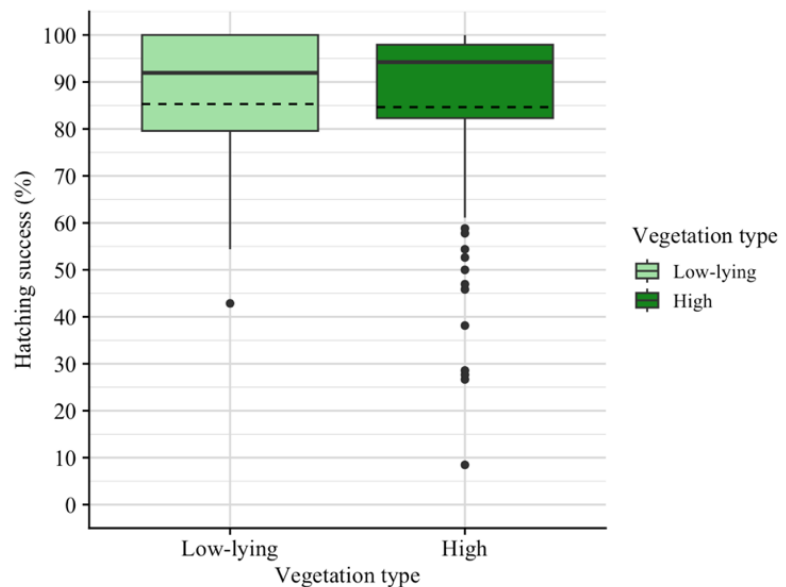
Fig. 9. Box plot showing the effect of the four different coverage percentage categories of a) bare sand, b) low lying vegetation, c) medium vegetation, and d) high vegetation on the mean number of nests ($n=490$) per along Rio Oro and Pejeporro beaches, Osa Peninsula, Costa Rica in nesting season 2023-2024. The dashed line in a boxplot indicates the mean and the non-dashed line indicates the median per percentage of that specific category. Different letters indicate significant differences ($p < 0.05$) between percentage categories within the same vegetation type.

The average hatching success for all four nesting seasons 2020-2024 together was $83.5\% \pm 1.16$ ($n=367$, [Table 2](#)). No significant differences were found between the years (LM, $p=0.5568$). The mean hatching success in low-lying vegetation in season 2023-2024 was $85.30\% \pm 4.23$ ($n=17$) and in high vegetation $84.65\% \pm 2.19$ ($n=87$, [Fig. 10](#)). No significant effect was found of low-lying or high vegetation on hatching success (LM, $p=0.9032$).

Table 2. Table showing the mean hatching success of the green sea turtle per nesting season at Rio Oro and Pejeperro beaches, Osa Peninsula, Costa Rica. No significant differences were found between the four nesting seasons (LM, $p=0.5568$).

Nesting season	Mean ($\pm$ SD) hatching success (%)	Sample size
2020-2021	82.8 ± 2.26	72
2021-2022	85.9 ± 1.52	122
2022-2023	81.9 ± 3.76	61
2023-2024	82.3 ± 2.35	112
Mean over all nesting seasons	$83.5 \pm 1.16\%$	376

Fig. 10. Box plot showing the effect of low-lying ($n=17$) or high ($n=87$) vegetation on hatching success at Rio Oro and Pejeperro beaches, Osa Peninsula, Costa Rica in nesting season 2023-2024. The dashed line in a boxplot indicates the mean and the non-dashed line indicates the median per percentage category. No significant differences in hatching success were found between low-lying or high vegetation (LM, $p=0.9032$).

