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Nesting habitat preferences and hatching success of green turtles, *Chelonia mydas*, in São Tomé Island, West Africa

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Mestrado em Biologia Marinha

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(Edilaine Cristina Bandeira Cunha)

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Contents

Abstract

Female sea turtles need sandy beaches to develop and incubate their eggs and guarantee the next generation's success. During the nesting process, the female becomes vulnerable to the environmental condition of the beach and predators. In addition, the location of the nests can affect the reproductive success and survival of their offspring. The present study conducted on Jalé beach in the south of São Tomé Island analysed the density and the distribution of Green turtle's (*Chelonia mydas*) nests along the beach and the effects of site selection on hatching success. Habitat features (such as bathymetry and coastal habitat), moon phases and tides influence on female emergences were analysed. We found a positive correlation between some of these factors on Green turtle's emergences. Green turtles nesting on Jalé beach also seemed to prefer to nest between the vegetation border and the open beach, avoiding the vegetation. In addition, it was verified if there was a pattern between the nest location and the distance to the high tide line. We observed a minimal distance between the nest and the high tide line, suggesting a small risk of the nests being washed by waves or loss by beach erosion caused by spring tides. No correlation was found between the microhabitats at the nest location and the hatching success. This study revealed that the main environmental factors that may influence Green turtles nesting site preferences, providing crucial information for future studies, better management related to climate change, and the rapid growth of tourism-related developments on the coast of Jalé beach.

Resumo

As tartarugas marinhas possuem um ciclo de vida complexo, são os únicos répteis capazes de migrarem centenas milhares de quilómetros durante todo o seu longo ciclo de vida, cruzando fronteiras de vários países, sendo por isso protegidas por vários protocolos internacionais. Um dos períodos mais vulnerais para o ciclo vida das fêmeas ocorre durante a época de desova, no qual a fêmea regressa à sua praia de origem para depositar os seus ovos. Todo processo de desova, além de deixar as fêmeas vulneráveis a predadores, deverá ser bem-sucedido para garantir o sucesso da próxima geração da espécie. O desenvolvimento e posterior sucesso dos filhotes, podem ser afetados pelo local de desova escolhido pelas fêmeas, sendo necessário que o habitat de nidificação reúna condições necessárias para a incubação e o menor risco de mortalidade.

O presente estudo foi realizado na ilha de São Tomé, situado no arquipélago de São Tomé e Príncipe, no Golfo da Guiné. A ilha de São Tomé, abriga desova de quatro espécies de tartaruga marinha, a tartaruga-de-couro (*Dermochelys coriacea*), de pente (*Eretmochelys imbricata*), oliva (*Lepidochelys olivacea*) e a mais abundante a verde (*Chelonia mydas*). A população da tartaruga-verde na ilha de São Tomé desova entre o período de julho a abril, e estima-se uma média de 683 desovas por ano (variando entre 250-1177 ninhos entre 2015 e 2021). O maior número de desovas da tartaruga-verde, ocorre na Praia Jalé no sul da ilha, contando com cerca de 40% das desovas de toda a ilha em apenas 1,4 km de praia, sendo por isso o sítio de nidificação mais importante para esta espécie. Este estudo será o primeiro a avaliar a densidade e distribuição da nidificação da tartaruga-verde na praia Jalé. Foram avaliados vários fatores que podem influenciar o processo de desova e as preferências de microhabitat, e como as variáveis da praia podem afetar o sucesso reprodutivo dos filhotes. Além disso, na praia Jalé atualmente tem ocorrido um aumento no desenvolvimento de infraestruturas ligadas ao turismo ao longo da praia, sendo também avaliado o impacto deste desenvolvimento e como o mesmo influencia o processo de desova das tartarugas-verdes.

Este estudo foi realizado na praia Jalé, entre 28 de setembro de 2020 e 05 de janeiro de 2021. A praia foi dividida em 3 zonas, Jalé 1, Jalé 2 e Jalé 3, divisão adotada pelo Programa Tatô, baseada nas características (tais como o habitat costeiro até à linha da batimetria dos 10 m e pela densidade de vegetação) distinta em cada zona. A zona da Jalé 1, tem 400 m, sendo o habitat costeiro caracterizado por um fundo rochoso e com um declive menos acentuado até à batimetria dos 10 m, e a praia caracterizada por uma floresta densa (vegetação composta

por coqueiros e caroceiros). A zona da Jalé 2, tem 300 m, sendo o habitat costeiro caracterizado por um fundo rochoso expostos durante a maré baixa e uma outra parte composta por um fundo arenoso. Esta zona possui uma distância menor da linha de costa à da batimetria dos 10 m em relação à Jalé 1. A praia é caracterizada por uma vegetação menos densa e principalmente pela presença de infraestruturas turísticas. A zona da Jalé 3, tem 700 m, sendo a zona costeira caracterizada por um fundo arenoso e uma distância à batimetria dos 10 m ainda menor quando comparada com as outras duas zonas. Possui uma vegetação densa, compostas por coqueiros e caroceiros, que se concentra junto a uma parede rochosa que delimita a zona.

Durante a monitorização, foram registados também as fases da lua e as marés durante cada ocorrência. Todas as ocorrências foram classificadas como desova, quando a tartaruga efetivamente conseguiu colocar o seu ninho, ou numa desistência, quando a tartaruga por alguma razão desistiu de desovar. Para cada desova, foi classificado o microhabitat no local do ninho: praia aberta (área exposta ao sol durante todo o dia); linha da vegetação (área parcialmente expostas ao sol) e vegetação (área não exposta ao sol). Foi ainda medida a distância da linha de maré alta até o ninho e a distância dos ninhos até à linha de vegetação. Após o período de incubação, os ninhos foram analisados e registado o número de ovos eclodidos, de modo a calcular o sucesso de cada ninho, sendo também avaliado o sucesso de cada ninho face às características do microhabitat em cada ninho.

Ao analisar as diferentes fases da lua, verificou-se que houve um maior número de ocorrências durante os quartos da lua. Este maior número de ocorrências no quarto da lua poderá estar relacionado com a influência da lua sobre o ciclo da maré, uma vez que são geradas marés mortas, marés mais moderadas e provavelmente mais propicias para as tartarugas-verdes na praia Jalé.

Em relação à distribuição dos ninhos, os resultados revelaram que a ocorrência da tartaruga verde na zonas da praia é primeiramente afetada pelo tipo habitat costeiro, que influencia o local para as tartarugas saírem na praia, sendo o fundo arenoso o mais propicio para estas ocorrências, logo foram registados uma maior ocorrências entre a zona da Jalé 2 e Jalé 3.

A análise do microhabitat demostrou que a tartaruga-verde na praia Jalé desova preferencialmente entre áreas da praia aberta e a linha da vegetação. Através da análise da localização do ninho, verificou-se uma distância mínima entre o ninho e a distância à linha da maré alta. Os ninhos localizados na praia aberta estão sempre acima da linha da maré alta,

resultando em ninhos com menor risco de sofrerem risco de erosão ou inundação. Na área da vegetação, verificou-se um número reduzido de ninhos que pode ser explicado pelo limitado acesso das tartarugas a esta área. No entanto mesmo quando alcançando áreas com maior vegetação não ocorreram desovas, provavelmente pela maior probabilidade de encontrar raízes e pedras que dificultam a construção dos ninhos.

Para o estudo do sucesso dos ninhos, a taxa de eclosão foi considerada alta (88%) em relação a outros estudos. Os ninhos na linha de vegetação e na praia aberta apresentaram ambos altas taxas de eclosão, sugerindo que o microhabitat pode ser influenciado por fatores abióticos (o tamanho dos grãos de areia, a temperatura e a humidade) nas diferentes áreas.

Os resultados deste estudo, revelaram as principais variáveis ambientais que influenciaram na preferência do local de desova da tartaruga-verde e a densidade de distribuição dos ninhos ao longo da praia, assim como a taxa de sucesso dos ninhos desta espécie. Sugere-se que estudos futuros na região, possam focar-se no sex-ratio dos filhotes, nos fatores abióticos da praia que possam influenciar o seu desenvolvimento, e nas ameaças futuras relacionadas com os efeitos climáticos, e com o rápido desenvolvimento turístico na costa, com o objetivo de permitir traçar as melhores estratégias para a redução desses impactos.

Palavras-chave: *Chelonia mydas*, ninho, praia Jalé, tartaruga-verde, microhabitat

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CHAPTER 1: Introduction

1. General Remarks on Sea Turtles

1.1. Adaptations and taxonomic classification

For hundreds of millions of years, turtles have inhabited the earth, with a rich fossil record since the Early Cretaceous (Jones et al., 2012; Naro-Maciel et al., 2008). Several changes occurred while in the sea, such as paddle-shaped limbs so they could swim, reduction to one or two forelimb claws used by males to grab the females during copulation, lachrymal glands enlarged and modified to remove excess salt, low-domed, streamlines carapaces to maximize buoyancy and agility in the water and well-developed pectoral muscles for swimming (Pritchard, 1997; Martín-del-Campo and García-Gasca, 2019; Meylan and Meylan, 1999), but they cannot retract their heads into their shells like their terrestrial counterparts (Meylan and Meylan, 1999).

Sea turtles are marine ectothermic reptiles with pulmonary respiration. Therefore, they need to come to the surface to inhale the air before prolonged immersion (Meylan and Meylan, 1999; Williard et al., 2013) and depend on the environmental temperature and sun exposure to regulate body temperature (Pike, 2008; Williard et al., 2013). They possess an exoskeleton or carapace that protects vital organs (Hays, 2008; Pritchard, 2017).

Only seven sea turtle species have survived in the oceans until today, representing two families, Cheloniidae and Dermochelyidae, part of the order Testudines and the suborder Cryptodira (Fig.1) (Hendrickson, 1980; Meylan and Meylan, 1999). The most ancient is the Dermochelyidae family with a single species, *Dermochelys coriacea* (Leatherback), the older lineage relative to the other sea turtles (Duchene et al., 2012; Jensen et al., 2013). All other six species belong to the Cheloniidae family: *Eretmochelys imbricata* (Hawksbill), *Caretta caretta* (Loggerhead), *Chelonia mydas* (Green), *Natator depressus* (Flatback), *Lepidochelys kempii* (Kemp's Ridley) and *Lepidochelys olivacea* (Olive Ridley) (Duchene et al., 2012; Meylan and Meylan, 1999).