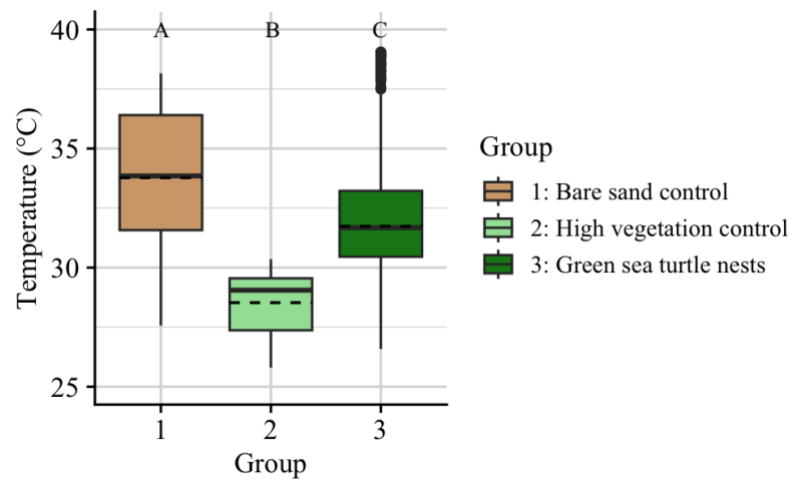


Temperature

The mean temperatures from both control loggers in different vegetation types differed significantly from the mean temperatures within the green sea turtle nests over the whole incubation period in nesting season 2023-2024 (LM, $p < 2.2e-16$, [Fig. 11](#)). Only green sea turtle nest surrounded by high vegetation were used in this analysis ([Table 1](#)). There was a significantly higher temperature in the bare sand control ($33.8^{\circ}\text{C} \pm 0.04$), and significantly lower in the high vegetation control ($28.5^{\circ}\text{C} \pm 0.02$) compared to green sea turtle nests ($31.8^{\circ}\text{C} \pm 0.02$) in high vegetation (Tukey, $p < 0.0001$, $p < 0.0001$, respectively). Moreover,

there was a significantly higher temperature in the bare sand control ($33.8^{\circ}\text{C} \pm 0.04$) compared to high vegetation control ($28.5^{\circ}\text{C} \pm 0.02$) (Tukey, $p < .0001$).¹³ The average temperature in a green sea turtle nest during the incubation period was $31.79^{\circ}\text{C} \pm 1.96$ and mean nest temperatures ranged from 29.90°C to 33.81°C (Table 3, Fig. S2). 9 out of 10 nests had a mean temperature in the sex determining period leading that led to an estimated PSR of 100% female hatchlings (Table 3).

Fig. 11. Box plot showing the effect of three different locations of temperature logger, bare sand ($n=4$), high vegetation ($n=3$), and within green sea turtle nest placed in high vegetation ($n=9$) on temperature in nesting season 2023-2024. The dashed line in a boxplot indicates the mean and the non-dashed line indicates the median per percentage category. Different letters indicate significant differences ($p < 0.05$) between groups.

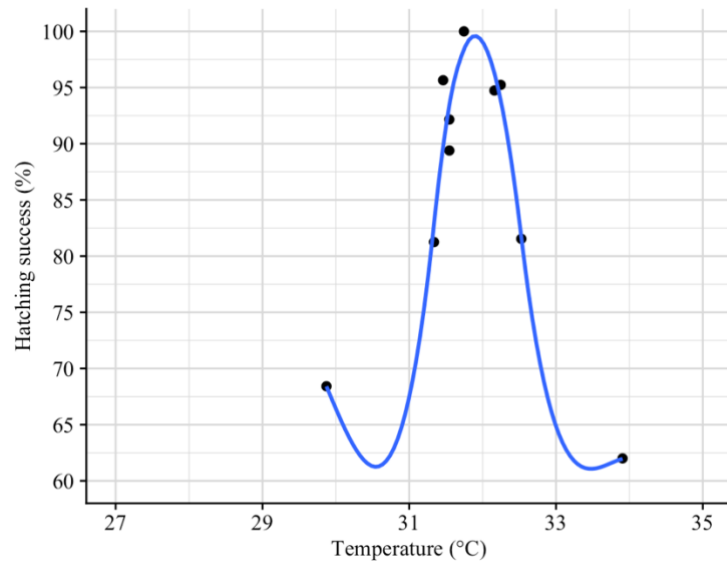


All nests were found to have a hatching success above 60%, where the nest with the highest temperature and the nest with the lowest temperature also corresponded with the lowest hatching success (Table 3). The Spearman's rank correlation coefficient between whole incubation period and hatching success was $\rho = 0.067$ (Fig. 12). There was no statistically significant relationship between the variables ($p = 0.8648$). However, a trend is visible that with a mean temperature between 31.50°C and 32.54°C , the hatching success remains above 80%, where below those temperatures hatching success seems to decrease.

Table 3. Table presenting the mean incubation temperature, the range of temperatures observed, the hatching success rate, the estimated PSR as percentage female hatchlings, and the type of vegetation surrounding each green sea turtle nest of 10 nests at Rio Oro and Pejeperro beaches, Osa Peninsula, Costa Rica.

Nest number	Mean ($\pm$ SD) nest temperature ($^{\circ}$ C) during sex-determining period	Mean ($\pm$ SD) nest temperature ($^{\circ}$ C) during entire incubation	Temperature range ($^{\circ}$ C)	Hatching success (%)	Estimated PSR (%) (Standora & Spotila, 1985)
1	32.81 $\pm$ 1.91	32.39 $\pm$ 2.08	28.2 – 36.0	96.38	100
2	32.25 $\pm$ 1.70	31.50 $\pm$ 2.18	27.7 – 35.1	89.39	100
3	33.57 $\pm$ 0.79	32.54 $\pm$ 1.47	29.2 – 35.0	81.54	100
4	32.18 $\pm$ 0.65	31.55 $\pm$ 1.18	29.6 – 33.5	92.15	100
5	34.64 $\pm$ 0.51	33.81 $\pm$ 1.22	30.8 – 39.1	62.00	100
6	32.58 $\pm$ 0.61	32.16 $\pm$ 0.91	29.6 – 34.1	94.73	100
7	28.82 $\pm$ 0.73	29.89 $\pm$ 2.41	26.6 – 34.8	68.42	8
8	33.33 $\pm$ 1.10	31.83 $\pm$ 1.77	28.2 – 35.2	100	100
9	31.95 $\pm$ 1.18	31.46 $\pm$ 1.38	29.7 – 34.0	96.49	100
10	31.49 $\pm$ 0.45	31.33 $\pm$ 0.48	30.4 – 32.3	81.25	100

Fig. 12. Scatterplot shows the hatching success (in %) for different mean temperatures over the whole incubation across ten green sea turtle nests. The LOESS smoothing line (blue line) illustrates the trend in the data, highlighting the potential non-linear relationship between temperature and hatching success. Statistical analysis using Spearman's rank correlation indicated that the relationship between temperature and hatching success is not statistically significant ($p > 0.05$). Each point represents data from a single nest.



Discussion

Green sea turtles are endangered marine reptiles, crucial for the health of our marine and coastal ecosystems. Nest site selection is a key factor influencing their reproductive success, which is defined as the laying of nests in zones that are not randomly chosen within a certain area. This study aimed to investigate the influence of beach slope and vegetation on nest site selection of green sea turtles at two important nesting beaches, Rio Oro and Pejeperro, on the Osa Peninsula in Costa Rica. This study found that beach slope did not significantly predict nest site selection. However, vegetation played a crucial role. Green sea turtles preferred to nest in areas with bare sand and low-lying vegetation coverage of less than 75% and high vegetation coverage of more than 25%. Additionally, vegetation was found to help reduce temperature, which is particularly important in the face of rising temperatures due to climate change. These findings highlight the importance of maintaining natural vegetation cover at green sea turtle nesting beaches. Moreover, this study found a highly skewed female sex ratio, indicating a risk of population feminization. Considering the numerous threats green sea turtles face on their nesting grounds, robust conservation measures like intensive beach patrolling, nest relocation, and irrigation are essential. Alongside coastal conservation efforts, it is also critical to implement offshore conservation strategies to ensure the comprehensive protection of these endangered species throughout their entire life cycle.