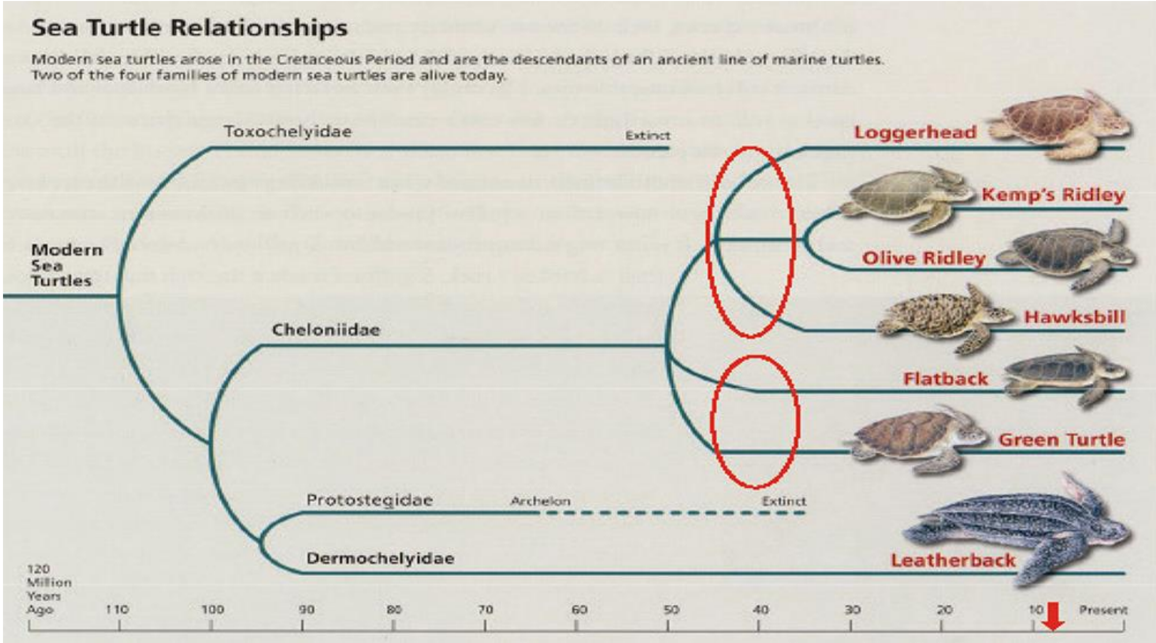

Figure 1. The evolution of the modern sea turtles (from J. Spotila, Sea Turtles: A Complete Guide to their Biology, Behaviour and Conservation)

Kemp's Ridley appears as a consequence of Panamanian separation from Olive Ridley after the formation of the isthmus as a land barrier (Duchene et al., 2012; Hendrickson, 1980). Although these seven species are recognised, different molecular studies support different groupings within Cheloniidae (Meylan and Meylan, 1999). One such example is the placement of Flatback with data supporting it as the sister taxon to a clade, including the three genera *Eretmochelys*, *Caretta*, and *Lepidochelys* (Dutton et al., 1996), or alternatively to *Chelonia* only (Naro-Maciel et al., 2008). The possibility of an eight extant species *Chelonia agassizii* (Black Sea turtle) (Dutton et al., 1996; Meylan and Meylan, 1999) was not supported with no species-level or evolutionary distinctiveness of Green turtles (Bowen et al., 1991; Seminoff et al., 2015).

1.2. Distribution and foraging behaviour

The widely recognized seven turtle species show considerable differences in their ecological niches, carrying out large migrations through several underwater habitats (Hendrickson, 1980). Within the *Lepidochelys* family, Olive Ridley occurs in the East and West Pacific and Indian Oceans and both sides of the Atlantic (Bowen et al., 1991). The Kemp’s Ridley is mainly restricted in range to the Gulf of Mexico and the North Atlantic. The Flatback turtle species is endemic to the Australian continental shelf. Green turtles are widely distributed in the tropical to temperate waters of the Atlantic, Indian, and Pacific Oceans (Okamoto and Kamezaki, 2014). Hawksbill turtles are the most tropical of all sea turtles, whereas Leatherback turtles undertake extensive migrations between tropical, temperate to boreal waters and, sometimes, in polar waters (Meylan and Meylan, 1999).

Turtle foraging areas occurs over a wide geographic range, within 20°C of average sea surface temperature and on coastal areas, but each sea turtle's species use the habitat in a distinct strategy, specialised into different niches that provide a minimum of direct competition (Miller, 1997; Pritchard, 1976). The Leatherback turtle is the largest sea turtle species with a diet consisting of medusae and other gelatinous plankton (Hendrickson, 1980) in the open ocean. Still, they also feed closer to shore (Fritsches and Warrant, 2013). Hawksbill turtle most frequently occurs in hard-bottomed and reef habitats containing sponges (Hendrickson, 1980). Green Turtles inhabit both pelagic and neritic environments on clear coral reefs, algae, and seagrass, with routine dives to 20 m and shallower (Bass et al., 2006; Fritsches and Warrant, 2013). Loggerheads are carnivorous, regularly found on benthic environments, sandy reefs, and in shallow bays, ingesting a wide range of invertebrates such as crabs and molluscs (Bjorndal, 1997; Miller et al., 2003; Pritchard, 1976). Kemp's Ridley occurs in shallow bays with sandy and/or muddy bottoms with abundant crabs and molluscs (Musick and Limpus, 1997). Olive Ridley is one of the smallest species, and they feed both in deep water and pelagic habitats, ingesting tunicates, fish, molluscs and crustaceans (Bjorndal, 1997).

1.3. Life cycle

Sea turtle species share a common life cycle composed of a series of different stages (Fig. 2). Sea turtles have an oviparous reproductive strategy and thus depend on suitable terrestrial nesting environments (Brock et al., 2009; Hays, 2008; Miller et al., 2003). Therefore, sea turtles must begin their life on land (Fritsches and Warrant, 2013). As adult females, sea turtles dig a nest cavity on sandy, ocean-facing beaches (Heppell et al., 2002; Witherington, 1992). The hatchlings in an undisturbed nest appear at the surface about two months after the eggs were laid. Sea turtle hatchlings spend another 3-5 days in the nest absorbing their yolk, attached by an umbilical cord to their abdomen, to provide the energy they need (Miller et al., 2003). While they are still in the nest, hatchlings spend two or three days digging in, trying to get out of the nest, in a coordinated effort, gradually raising the sand until they get very close to the surface (Miller et al., 2003). Hatchlings always emerge from their nests at night, avoiding the high temperature of the sand (Bourgeois et al., 2009; Lohmann and Lohmann, 1996) and predators (Lohmann and Lohmann, 1996; Pritchard, 1976). Sea turtle hatchlings can perceive and respond to several environmental cues, including magnetic field intensity, magnetic inclination angle, visual cues, water temperature gradients, wave direction, and chemicals in the water (Lohmann et al., 2008; Lohmann and Lohmann, 1996).

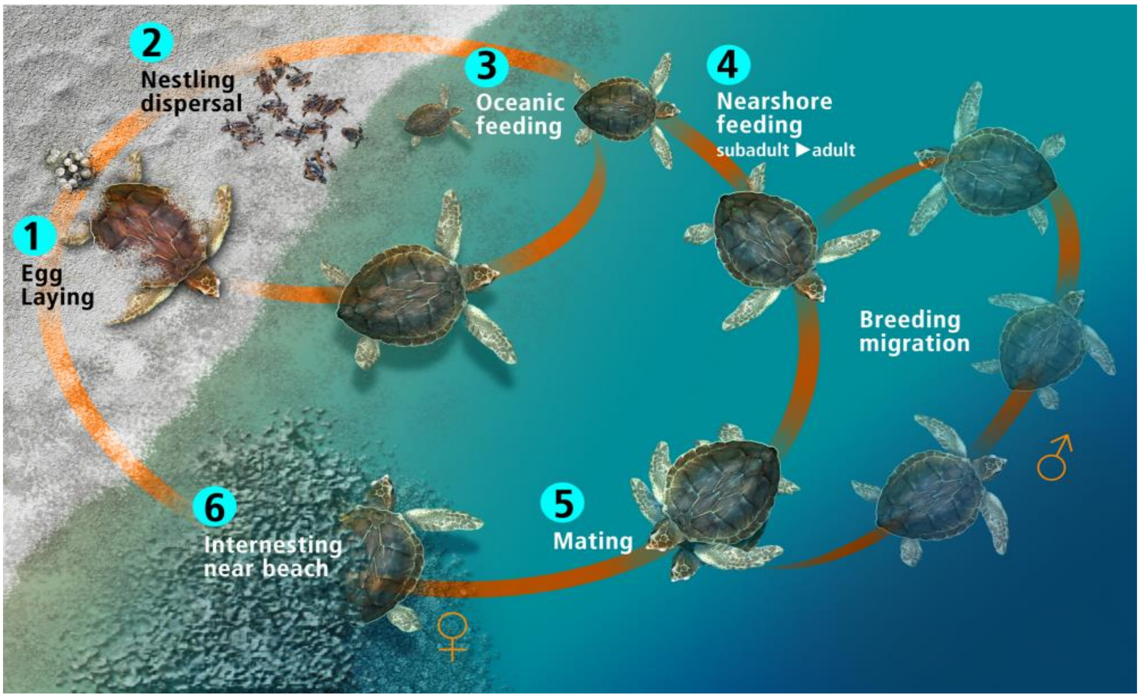

Figure 2. The general life cycle of sea turtles (Diagram by Kate Sweeney)

After leaving the nesting beach, the hatchlings begin an oceanic phase in oceanic gyre systems (Meylan and Meylan, 1999; Musick and Limpus, 1997). Their life stage after entering the ocean is the least understood, generally called "lost years" (Bass et al., 2006; Bjorndal et al., 2000; Bolten, 2003). Subsequently, after years the term hatchling is defined by the size or age, and it is named a juvenile or an "immature". The term subadult refers to the stage in which the turtle resides in neritic habitats (Musick and Limpus, 1997), and the transition from one phase to another varies depending on age and size (Bass et al., 2006; Musick and Limpus, 1997).

Once hatchlings emerge from their nests, there are differences between the areas of development and three patterns can be established: (1) distinguished by developing entirely within the neritic zone (Flatback turtle); (2) early oceanic juvenile stage and later neritic juvenile development (Green turtle, Hawksbill, Loggerhead and Kemp's Ridley) and (3) complete development in the oceanic zone (Leatherback and Olive Ridley) (Bolten, 2003).

Type 1 pattern, the turtle spends all life stages in the neritic zone (including post-hatchlings), feeding on the surface, and after increase the ability to dive, they can also develop a benthic feeding strategy (Bolten, 2003). Type 2 pattern, the individuals change their habitat during their development, taking up different feeding grounds during the juvenile stages, one in the oceanic zone followed by the neritic zone, other species switch from area depending on size. Loggerheads leave the neritic zone at approximately 46 cm, while Green Turtles and Hawksbill do it at ca. 20 cm (Bolten, 2003). Type 3 pattern consists of turtles that spend all stages at the oceanic zone (excluding post-hatchlings). However, the Olive Ridley turtles show either a Type 2 or a Type 3 pattern, they may change patterns depending on the availability of resources (Bolten, 2003).

1.4. Reproduction cycles

Sexual maturity is reached at different ages and sizes and varies between species and individuals of the same species (Hirth, 1997). Upon reaching maturity, male and female sea turtles develop different sexual characteristics. Adult males develop secondary sexual characteristics, a large and muscular prehensile tail that extends well beyond the carapace. Immature or young males may not have yet developed long tails and could therefore be mistaken as small adult females (Wibbels, 1999).