Importance of Rio Oro and Pejeperro beaches for green sea turtle nesting

Results of the nesting analyses observed in this study mirror that of previous research on green sea turtle nesting beaches on the Pacific coast of Costa Rica, exhibiting peak nesting between November and March (Drake, 1996; Exley et al., 2022; Santidrián Tomillo et al., 2015) ([Fig. 4](#)). Analysing nest counts over the past two seasons reveals that Pejeperro and Rio Oro beaches stand out as important nesting sites for green sea turtles, compared to other

important nesting sites such as Veracruz in Mexico, and Akyatan in Turkey. There 1037 and 529 nests were recorded over two nesting seasons in the years 2008-2009 & 2005-2007, respectively (Turkozan et al., 2011; Zavaleta-Lizárraga & Morales-Mávil, 2013). The number of nests (854) found at Pejeperro and Rio Oro over two nesting seasons (2022-2024) is comparable, if not higher despite their smaller available nesting area. Together with the high average hatching success of 83.5% over four nesting seasons (2020-2024, [Table 2](#)), it underscores the vital role of Pejeperro and Rio Oro beaches as crucial nesting grounds for green sea turtles. Currently, historical nesting data for green sea turtles at Rio Oro and Pejeperro beaches is insufficient to estimate population size or observe trends in abundance. Daily patrolling efforts have only been implemented for the two most recent seasons, making year-to-year comparisons impossible. Maintaining intensive beach management is crucial to gather data on these significant nesting beaches. With data spanning multiple years, environmental disturbances can be identified promptly. For instance, shifts in nesting seasons could be detected which is important since it can be an indicator of environmental disturbances, such as increased temperatures (Parmesan, 2007). Therefore, extending this study to future nesting seasons is crucial to assess population size of green sea turtle nesting at the Osa Peninsula.

Effect of beach slope on nest site selection

Results show that nesting females prefer certain sectors along the beach ([Fig. 5](#)). In other studies, beach slope was found to be one of the most relevant morphological variables in determining sea turtle nest site selection (Cuevas et al., 2010; Fish et al., 2005; Wood & Bjorndal, 2000). However, in this study, it does not become clear whether the beach slope is a cue used for nest site selection in green sea turtles. No significant correlation was found between beach slope and frequency of nesting or activity without actual nesting ([Fig. 7](#)). The

average slope, ranging from 2.71° to 26.5° (Fig. 6) with a mean of $6.97 \pm 4.59^\circ$, falls within the preferred range of 3-30° (Natih et al., 2021). This implies that green sea turtles nesting at Pejeperro and Rio Oro beaches may exhibit a broader range of preferences for beach morphological features (Fuentes et al., 2013). Furthermore, the lack of a significant relationship between beach slope and number of nests suggests a preference for the current slope. Moreover, other factors may be more strongly influencing nest site selection, for example, human activity, currents, and the presence of predators, even though the physical conditions of the beaches can be appropriate for nesting (Zavaleta-Lizárraga & Morales-Mávil, 2013). Additionally, beach morphology is dynamic and it might change rapidly due to seasonal or storm-induced changes. Neighbouring beaches of Rio Oro and Pejeperro beaches already have been found to experience sea-level rise with serious implications for beach erosion and, therefore, decreasing nesting habitat (Beber et al., 2024). Also, on the Caribbean coast of Costa Rica, 18–24% of the sea turtles were unable to crawl in 20% of the beach length due to large dune scarps (Rivas et al., 2023). Dune scraps might also become a problem at the Osa Peninsula and will limit green sea turtles to nest in their preferred area. Hence, it is imperative to continually monitor beach profile alterations.

Effect of vegetation on nest site selection, temperature and hatching success

Vegetation was found to have a significant effect on nest site selection for green sea turtles (Fig. 9). When >75% of bare sand coverage was present, it led to a significant lower amount of green sea turtle nesting. Hence, indicating that a lower percentage of bare sand is preferred. When low-lying vegetation cover was >75%, the number of nests was significantly lower. Besides that, the number of green sea turtle nests significantly increased when high vegetation coverage increased. From these results, it can be said nesting is preferred where bare sand and low-lying vegetation coverage is low and where high vegetation coverage is

high, which is in agreement with other studies of green sea turtles in Costa Rica (Beber et al., 2024; Heredero Saura et al., 2022; Whitmore & Dutton, 1985; Zavaleta-Lizárraga & Morales-Mávil, 2013). Nesting within the vegetation has advantages such as lower vulnerability to coastal erosion, lower surface temperatures, wave runup, clutch inundation and subsequent mortality (Beber et al., 2024). However, it is important to consider the ecological trade-off, above the vegetation line nesting females might be more susceptible to parasite infestation rates from the present vegetation, and predators, such as white-nosed coati's (*Nasua nasua*), raccoons (*Procyon lotor*), black vultures (*Coragyps atratus*) and domestic dogs, which are common to be seen on Costa Rican beaches (Barquero-Edge, 2013). While previous studies, such as Santidrián Tomillo et al. (2015), have reported significantly higher hatching success in areas with denser vegetation, our findings did not demonstrate a significant effect of either low-lying or high vegetation on hatching success (Fig. 10). However, this lack of significance may be attributed to the fact that our study did not categorize the vegetation surrounding the nests at species level and therefore potentially obscuring any significant differences between vegetation types and hatching success. High vegetation did have a large significant effect on reducing nest temperature (Fig. 11), which is identical to what was found in hawksbill nests (Kamel, 2013). Sand temperature in bare sand was on average 5.3°C higher compared to sand temperature within high vegetation, at equal depths. Even the metabolic heating of the nest was not sufficient to remove the reducing effect of vegetation on temperature (Fig. 11), underscoring the crucial role of vegetation in lowering nest temperatures (Broderick et al., 2001). The findings that vegetation plays an important role in nest site selection and reducing nest temperatures highlight the importance of maintaining natural vegetation cover at green sea turtle nesting beaches, since the loss of native vegetation cover can have large negative consequences on green sea turtle fitness.

Effect of temperature on hatching success and primary sex ratio

The mean nest temperatures ranged from 29.90°C to 33.81°C ([Table 3](#)) and lies within the thermal tolerance range of 25°C- 35°C for successful incubation of green sea turtles (Howard et al., 2014). One out of ten nests had temperatures rising to greater than 35°C, which also corresponded with the lowest hatching success, indicating that heat stress can explain the embryo mortality. However, contradictory to other studies (Fuentes et al., 2023; Howard et al., 2014), no significant correlation was found between mean nest temperature and hatching success ([Fig. 12](#)). Nevertheless, a trend can be seen that the highest and the lowest temperatures had the lowest hatching success. However, in general caution should be exercised when using hatching success data to evaluate certain environmental cues for nest site selection, since other factors such as grain size, humidity, salinity and nest depth can all influence both temperature and hatching success (Bustard & Greenham, 1968; Turkozan et al., 2011).