Before mating, sea turtles accumulate energy in their foraging areas (Miller et al., 2003). The males require lower levels of fat deposition for breeding and may breed every year. Meanwhile, females accumulate energy reserves to support vitellogenesis and other reproductive efforts (such as migration to breeding beaches, oviposition) over several years depending on the habitat (Miller et al., 2003). Females show strong site fidelity to courtship and foraging areas, while males may travel considerable distances searching for potential mates (Hamann et al., 2003; Miller, 1997). After mating, the females migrate to the inter-nesting areas near the nesting beach (Miller, 1997). The reproductive phase varies depending on physiological factors (such as age, weight, health, and reproductive history) and exogenous factors (e.g., migratory distance, available resources at the foraging area).

1.5. Nest activity and process

The general nesting process is essentially the same in all species of sea turtles (Witherington and Martin, 2003). First, the females choose a dry sand area out of reach of the highest tide (Witherington and Martin, 2003). Then, the process consists of a succession of steps: 1) emerge from the ocean, 2) select a nesting site by digging a body pit, 3) dig an egg chamber within the body pit, 4) deposit eggs inside of the egg chamber, 5) cover the eggs using rear flippers, 6) camouflage the nesting site, and 7) make a return crawl to the ocean (Fig. 3) (Witherington et al., 2011). Sometimes, a female turtle does not successfully dig a nest chamber within the body pit, possibly due to the sand's quality, texture, temperature or any other disturbance. Whatever the reason(s) for aborting the nesting attempt, the turtle usually returns to the nest the same night or the following night and, frequently, returns to the same beach (Miller, 1997).

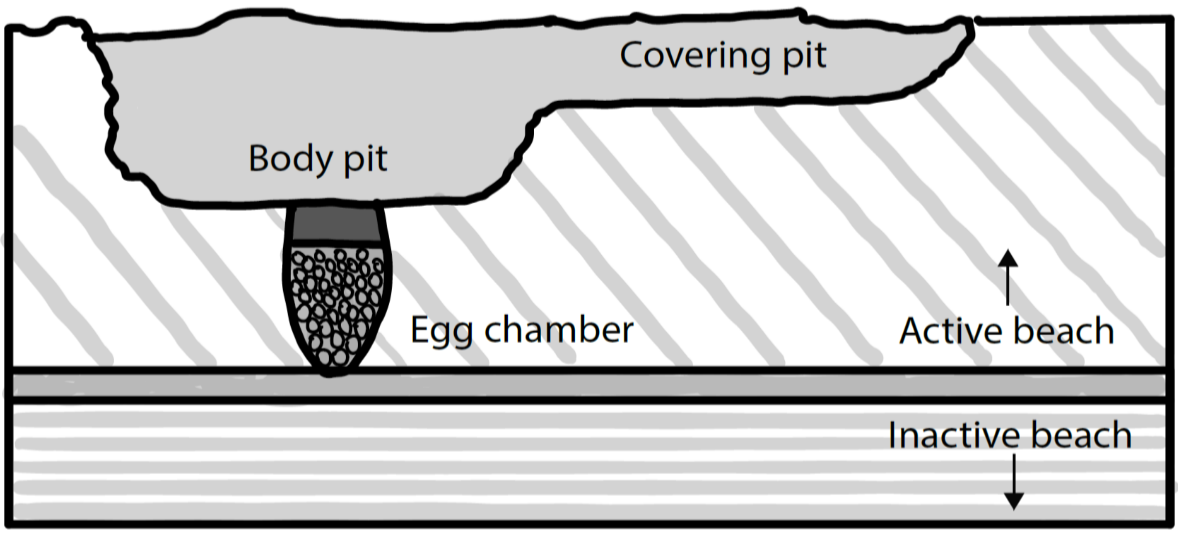

Figure 3. Diagram of egg chamber of sea turtles (adapted from http://www.scistp.org/nests/morphology.php).

While nesting, sea turtles release viscous "tears" from their eyes, which helps eliminate excess salt from their body and keep the eyes free of sand (Meylan and Meylan, 1999). The eggs are dropped in batches of between one and four, and when laid, are surrounded by thick clear mucus. The number of eggs laid varies from species to species and sometimes from one population to another; in many areas, the average clutch has between 100 and 130 eggs (Martín-del-Campo and García-Gasca, 2019). When oviposition is complete, the nest chamber is filled with scraping moist sand near the opening, with alternate movements of the hind flippers (Miller, 1997). At the end of the process, the nest site should be at least one meter behind the turtle and the body pit far from the eggs. This behaviour is interpreted to be a camouflage of the nest, presumably from predators. The amount of time required to complete the nesting process varies among the species and can vary from 1 to 3 hours (Miller et al., 2003).

As soon as the turtles emerge from the sea into land, the identification of the species can be made by their track markings since the style of the gait, and the width of the track provides a clear indication of the species that have attempted to nest. In a symmetrical track, the front flippers move synchronously used by Green Turtle, Flatback, Leatherback (Eckert et al., 1999). And an asymmetrical track, only one front flipper moves at a time used by Olive Ridley, Kemp's Ridley, Hawksbill and Loggerhead (Miller et al., 2003). To avoid lethal temperatures during the day, most females prefer nesting at night (Witherington and Martin, 2003), although Kemp's Ridley and Flatback turtle may nest during the day (Meylan and Meylan, 1999; Witt et al., 2009). In some regions, the genus *Lepidochelys* exhibit mass nesting in which groups of 100 to 10,000 or more females emerge simultaneously to lay eggs, an event called "arribada" (Hamann et al., 2003).

1.5.1. Nest site selection and environment

Adult females exhibit a substantial degree of fidelity to return to the area of beach at which they were hatched, called "natal homing". This behaviour was confirmed after obtaining mitochondrial DNA (mtDNA) sequences from females of control regions, where the results revealed an intra- and intergroup variation (Allard et al., 1994). This fidelity to return to the same beach is not exact, the explanation for this ability is that the turtles imprint the geomagnetic signature of their natal beach as a hatchling and use this information to return as adults (Brothers and Lohmann, 2015).

Upon arrival at their breeding grounds, the females choose a specific site on a beach to nest. The mechanisms by which a nesting site will be selected are poorly understood (Mortimer, 1995). Turtle species share distinct nesting requirements; however, there is inter- and intraspecific variation in preference in terms of specific physical features of a beach, such as width, slope, orientation, and vegetation (Garmestani et al., 2000; Karavas et al., 2005; Witherington and Martin, 2003). For example, Leatherback prefers deep and open underwater access and a relatively steep (often windward) beach profile; Hawksbill usually chooses shallow, coral strewn habitats with dense vegetation and low-profile beaches (Karavas et al., 2005). Olive Ridley nesting areas take place on beaches near coastal lagoons or estuaries (Diez and Ottenwalder, 1999).

Several studies have shown that nest location and the nesting season can have an essential influence on hatchlings (Wibbels et al., 2003), including emergence success, sex ratio, size and vulnerability to nest predators (Miller et al., 2003; Sarahaizad et al.,2012). When the nest is close to the waterline, increased the risk from inundation or egg loss for erosion, when close to the vegetation, the threat of invasion of the roots, and hatchling misorientation (Forley et al,.2006; Wood et al., 2000). Nests laid under dense vegetation are likely to be cooler and thus more likely to produce more males than those laid in the open zone, which is expected to be warmer and thus produce more females (Godfrey and Mrosovsky, 1999).

2. Species of interest

2.1. Morphology

Green sea turtles (*Chelonia mydas* Linnaeus, 1758) are the largest of the hard-shelled species. The name originates from the green colour of the fat located beneath its carapace and not the colour of its carapace. The colour of the carapace of this species is extremely variable. It can vary even for turtles of the same size and from the same population except in the hatchling stage, in which they have dark blue-black (Hirth, 1997). As the turtle grows, it is sometimes shaded with olive, pale to dark greens or yellow with large blotches of dark brown (Fig. 4) (Pritchard, 1976; Seminoff et al., 2015, Hirth, 1997). The plastron is yellowish in adults and is pure white in hatchlings (Hirth, 1997). The adult curved carapace length (CCL) is on average 1 m and weighs 200 kg (Hirth, 1997). The largest specimen known is from Suriname with 1.25 m curved carapace length (CCL) and weighed 385 kg (Pritchard, 1976).

Figure 4. Juveniles of Green turtle (*Chelonia mydas*) in São Tomé Island. (Photo by Cristina Bandeira).

2.2. Distribution and habitat

Green turtles are widely distributed around the world (Domiciano et al., 2017). These species nest in over 80 countries and live in the coastal areas of more than 140 countries (Fig. 5) (Seminoff et al., 2015).

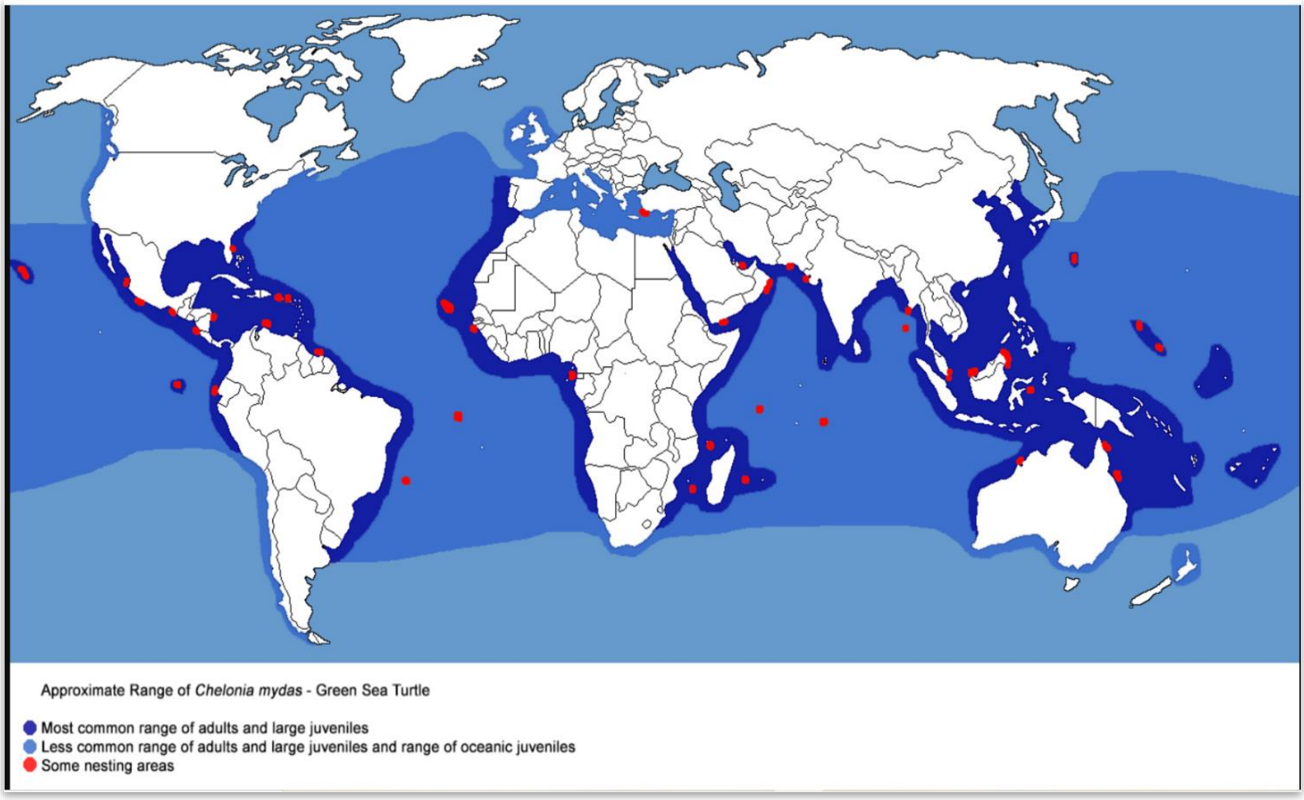

Figure 5. Distribution map of Green turtles around the world (*Chelonia mydas*) (From http://www.californiaherps.com/turtles/pages/c.mydas.html)

It is common for them to stay near the coastline and around islands, living in bays and protected shores, especially in areas with seagrass beds, primarily of tropical, subtropical and temperate regions of the Atlantic, Pacific, and Indian Oceans, they are rarely observed in the open ocean (Eckert et al., 1999; Hirth, 1997; Seminoff et al., 2015). In addition to coastal foraging areas, oceanic habitats are used by oceanic-stage juveniles, migrating adults. Despite these habitat uses by green turtles, much remains to be learned about how oceanography affects juvenile survival, adult migration, and prey availability (Musick and Limpus, 1997).

2.3. Life Cycle and Diet

Upon leaving the nesting beach and entering the marine environment, post-hatchling green turtles begin an oceanic juvenile phase during which time they are presumed to primarily inhabit areas where surface waters converge to form local downwelling, resulting in linear accumulations of floating material (Musick and Limpus, 1997; Seminoff et al., 2015). Food items documented for a limited number of stranded post-hatchling green turtles have included predominantly *Sargassum sp.* and associated hydroids, bryozoans, polychaetes, gastropods, as well as cnidarians and other pelagic invertebrates, fish eggs, and debris. In the eastern Pacific Ocean, Green turtles forage a larger proportion of invertebrate foods, with omnivorous diets reported in turtles throughout the region (Bjorndal, 1997; Musick and Limpus, 1997; Seminoff et al., 2015).

Oceanic-stage juvenile green turtles appear to use oceanic developmental habitats and move with the predominant ocean gyres for several years before inhabiting the neritic foraging and nesting habitats (Bass et al., 2006; Bjorndal, 1997). After entering the neritic habitat, the juvenile shift from omnivore to primarily herbivorous, foraging on seagrasses and/or marine algae, although some populations appear to forage heavily on invertebrates (Gorga, 2011; Musick and Limpus, 1997; Pritchard, 1976).

2.4. Nesting and Egg Development

The nesting process of the Green turtle does not vary significantly from one part of the world to another. Females migrate from foraging areas to their natal beaches every 2–4 years and show a high degree of nest site fidelity (Seminoff et al., 2015). The average ovipositing is three clutches (Miller, 1997) at 10- to 17-day intervals and remain near the nesting beach during the inter-nesting period (Hirth, 1997). Mean laid eggs per season varies greatly among Green turtle populations, but on average is approximately 550 eggs season (Hirth, 1997).

Studies of beach selection of this species described that Green turtle tends to prefer a beach with clean access to the offshore (Wood et al., 2000), choosing an area of the beach with vegetation behind the open sand (Wood et al., 2000)

3. Sea turtle conservation and threats

The conservation of marine biodiversity is recognized as a critical need (Bjorndal et al., 2000). Because they spend discrete portions of their life in a variety of marine habitats, they are vulnerable at multiple life stages: as eggs on the beach, as hatchlings in the open ocean gyres, as juveniles in nearshore waters, and as adults migrating between feeding and nesting grounds (Milton and Lutz, 2003).

Around the world, the survival of the sea turtle population reflects the effects of natural and anthropogenic stressors that include: (1) environmental variability (salinity, pollution, temperature), (2) physiological factors (hypoxia, acid-base imbalance, nutritional status), physical factors (trauma), and (3) biological factors (toxic blooms, parasite burden, disease), terrestrial habitat loss, terrestrial and aquatic habitat degradation, and direct and indirect fisheries (Eckert, 1999; Marcovaldi and Thomé, 1999). New threats include increases in egg incubation temperatures (further skewing sex ratios, which are defined by incubation temperature) caused by: (1) global warming, habitat loss due to increased coastal development (e.g., armouring, artificial lighting, and recreational activities) (Marcovaldi and Thomé, 1999; Wibbels, 1999), (2) accumulation of pollutants (e.g. plastics, heavy metals, environmental estrogen, and oil products in the pelagic nursery) and (3) demersal coastal habitats threatening juvenile and adult sea turtles of all species (Domiciano et al., 2017; Eckert, 1999).

As a result of these threats, all seven species of sea turtles are included on the IUCN Red List of Threatened Species <https://www.iucnredlist.org/>. Kemp's Ridley and the Hawksbill are considered Critically Endangered; the Green turtle is listed as Endangered; and Loggerhead, Olive Ridley's, Leatherbacks are considered Vulnerable and Flatbacks data deficient. Because of their precarious status, sea turtles have been afforded protection by local, state, provincial, and national laws and by international treaties.

3.1. Challenges in marine turtle conservation in São Tomé and Príncipe

Four species regularly nest on São Tomé and Príncipe, the Green (*C. mydas*), Olive Ridley (*L. olivacea*), Hawksbill (*E. imbricata*) and Leatherback (*D. coriacea*), and green and hawksbill juveniles inhabit the foraging areas around the island. A fifth species, the Loggerhead (*C. caretta*) it can be also found sporadically at sea (Ferreira and Vieira, 2019).

The northern and eastern coastal regions host the largest human settlements, while the southern (especially southwestern) coastal development is smaller (Formia et al., 2003). The apparent lack of nesting on the western coast is likely due to the rocky substrate of the beaches stretching the length of the coastline.

São Tomé and Príncipe is an underdeveloped nation (Costa Alegre, 2009), and like many coastal nations, sea turtles are considered significant sources of food and income. Directed hunting of sea turtles as a survival livelihood contributes to an illegal trade network and is a significant threat to the conservation of sea turtles in São Tomé and Príncipe. From 2002 to 2003, the use of Hawksbill turtle carapace to manufacture handicrafts and jewellery was the most significant driver of the indiscriminate harvest and decline of this species (Graff, 1996; Ferreira and Vieira, 2019). The green turtle population on this archipelago were classified as low risk, however, there are several threats to their population (Wallace et al. 2011) (Fig. 6)

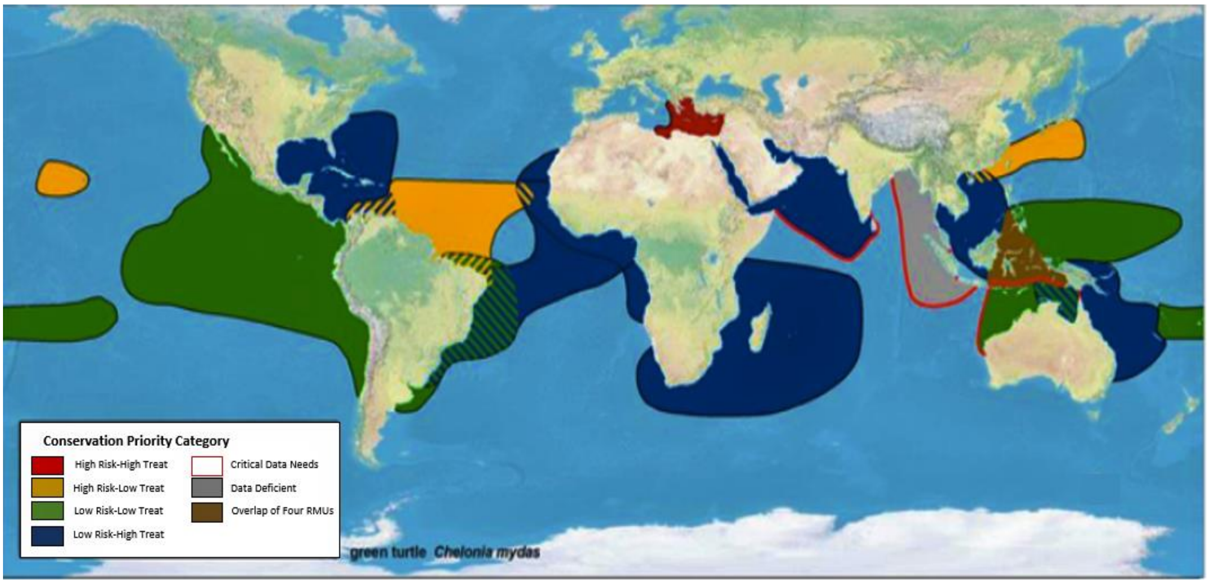

Figure 6. Regional Management Units of green turtles' population worldwide (from Wallace et al. 2011).

The first conservation initiatives in the country were in 1998, then efforts were made to create a law to protect sea turtles, but it was only in 2014 that a national law decree protecting sea turtles and criminalizing the consumptive use of sea turtles and their by-products in São Tomé and Príncipe was adopted (Law Decree nº 8/2014) (Formia et al., 2003; Vieira et al., 2016). But five years before the national law was officialised, the regional government of Príncipe, which has an administrative autonomy, implemented the law to protect sea turtles on the island (Law Decree nº 03/2009), which may have encouraged the national government to act (Ferreira and Vieira, 2019; Ferreira-Airaud et al. in press.). Moreover, directs threats to sea turtles still include hunting by fishermen through artisanal fisheries or exploited meat and eggs from adult females during the nesting season; indirect threats include sand extraction for construction work, both on-site and offshore, that affects the availability of sand on several beaches on São Tomé, especially on the northern shore (Hancock, 2020; Vieira et al., 2016; Ferreira and Vieira, 2019). Also, along the coast, it is possible to observe the growth of buildings related to ecotourism and an increase in disturbances on the nesting beaches. The growth in this sector can aggravate threats to coastal environments and biodiversity. Among the most obvious results of building development along the coast are physical barriers and the increase of artificial light (Varela-Acevedo et al., 2009). Nowadays, sea turtle research and conservation in São Tomé Island is developed by the NGO Programa Tatô, created in 1998. This NGO combines research, ecological monitoring, protection of critical sites, environmental education, advocacy, community-based ecotourism, reconversion of former poachers and traders, and development of alternative livelihoods (Ferreira-Airaud et al. in press; Ferreira and Vieira, 2019; Vieira et al., 2016).