Nine out of ten temperature loggers resulted in a PSR of 100% female hatchings ([Table 3](#)). This emphasizes that warming temperatures are threatening to cause unsustainable levels of feminization in the green sea turtle populations at the Osa Peninsula. Highly skewed sex ratios from climate warming could reduce genetic diversity, the effective population size and increase the potential for inbreeding (Heppell et al., 2022). However, it has been suggested that a female-biased sex-ratio may enhance population growth (Laloë et al., 2014), as few males can fertilize many females. Meaning that these unbalanced PSRs are found to translate into a more balanced operational sex ratio (OSR), which is the sex ratio of adult individuals that reproduce in a given season. Moreover, females can only mate about every three years, and store sperm throughout the season, males are able to mate every year (Fitzsimmons, 1998). So, an adult population that is female-biased can have equal numbers of reproductive

individuals of each sex in a given year (Santidrián Tomillo & Spotila, 2020) and therefore a highly female biased sex ratio is not directly alarming. However, the low generation turnover rate may lead to a delayed impact of lack of males. Eventually, the few remaining males may no longer be able to fertilize enough females to sustain the populations (Jensen et al., 2018), and the population might go extinct, especially if temperatures keep rising under climate change. Currently, there is still a knowledge gap on what PSR could still be translating in a balanced OSR on population level (Santidrián Tomillo, 2022). Therefore, it is important to examine the fertility rate of eggs over time, since that could be an indication for the lack of males in populations (Santidrián Tomillo, 2022). Overall, together with concerns over the immediate threats of climate change to sea turtle populations, we need to learn more about how sea turtles and other TSD species can cope with rapid climate change.

Applications to conservations

Multiple direct and indirect anthropogenic effects are causing declines in green sea turtle populations. These include incidental captures by fisheries, habitat degradation, marine pollution, coastal development, harvest of eggs for consumption and commercial purposes, and human disturbances. Climate change exacerbates these pressures through sea-level rise, increased cyclonic activity, and elevated sand temperatures. Many of these threats reduce the availability of suitable nesting habitats, complicating green sea turtles' access to their preferred nesting sites (Beber et al., 2024; Fuentes et al., 2011b; Santos et al., 2017). Additionally, by 2100, global air temperatures could rise by 2.6 °C and 4.8 °C (IPCC, 2013), likely increasing sand temperatures higher at all nest depths (Santos et al., 2017). Given these threats to green sea turtle nesting beaches and their importance, conserving and protecting them is essential. Debate over the optimum methods for conserving dwindling populations of endangered green sea turtles has been a concern of the conservation community for decades.

Some studies say that currently it does not seem reasonable to intervene with nests that can successfully incubate on the beach, as the impact of these mitigation actions is not yet understood (Santidrián Tomillo, 2022). However, the multiple threats (locally and globally) that threaten most populations worldwide, may tip the balance toward extinction. Intensive beach management has been found to be a successful strategy to increase the impact of sea turtle conservation efforts (García et al., 2003). This includes intensive beach patrolling to monitor sea turtle activity and adequate nest reburial if nests are being laid in areas with low hatching success, such as areas related with high temperatures or high risk to erosion. Furthermore, nest shading and irrigation have been demonstrated to be a viable approach for conservation practitioners to partially alleviate the impacts of climate change on nesting sea turtles by reducing incubation temperatures, increasing hatching success, and balancing offspring sex ratios (Hill et al., 2015; Jourdan & Fuentes, 2015; Laloë et al., 2014). However, the impacts of shading and irrigation depend highly on the thermal environmental conditions of the nesting beach and therefore can vary significantly between nesting sites (Laloë et al., 2014). Nevertheless, due to the extremely high female biased sex-ratio found here in this study, implementing these conservation strategies could contribute positively to the fitness of the green sea turtle population.

As aforementioned, the green sea turtle's life cycle is highly complex and is found to face threats at all life stages. Therefore, not only coastal conservation is necessary to sustain a healthy green sea turtle population but also offshore conservation measurements are necessary. Marine Protected Area's (MPA's) have been found to be able to recover sea turtle populations (Nel et al., 2013; Shaver et al., 2020). Effective MPAs should include important foraging grounds, migratory routes, and developmental habitats to ensure comprehensive protection. By restricting activities such as commercial fishing, particularly practices that

result in high bycatch rates, MPAs can significantly reduce incidental green sea turtle capture and mortality. However, the success of MPAs requires strong enforcement, monitoring, and community engagement to ensure compliance and adaptability to emerging threats (Nel et al., 2013).