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CHAPTER 2: Nesting habitat preferences and hatching success of green turtles, *Chelonia mydas*, in São Tomé Island, West Africa

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Abstract

Sea turtles deposit their eggs on the sandy beaches, where they develop without parental care, and the survival and success of the hatchings depend on environmental conditions. We describe the nesting habitat preference for the green turtles (*Chelonia mydas*) and its impact on the hatching success at Jalé beach, in the south of São Tomé island. We recorded the spatial distribution of 315 emergences and monitored 50 nests during the oviposition. Overall, green turtles tended to emerge on zones of the beach with an accessible offshore approach, relatively free of rocks, particularly during the neap tides. The females tended to nest between the vegetation line and the open beach, establishing a minimum distance above the high tide line. The hatching success was not significantly different between the microhabitats. This study allowed us to identify the beach characteristics that affect the nesting process, understand how threats can affect the female nesting and the best way to minimize the future impacts.

Keywords: Green turtles, São Tomé, nest site selection, hatching success

1. Introduction

The key to the successful conservation of vulnerable species begins with understanding current populations status, predicting the viability of wild populations, and their connection with the environment (Ceriani et al., 2019; de Witt, 2014; Roark et al., 2009). Population monitoring is complicated for highly migratory species like sea turtles due to their extensive distribution and migratory nature (Ceriani et al., 2019; Eckert et al., 1999).

Sea turtles are considered one of the most fascinating migratory groups of animals. Most sea turtles' species have a circumglobally distribution across tropical and subtropical waters (Jensen et al., 2013); some species can be found on the artic circle (Meylan and Meylan, 1999). All sea turtles appear to exhibit migratory behaviour (Meylan and Meylan, 1999), between foraging grounds and nesting beaches (Eckert, 1999; FitzSimmons et al., 1997), creating a complex network of migrations routes (Jensen et al., 2013), spending most of their life in water (Matsuzawa et al., 2002), but they do spend a significant proportion of their time on the surface to breath (Fritsches and Warrant, 2013)

As oviparous reptiles, all sea turtle species need sandy beaches to successfully incubate their eggs (Wood and Bjorndal, 2000; Zavaleta-Lizárraga et al.,2013). Females emerge in the same geographic area where they emerged as hatchlings for laying eggs (Brock et al., 2009; Karavas et al., 2005; Serafini et al., 2009). When female sea turtles emerge at the beach, they generally enter in a mixed habitat (Bjorndal and Bolten, 1992), surrounded by a variety of environments (Patrício et al., 2018; Wood and Bjorndal, 2000). The nest site locations represent a crucial aspect of their reproductive biology (Bjorndal and Bolten, 1992; Hays and Speakman, 1993). The nesting location chosen by a female sea turtle is influenced by biotic and abiotic factors which can (1) reduce the viability of the embryos, (2) prevent the eggs from successfully hatching (Bjorndal and Bolten, 1992; Serafini et al., 2009; Spencer, 2002), and (3) influence its survival (Bjorndal and Bolten, 1992; Sampaio, 2018; Serafini et al., 2009; Wang and Cheng, 1999).

However, the mechanisms the female sea turtle uses to decide which specific beach or site on a beach to nest (Wood and Bjorndal, 2000), is still not fully understood (Heithaus, 2013; Wang and Cheng, 1999). Species-specific differences occur in parameters such as nesting strategy (aggregated vs. solitary), size at first reproduction, average clutch size, details of the nest size and construction (Eckert, 1999). Nest site selection may vary at an inter-specific and intra-specific level (Domiciano et al., 2017; Fish et al., 2005), including several physical features such as beach profile topography (e.g., beach width, length and slope) (Fish et al., 2005), the presence (or absence) of beach vegetation usually beyond the beach (Meylan and Meylan, 1999; Serafini et al., 2009; Wang and Cheng, 1999; Wood and Bjorndal, 2000), the structural properties of the sand (e.g. compressibility, grain size, sand colour and organic content) (Karavas et al., 2005) also by anthropogenic factors such as light pollution and human disturbance (Silva et al., 2020; Wang and Cheng, 1999).

The dynamic of the coastal areas turn this environment unpredictable for an optimal hatching success (Karavas et al., 2005), and the consequences of a non-ideal nest placement imply nest loss by tidal inundation or erosion when placed at a short distance from the sea (Kamel and Mrosovsky, 2004; Roark et al., 2009). While near the vegetation, the egg chambers are less vulnerable to collapse compared to the open beach, the roots can penetrate the egg chamber and rot the eggs or create deformities in the hatchlings and hatchling misorientation (Bjorndal and Bolten, 1992; Wang and Cheng, 1999; Wood and Bjorndal, 2000). For example, Green turtles (*Chelonia mydas*) preferred a beach with easy offshore access and nest above the high spring tide near the vegetation. On the other hand, studies on hawksbills (*Eretmochelys imbricata*) have detected a more selective nesting behaviour, with individual females systematically nesting far away from the high tide line and closer to vegetation (Pfaller et al., 2009; Serafini et al., 2009).

The hatching success is the proportion of eggs from which hatchlings emerge in the nest chamber, and the emergence success is the proportion of hatchlings emerging from the nest chamber and reaching the beach's surface (Zárate et al., 2013). The sand characteristics interact with the hatching and emergence success: temperature, moisture, sand structure and composition and salinity can affect embryonic development by altering nest conditions. For example, increased salinity within the nest environment decreases water availability and consequently egg desiccation (Veelenturf et al., 2021); high nest temperatures can reduce emergence success by limiting the ability of hatchlings to reach the surface (Matsuzawa et al., 2002), also influencing sex determination, wherein higher incubation temperatures produce more female, whereas lower incubation temperatures produce more males (Mortimer, 1990; Wang and Cheng, 1999)

All sea turtles are threatened with extinction. Green turtles have been heavily affected by human exploitation and, globally, are considered ‘Endangered’ and listed by the International Union for Conservation of Nature (IUCN). In São Tomé, according to Programa Tatô, the sea turtle conservation program, the number of the successful Green turtle nests vary across the coastal zone, with an annual variation between 49 to 1,177 nests per year in São Tomé only, with a greater incidence in the south, at Jalé beach (Ferreira-Airaud et al., in press). Despite being one of the areas of the country with the highest incidence of turtles, few studies have been carried out in this area. And with the increase of tourist facilities along the coastal zone of this beach, it becomes of great importance to assess the distribution of green turtles nesting in Jalé beach and how the pressure of human disturbance influences the distribution of the nests.

In this study, we analysed the nesting habitat preferences of Green turtles on the beach with the highest nest density for the species in the south of São Tomé island, Jalé Beach. The study's main objectives were: (1) to identify microhabitat variables associated with higher nest density and female emergence within a nesting area. (2) to evaluate the importance of specific habitat features, such as offshore bathymetry, beach extent, on sea turtle nest locations. (3) to determine the susceptibility of nesting events by human disturbance (presence of light, noises, etc.) under different beach conditions.

Following the set objectives, the following hypotheses will be tested:

Hypothesis 1: If nesting sites' choice occurs randomly, the microhabitat characteristics will not influence the number of nests present. The expectation is that different microhabitat characteristics will bear some influence in the selection of nest locations.

Hypothesis 2: If hypothesis 1 is rejected (microhabitat characteristics have relevance in nest locations choice), we postulate that locations with advantageous features will have more nests. This hypothesis allows us to determine the relative relevance of microhabitat characteristics.

Hypothesis 3: The more human disturbances in a location, the less probable is the establishment of nests. The expectation is that instabilities in the habitat will drive away from the turtles to more calm and undisturbed places.

2. Materials and methods

2.1. Study area

This study was conducted on Jalé Beach, south of São Tomé Island (Fig. 1). São Tomé is the main island part of an archipelago nation located in the Gulf of Guinea (Costa Alegre, 2009). The island has an area of 960 km^2 with a marine sovereignty zone of 1600km^2, made primarily of basalt, rise from a seabed platform formed by volcanic action, and a coastal zone characterized by rocky cliffs, estuaries, coral reefs, mangrove, and islets (Costa Alegre, 2009). São Tomé has a humid tropical climate, with rainfall seasons between October and May, with an exceptional period where the temperature and precipitation decrease, corresponding to the "gravana" (Costa Alegre, 2009; Giardino et al., 2011). Jalé Beach has 1400 m long and is characterized by coconut palms (*Cocos nucifera*), beach almonds (*Terminalia catappa*). Four species of sea turtles' nest on Jalé beach, the Green turtle, the Leatherback, the Hawksbill and sporadic emergences of the Olive Ridley. On Jalé beach, there is touristic activity due with the communitarian lodge Jalé Ecolodge, housing one restaurant and three bungalows and a further ecolodge under construction with four bungalows.

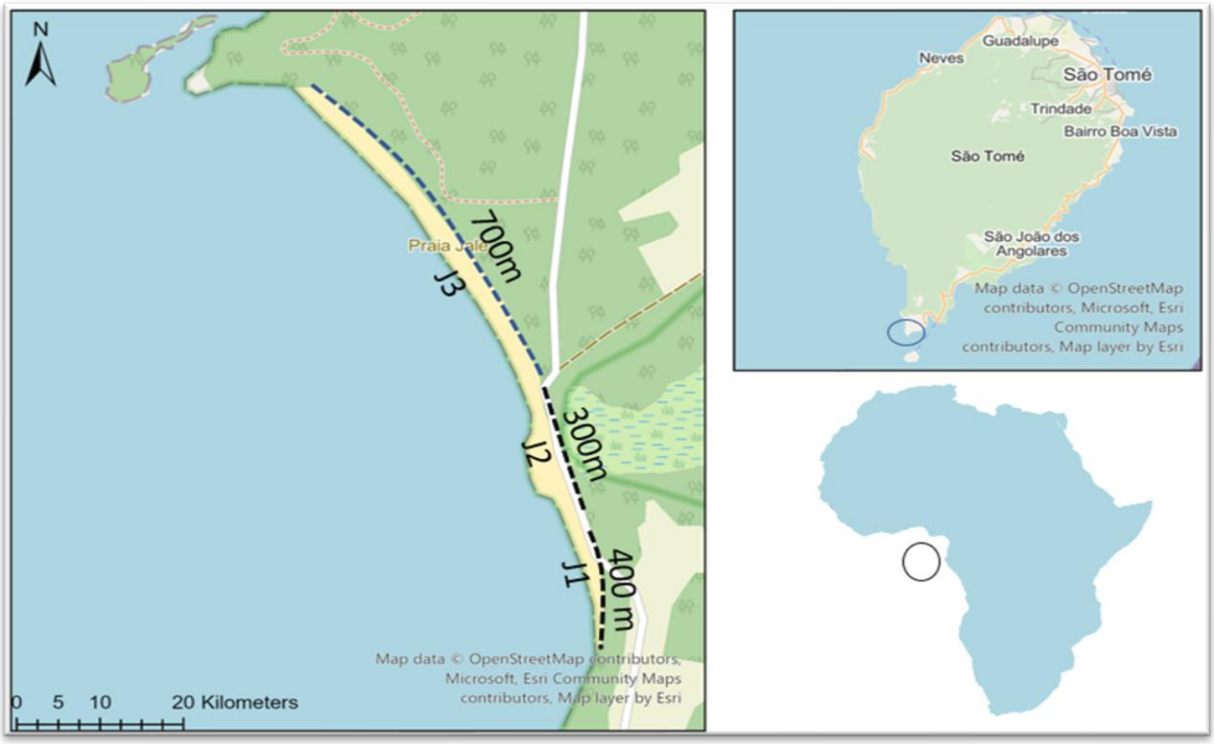

Figure 1. Locations of Jalé beach (0° 2'24.61"N, 6°30'44.76"E), south of São Tomé. The nesting beach is divided into three sections: J1-Jalé 1; J2-Jalé 2; J3-Jalé 3. Black circles represent the bungalows distributed along the beach.

2.2. Nest monitoring.

The Green turtle nesting season in São Tomé island occurs every year, between July and April, with a nesting peak from December to January (Ferreira and Vieira, 2019). Fieldwork was carried out on Jalé Beach from September to January to study sea turtle nesting activity distribution throughout the beach and characterise the nesting habitat. The monitoring was divided into night patrol, starting between 6 pm until midnight, walking near the high tide line, checking each track to confirm the presence of turtles on the beach. When a nesting turtle was encountered, morphometric data and environmental variables were recorded. Only females that were laying, covering or camouflaging nests were counted. The curved carapace length (CCL) was measured from the notch at the anterior of the carapace to the tip of the last posterior marginal scute using a flexible measuring tape.

2.3. Beach characterization.

The study area was divided into three different zones: (1) Jalé 1 (400 m) south zone of the beach, characterized by dense vegetation, a steep slope, and with rocky segments; (2) Jalé 2 (300 m) zone composed of bungalows, with less dense vegetation; and (3) Jalé 3 (700 m) north zone of the beach with a large density of vegetation, but concentrated in rock walls that delimit the beach (Fig. 1). Along the beach width, the distribution of the nests was classified according to three microhabitats: “vegetation”, “vegetation border”, and “open beach” (Fig. 2). The “vegetation” habitat encompassed all the beach with a densely vegetated area, consisting primarily of high and dense trees above the beach berm, shaded throughout most of the day. The “vegetation border” is near to the vegetation and experienced partial shade. “Open beach” is characterized by open sand area, from >1m of vegetation to the high tide line, with no vegetative cover or perhaps some sparse beach grasses and exposed to the sun throughout all day long, also characterized by formations of beach scarp.

A total of seven variables were assessed: (1) bathymetry (from a previously existing map provided by Programa Tatô), (2) coastal habitat; (3) effects of tides cycles, we used the website Hidrografico, to obtain tide height in each night ; (4) moon phases (± three days from the date of the first quarter, full moon, last quarter or new moon, giving eight days per quarter); (5) distance from vegetation (DV), defined as the distance from the nest to the line of vegetation; (6) distance from current high tide line (DW) which is the distance from the nest to the current waterline at the moment of egg-laying (7) presence or absence of human construction (considering presence or absence of artificial light or any other perturbation during nesting activity).

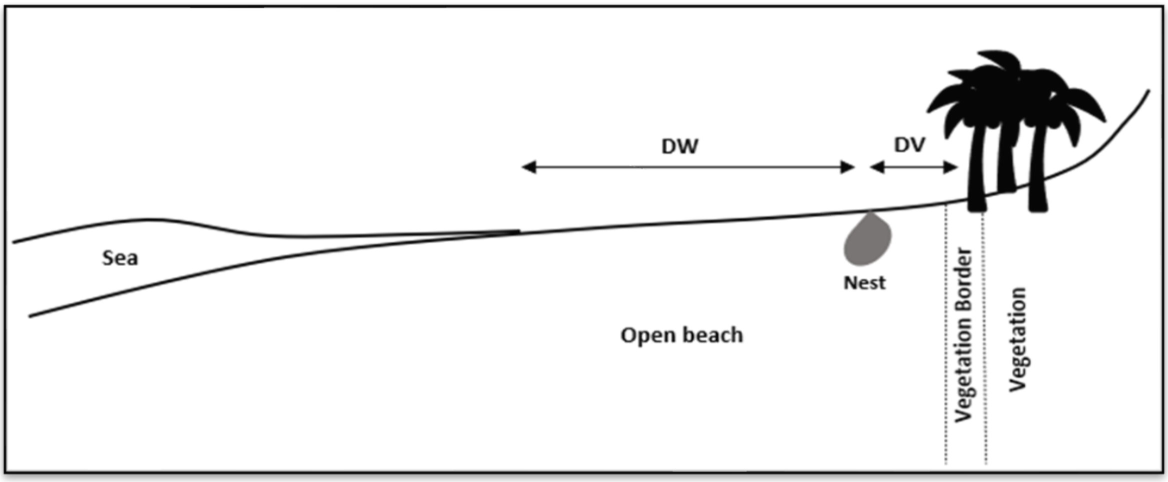

Figure 2. Schematic of the microhabitat classification at Jalé beach. Distance from current high tide line (DW), distance from vegetation line (DV).

2.4. Hatching success

The following morning, after hatchlings emerged during the night, the number of empty eggshells, unhatched eggs, and dead and living hatchlings remained in the egg chamber was determined. Hatching success was defined as the number of hatched eggs divided by the total number of eggs.

2.5. Data analyses

A chi-square test of independence was used to test the effects of the moon phases (New Moon, Full Moon, Quarter Moon) whether or not each one affected the number of successful nesting and false crawls. A chi-square test of independence was used to test the effect of beach microhabitats (vegetation border, vegetation, open beach) and if the tides' cycle affected the location of the monitored nests (n=50). To test the effect of microhabitat type and sun exposure on the hatching success and incubation period, a one-way ANOVAs was run with two levels of microhabitats (vegetation border, open beach) and of exposure (sun, sheltered) as fixed factors and either hatching success or incubation period as the dependent factor. The vegetation location was not included because limited sample. Assumptions were tested using Shapiro's and Levene's tests for normality and homoscedasticity, respectively. A Spearman rank correlation was used to test the degree of association between hatching success and distance to either high tide line or vegetation.

3. Results

3.1. Nesting activities

In Jalé beach, south of São Tomé Island, 632 emergences (41% of the total Green turtle emergences) were recorded during the nesting season, from which 46% (n=288) resulted in successful nests and 54% (n=344) resulted in false crawls.

The survey conducted in Praia Jalé, the objective of this present study, lasted 99 days, from the 28th of September until the 5th of January. During the survey, 315 emergences were recorded from which 42.8 % resulted in successful nesting events (n=135) and 57.1 % resulted in false crawls (n=180), with an average of 1.3 nests per night (ranging from 0 to 7). Out of the 135 successful nesting events, 50 nests were analysed (36%) until hatchlings emerged from the nest and were also used to assess the microhabitat preferences.

3.2. Effects of moon phases

Out of 315 observed emergences, 20,2% (n=64) occurred during the full moon, 12,9% (n=41) during the new moon and 66,7% (n=210) during the quarter of the moon (34,1 %, n=108 corresponding to the first quarter and 32.5%, n=102 corresponding to the last quarter) (Fig.3). There was a significant correlation between the phases of the moon with successful nesting or false crawls (χ (2) = 9.4279; p = 0.024).

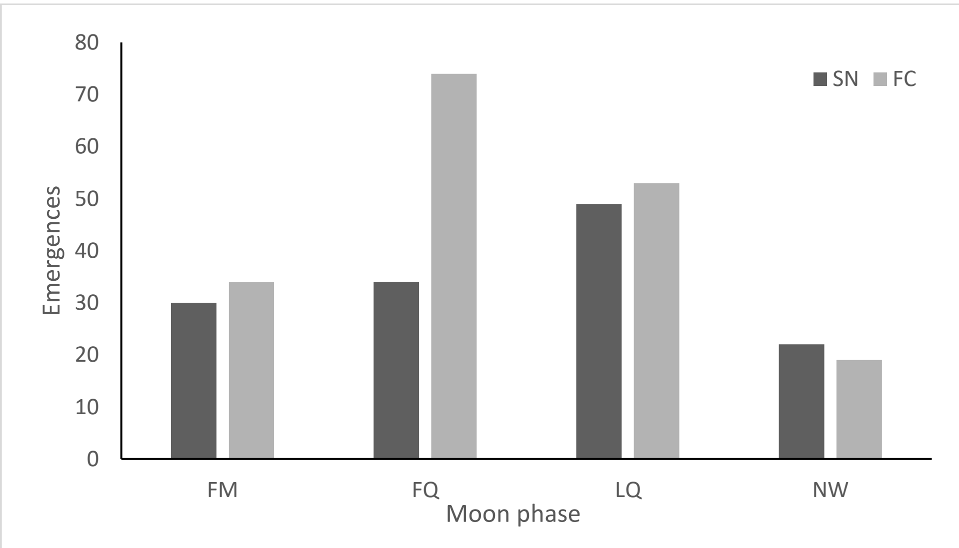

Figure 3. Green turtle (*Chelonia mydas*) emergences on the beach in relation with the moon phases, distinguished between SN: successful nesting; UN: unsuccessful nesting / false crawl. NW: New Moon; LQ: Last Quarter; FQ: First Quarter; FM: Full Moon.

3.3. Spatial distribution.

During the survey period, 135 of the emergences resulted in successful nesting (Fig 4). The nesting activities were significantly different between beaches (χ (2) = 22.711, p > 0.001). In the area of Jalé 1 (length 400 m) it was registered 14% (n=19) of successful nests, a total of 0.05 nests per meter, while in Jalé 2 (length 300 m) occurred 41% (n=56) of the successful nests, 0.19 nests per meters; and in Jalé 3 (length 700 m) occurred 44% (n=60), 0.08 nests per meter.