Conclusion

The current study underscores the critical importance of Rio Oro and Pejeperro beaches as significant nesting sites for green sea turtles on the South Pacific coast of Costa Rica. Despite limited historical nesting data, the high number of nests recorded and the substantial hatching success rates highlight the essential role of these beaches for green sea turtle nesting. Beach slope did not exhibit a significant correlation with nest numbers, suggesting that this factor alone does not influence nest site selection in this study. Vegetation significantly impacted nest site selection, with green sea turtles preferring nesting in high vegetation (>2.5 m). Moreover, vegetation was found to play a crucial role in reducing sand temperature, which is particularly important given the current trend of rising temperatures due to climate change and extreme weather events. No significant correlation was found between mean nest temperature and hatching success, however, this study revealed a highly female-biased sex ratio among the studied green sea turtle populations, signalling potential risks of feminization due to warming temperatures. Further research is necessary on what PSR can translate into a balanced OSR on population level. Preserving natural vegetation along nesting beaches emerges as a critical strategy, as it plays an important role in nest site selection by green sea turtles and helps mitigate high nest temperatures, thereby reducing the proportion of female hatchlings. To effectively mitigate the threats facing green sea turtles, it is necessary to implement effective coastal conservation strategies on nesting beaches, including intensive beach management, adequate nest reburial, nest shading, and irrigation. Given the complexity

of the green sea turtle's life cycle and the several threats encountered at every life stage, these coastal efforts alone are insufficient. It is equally crucial to adopt offshore conservation measures to ensure the sustainability of healthy green sea turtle populations. Only through a holistic approach that addresses both coastal and offshore threats, we can better ensure the survival of these important marine animals."