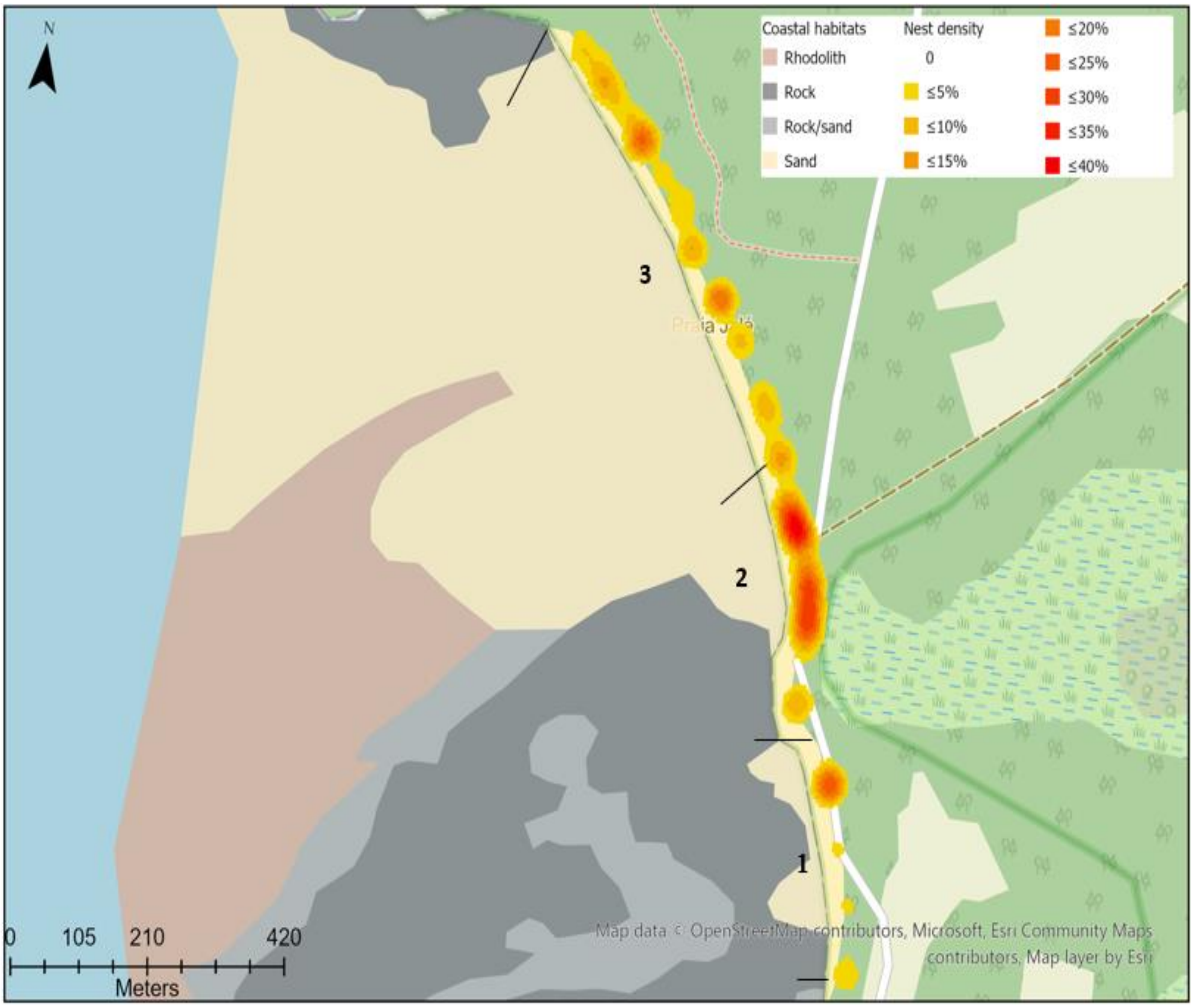

Figure 4. Kernel nest density (ArcGIS) successful Green turtle (*Chelonia mydas*) nesting events on Jalé beach during the surveys (n= 135).

3.4. Microhabitat preferences and tides.

From the 135 nests that occurred on Jalé Beach, 50 nests were used to analyse the beach microhabitat preferences. Of these, 52% (n=26) were found at the vegetation border, 46% on the open sand (n=23), and only one nesting event occurred in the vegetation (2%) (Fig.5a). There was no significant correlation between nesting events and microhabitat preference and tides (Neap or spring; χ (2) = 1.4295, df = 2, p-value = 0.4893).

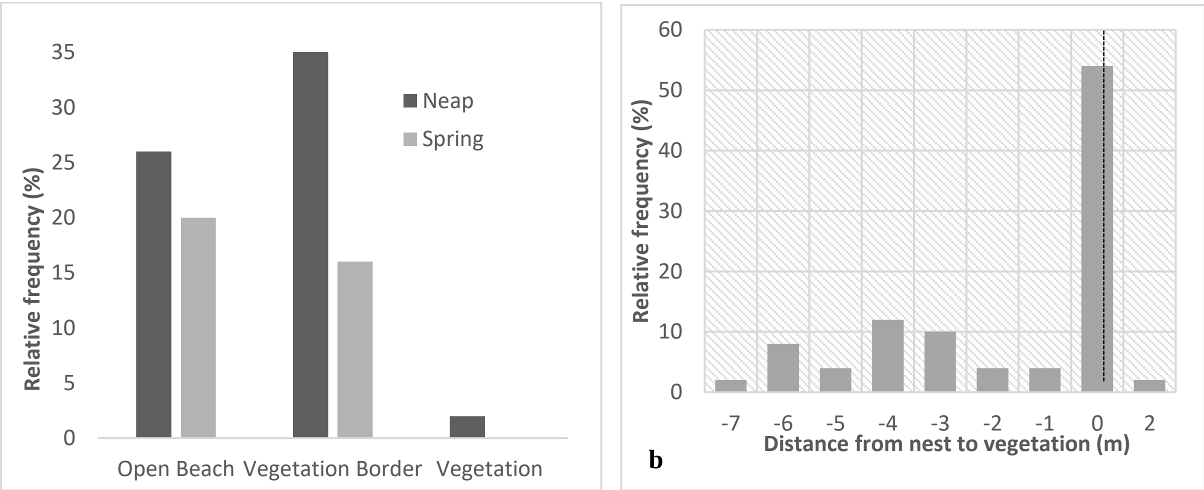

Figure 5. a) Distribution of the nests in different zones along the beach profile in relation to the tidal cycle, b) frequency distribution of nest-to-vegetation distance in Jalé Beach for Green turtles.

The minimum distance from the nest at the open beach to the vegetation border was 1 m, and the maximum was 7 m while the average was 2 m (Fig 5b). Most of the nests, 52% (n=26), occurred at 0 m (vegetation border) and the nest on the vegetation was 2 m from the vegetation border.

The minimum distance of the nests from the high tide line was 10 m (2%, n= 1), while the maximum was 47 m (4%, n=2), with an average of 20.3 m (Table 1). The highest frequency occurred at 18 m (14%, n=14). In all three sections of the beach, 92% of the nests were located near the high tide line between 10 and 30 meters.

Table 1. Nest location at each beach zone in relation to the distance from the high tide line

Location	Distance of nest to the high tide line			**Total**
	< 10m (%)	10-30m (%)	> 30m (%)	
Jalé 1	0	6 (12%)	0	6
Jalé 2	0	19 (38%)	1 (2%)	20
Jalé 3	1 (2%)	21 (42%)	2 (4%)	24
Total	1 (2%)	46 (92%)	3 (6%)	**50**

3.5. Habitat Features.

For each nest, its location was compared with habitat features such as bathymetry (distance from the coast to the 10 m isobath) and coastal habitat (sand, rhodolite, rocks): 1) on Jalé 1 (with only 19 successful nesting events from the 135), the mean of bathymetry was 570 m, and the coastal habitat is characterized by rocks; 2) on Jalé 2 the mean of bathymetry was 370 m, and the coastal habitat at this area is characterized half by stones and the other half with a sandy bottom; 3) on Jalé 3 the mean of bathymetry was 300 m, and the coastal habitat is predominantly characterized by a sandy bottom.

3.6. Hatching success.

The average hatching success of the successful nesting events was 88% (n= 44, SD=15.6, range 2-99). The average incubation period was 76 days (n=44, SD=10.4, range = 59-109). A total of 6 nests (from the 50 analysed) were predated, or the location of the nests on the beach was lost and was not included in these analyses.

The correlation between the incubation period and the hatching success is showed in Figure 6.

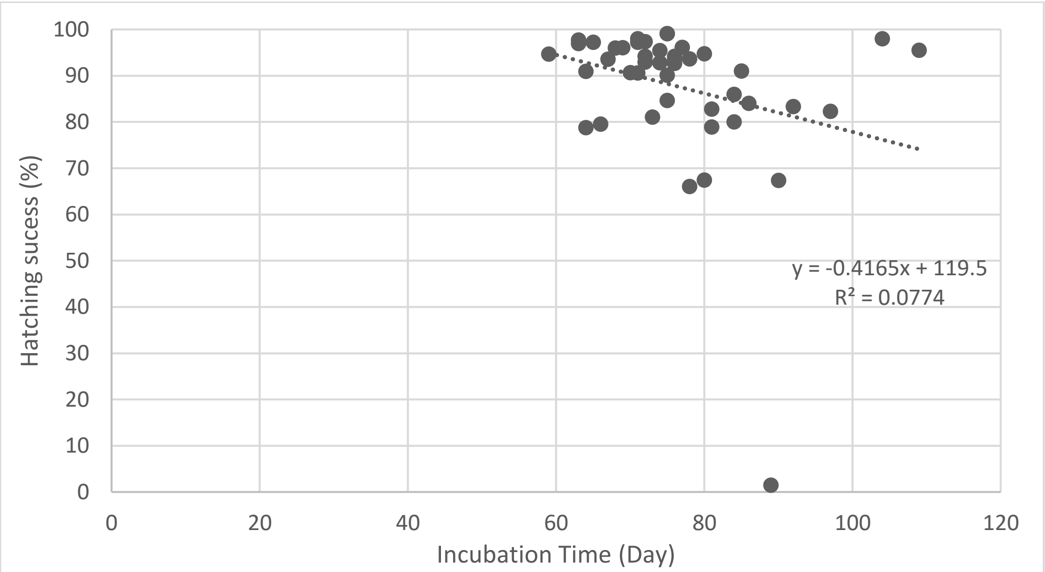

Figure 6. Correlation between hatching success (%) and incubation period (days).

The correlation between the nest location (vegetation, vegetation border and open beach) and the nest exposure (sun or shaded) was analysed with the hatching success and the incubation period. No significant effect of the nest location on the hatching success was found (one way-Anova, $p > 0.05$), as well as no significant impact on the incubation period (one way- Anova, $p > 0.05$).

No significant effect of the nest exposure on the hatching success was found (one-way Anova, $p > 0.05$) or with the incubation period (one-way Anova, $p > 0.05$).

Table 2. Hatching success and Incubation period at the different beach zones: vegetation, open beach, and border of vegetation.

Area	N. of nest	Mean hatching success (%)	Mean Incubation period (Day)
Vegetation	1	79	64
Vegetation border	25	91	76
Open beach	18	84	78

No correlation was found between the hatching success and the distance of the nest to the vegetation border (rho = 0.19, $p > 0.05$, n=50; Fig. 7a), as well as no correlation was found with the distance of the nest to the high tide line (rho = -0.27, $p > 0.05$, n = 50; Fig. 7b).

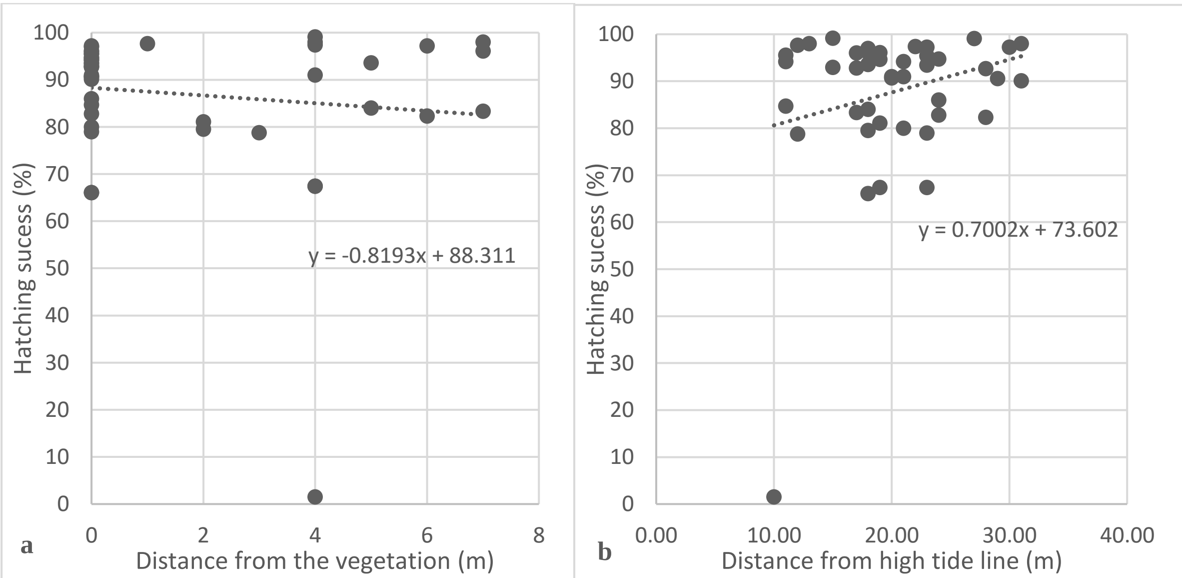

Figure 7. Regression between (a) hatching success and distance of the nest to the vegetation and (b) hatching success and distance to the high tide line.

4. Discussion

This study explored the nest distribution of the Green turtle at Jalé beach, south of São Tomé Island, one of the most important nesting beaches for the species in São Tomé Island. The objective of this study was to understand if the nesting process of this species has a pattern and to verify if there are environmental variables that influence this behaviour. The beach environment can be challenging for sea turtles to orient, avoid predators and guarantee a successful nesting (Wood and Bjorndal, 2000; Bjorndal and Bolten, 1992).

4.1. Effect of moon phases

To minimize the exposure to unfavourable environmental conditions and reduce the energetic and physiological stress, even before the female emerges on the beach, they may wait for the optimal environmental conditions such as moon phase or tidal patterns (Law et al., 2010; Azanza et al.,2003). Our results revealed a significant difference between the number of nesting events during different moon phases, showing that Green turtles prefer to nest during the quarter moon. Studies suggested that sea turtles' preference to emerge at a particular phase of the moon varies from region and could be related to the effect of moon illumination and the influence of the phase of the moon on the tide cycles (Law et al., 2010). Sea turtles may prefer to emerge during dark nights (New Moon) to avoid predators (Nakamura et al., 2019; Pinou et a., 2009), in which the moon illumination during the full moon may affect sea turtles' vision as well as the visibility of the nesting beach (Law et al. 2010), making the presence of scrubland, trees or cliffs, may discourage turtles from nesting (Ferreira 2012).

We believe that the preference of green turtles in Jalé beach to emerge around the moon quarter instead of at the new moon or full moon may be related to the amplitude of the tide (Nakamura et al., 2019). The first and last quarters have similar tidal patterns during the quarter, producing moderate tides known as neap tides. We hypothesize that the sea turtle preference emerges at smaller tide amplitudes to facilitate access of the Green turtles to the sandy beach (Azanza et al.,2003; Nakamura et al., 2019); however, more detailed data throughout a whole season may reveal different results.

4.2. Microhabitat preferences

In Jalé beach, Green turtle females showed no preferences for the open sand or the vegetation border. Still, a prevalence to nest above the high tide mark similar to Patricio et.,al (2018), although avoiding to nest in the vegetation which differs from other studies, where Green turtles have clear preferences for vegetation (Azanza et al., 2003; Turkozan et al., 2011) or shrubland/open sand (Ferreira 2012).

The absence of nests in the vegetation may be related to the difficulties of access. However, even when the female manages to approach these areas, the chances of finding unfavourable habitats are high (Serafini et al., 2009), such as the presence of rocks or roots (Wood and Bjorndal, 2000) and dry sand or more compact (Sarahaizad et al., 2012). However, these results should be interpreted cautiously: it could represent either a false pattern due to the low numbers of nests in vegetated areas or a true pattern.

The variation of the beach width during tide's circles did not significantly affect the nesting selection by Green turtles. The females place their nests within a minimum distance of 10 m from the high tide line. Therefore, most of the time crawled a long-distance and found an appropriate area to nest (Sarahaizad, 2012). Females may be looking for a nest site that will not be affected by the high tides (Wood and Bjorndal, 2000) or prefer a moist substrate to facilitate eggs chamber construction (Garmestani et al.,2000; Miller, 1997).

4.3. Habitat features

The differences found in the nest densities along Jalé beach showed that the preference to nest on a particular beach zone could be related to the variation in the habitat features between the three sections of the beach (Jalé 1, 2 and 3). Our results showed that Green turtles tended to nest on beaches with the deepest offshore approach to the beach (small distances from the coast to the 10 m isobath) (Hays et al. 1995), which differed from the results showed by Ferreira (2012) where Green turtles tended to nest in shallow offshore approach. In addition, Jalé 1 is characterized by shallow offshore approaches but has a lower nesting density, which may be related to the presence of rocks in all offshore approaches to this area. In comparison, zones free from rocks and with sandy offshore approaches had high nest density, suggesting that the depth of offshore entrances does not seem to be determinant for the nest site selection of Green turtles on Jalé beach. Similar results showed Green turtles that tended to emerge on beaches with an accessible offshore approach, relatively free of rock as described by Mortimer (1990).

Human Influences. The presence of human activity may influence turtle decision for nesting, reducing nesting close to the buildings or artificial lights (Halls et al., 2018; Salmon 1995; Zavaleta-Lizárraga et al., 2013). However, during the surveys in Jalé beach, the higher nest density was observed close to the buildings (most of which occasionally occupied during the surveys) in the zone of Jalé 2. Similar results were described by Salmon (1995a), and even during human disturbances, turtles still laid their clutches successfully. Nevertheless, in Jalé beach the false crawls rate is extremely high (more than 50% of the total emergences) which may suggest that these buildings and the presence of people can persuade turtles from nesting the first time they come ashore to nest (Ferreira and Vieira, 2019).

Nonetheless, the artificial lights may also affect the hatchling emergence, driving the hatchlings to the light source (misorientation) or paths non-directed to the ocean (disorientation), eventually causing their death by desiccation or predation (Salmon et al., 1995b).

4.4. Hatching success

Many studies have evaluated nest site selection by sea turtles and its relationship to hatching success (Fowler, 1979; Sampaio, 2018; Santos et al., 2017; Serafini et al., 2009; Veelenturf et al., 2021; Zárate et al., 2013). Globally Green sea turtle hatching success is approximately 60-90% (Veelenturf et al., 2021), indicating our results are within the success range. The hatching success was not significantly different between nest locations; however, the advantage of the various sites may be related to other abiotic factors, such as sand moisture, sand grain, temperature and rain (Wood and Bjorndal, 2000; Patricio et al., 2017). Nests in open beach areas may have less risk of desiccation because of the high moisture in the sand (Zavaleta-Lizárraga et al., 2013; Lovalar et al.,.2015). However, nests close to the high tide line have a bigger chance of being washed by waves and can decrease hatching success (Ackerman, 1997; Bonfim et al., 2021). Alternatively, supposing the waves do not entirely destruct the nests, and the hatchlings emerge close to the water, there is a significant increase in success rate as they are less vulnerable to predators and disorientation (Kamel and Mrosovsky, 2004). Nests close to the vegetation have a higher risk of root invasion, and the distance of hatchlings to reach the ocean increases (Patricio et al.,2018).

In our study, the recorded mean incubation period (76 days) was longer than found in other studies (Fowler, 1979; Patricio et al., 2017). This may be related to the high humidity caused by the rainy season coincident with the nesting season (Patricio et al., 2017). Although this has not been addressed in our study, the effect of rain on embryo development must be better understood to assess the variation between offspring in the response of sex ratio to mean incubation temperatures and incubation period.

4.5. Conclusions and limitations

With this study, we evaluated the population of green turtles at Jalé beach. We observed the number of variables and habitat features that may influence the distribution and the nest preferences of this species, as well as the hatchling success, providing information for future studies and better management on future threats related to climate change and the fast growth in tourism developments along the coast.

Green turtles nesting on Jalé beach usually prefer to emerge during the quarter moon, which in future management decisions can help to concentrate monitoring efforts during these quarter moon nights.

It was also found that Green turtles nested mainly between the vegetation line and the open beach area, above the high tide line, with high hatching success rates and high incubation periods. The different choices of microhabitat may not influence the hatching success as found for example by Patricio et al. (2018) but may affect the sex ratio determined by the environmental temperature (Zavaleta-Lizárraga et al., 2013; Matsuzawa et al., 2002; Katselidis et al.,2012). These results show us that there are still significant information gaps that need to be addressed to understand the sex ratio in this Green turtle nesting site and its contribution to the population. This should be studied with more detail also to understand the species sex-ratio in both São Tomé and Príncipe island and understand the contribution of each site for the population’s reproductive success and the impacts of future global warming expected to increase incubation temperatures (Ackerman, 1997; Patrício et al., 2017). The success of the nests' location within these habitats may also affect this sea turtle’s population, especially important in the context of habitat loss, either by environmental changes such as a sea-level rise or coastal development. Both threats may reduce the range of the nest sites available to females (Fish et al.,2005). In addition, female sea turtles return to their natal beaches, and alteration on those nesting areas may reduce the local nesting population's reproductive success and size (Fish et al.,2005).

Additionally, it was shown that the higher nesting density was in the zone with ecotourist buildings, which can be explained by the low occupation rate during the period of this present study. Future studies should analyse more in detail the relationship between successful nesting and false crawls and the possible causes during an entire season and a normal year of tourism occupation (since most of tourism was closed during this period due to Covid19) to understand the effective impacts on Green turtles successful nesting and to understand the high rates of false crawls. This may also negatively affect the hatching emergence since the presence of ecotourist buildings with artificial lights could interfere with hatchling orientation (Silva et al., 2020; Wang and Cheng, 1999).

Although our surveys took place over a short period of time and the sample was small, the results give us already an insight of Green turtles' preferences in Jalé Beach, south of São Tomé Island, and conservation and research priorities to better understand this population reproduction success.

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