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Supplementary

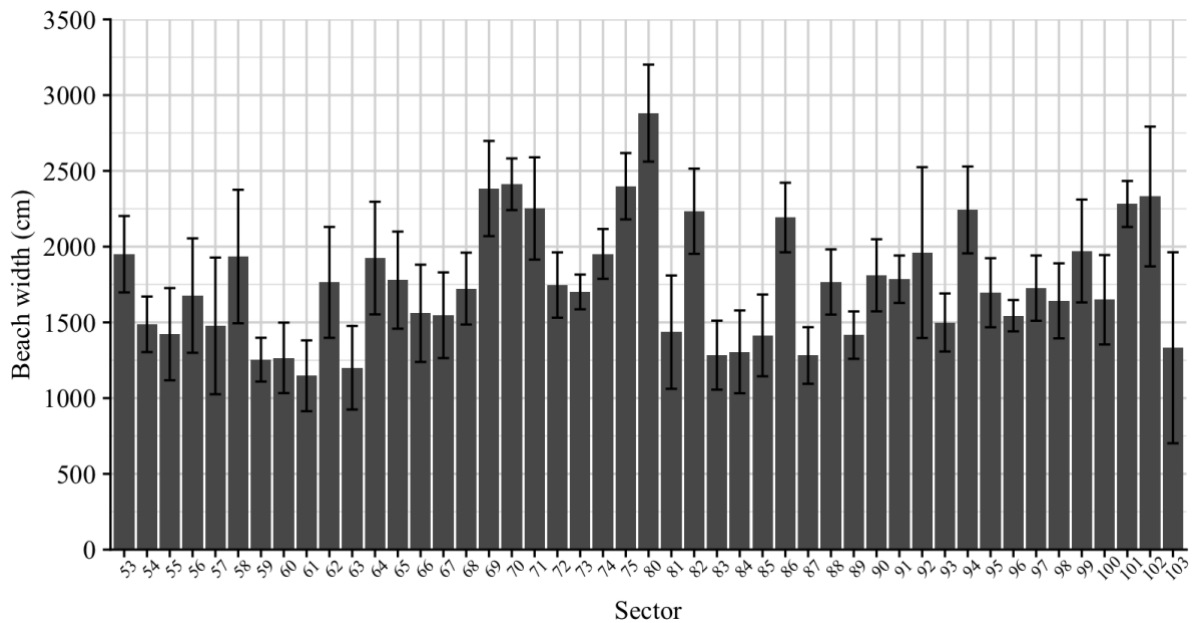


Fig. S1. Bar plot showing the mean beach width of each sector over a time period of four months (November 2023 till February 2024) along Rio Oro (sector 53-75) and Pejeperro (sector 80-103) beaches, Osa Peninsula, Costa Rica. Error bars represent the standard deviation.

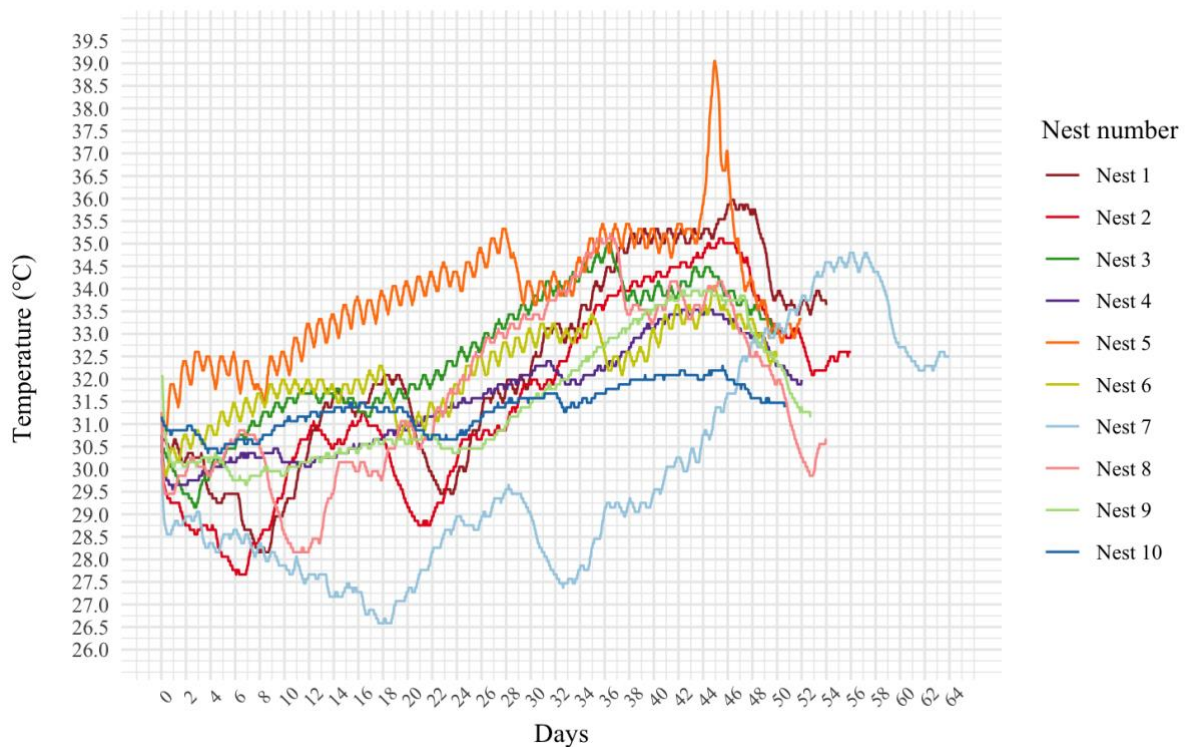


Fig. S2. Line plot showing the temperature over the whole incubation period for 10 green sea turtle nests at Rio Oro and Pejeperro beaches, Osa Peninsula, Costa Rica. Each line represents a single nest. Temperature loggers were placed in the centre of the nest, after the female laid 30 eggs.

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