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RESEARCH ARTICLE

Repeatability of Nest Site Selection of Green Turtles on Samandağ Beach

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Abstract: Nest site selection is a maternal effect on hatchling survival in species without parental care. Sea turtles depend on sandy beaches for nesting, and females perform nest site selection on the beach for the successful development of their embryos. This study investigated the repeatability of nest site selection on Samandağ beach, a major green turtle nesting beach in the Mediterranean. During the 2013 nesting season, the distance to the sea, distance to vegetation, horizontal distance, and nest depth of the first and second nests of females were recorded using a tagging method. Repeatability analysis was performed using the rtpR package in R. A total of 91 turtles were tagged, of which 36 were observed during their first and second nests. The highest repeatability was found for horizontal distance, followed by distance to vegetation and distance to the sea. In contrast, nest depth showed insignificant repeatability. This suggests that green turtles select nesting sites that are close to the vegetation line but far from the sea and sites that are very close in horizontal distance. The results of this study include the results of a single year nesting season. It is recommended that future studies be conducted in consecutive years to see the results of repeatability of nest site selection from year to year.

Anahtar kelimeler:

Chelonia mydas
Yeşil kaplumbağa
Yuva yeri seçimi
Tekrarlanabilirlik
Samandağ

Samandağ Kumsalında Yeşil Kaplumbağaların Yuva Yeri Seçiminin Tekrarlanabilirliği

Öz: Yuva yeri seçimi, ebeveyn bakımı olmayan türlerde yavruların hayatta kalması üzerinde maternal bir etkidir. Deniz kaplumbağaları yuva yapmak için kumsallara bağımlıdır ve dişiler embriyolarının başarılı bir şekilde gelişmesi için kumsalda yuva yeri seçimi yaparlar. Bu çalışmada, Akdeniz'de yeşil kaplumbağalar için önemli bir yuvalama kumsalı olan Samandağ kumsalında yuva yeri seçiminin tekrarlanabilirliği araştırılmıştır. 2013 yuvalama sezonu boyunca, dişilerin ilk ve ikinci yuvalarının denize uzaklığı, bitki örtüsüne uzaklığı, yatay mesafesi ve yuva derinliği etiketleme yöntemiyle kaydedilmiştir. Tekrarlanabilirlik analizi R programında rtpR paketi kullanılarak gerçekleştirilmiştir. Toplam 91 kaplumbağa etiketlenmiştir; bu kaplumbağaların 36'sı ilk ve ikinci yuvaları sırasında gözlemlenmiştir. En yüksek tekrarlanabilirlik yatay mesafede bulunmuş, bunu bitki örtüsüne olan mesafe ve denize olan mesafe izlemiştir. Buna karşılık, yuva derinliği önemsiz bir tekrarlanabilirlik göstermiştir. Bu da yeşil kaplumbağaların yuvalama alanı olarak bitki örtüsüne yakın ancak denizden uzak alanları ve yatay mesafe olarak birbirine çok yakın alanları seçtiğini göstermektedir. Bu çalışmanın sonuçları tek bir yıllık yuvalama sezonunun sonuçlarını içermektedir. Yuva yeri seçiminin yıldan yıla tekrarlanabilirliğinin sonuçlarını görmek için gelecekteki çalışmaların birbirini takip eden yıllarda yapılması tavsiye edilir.

Introduction

Nest site selection is a form of maternal influence that enhances the survival and variety of hatchlings' characteristics, which are subject to natural selection (Kamel and Mrosovsky, 2004). Sea turtles are a good example of these species and depend on sandy beaches for nesting. Most sea turtle species do not nest every year but typically lay two to four clutches every 2 to 4 years (Ehrhart, 1982). The female's nest site selection is critical to the successful incubation of eggs, as egg survival depends on the interaction of several factors, including temperature, moisture, salinity, tidal inundation, erosion, and predation. (Fowler, 1979; Yntema and Mrosovsky, 1980; McGehee, 1990; Sönmez and Yalçın Özdilek, 2013; Sönmez, 2018).

Nests close to the sea are more vulnerable to the risk of flooding and erosion (Sönmez and Yalçın Özdilek, 2013), while nests near or in vegetated areas may have roots that can affect the embryonic development (Kamel and Mrosovsky, 2004). Also, vegetated areas might increase

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the likelihood of predation (Sönmez, 2018). Furthermore, because sea turtles have temperature-dependent sex determination (Yntema and Mrosovsky, 1980), nest site selection may affect hatchling sex via incubation temperature (Heredero Saura et al., 2022). In addition to sex, incubation temperature can also affect the size and locomotor performance of sea turtle hatchlings (Booth, 2017). Consequently, nest site selection can affect hatchlings' health that may vary with changes in the external environment (Patricio et al., 2018). In the face of changing environmental conditions, the nest site selected by the mother can affect the successful incubation of eggs as well as the hatchlings' sex, fitness, and survivability.

Wilson (1998) describes nest site selection as the process of nesting in non-randomly selected locations, and sea turtles exhibit diverse patterns of nest site selection that may vary between species or locations. For instance, Kamel and Mrosovsky (2004) reported significant repeatability and individual consistency in leatherback sea turtle nest site selection on the French Guiana nesting beach. Researchers have also reported that green turtles in Costa Rica exhibit nest site repeatability and prefer areas close to vegetation, particularly under trees (Heredero Saura et al., 2022). Similarly, green turtles showed high repeatability in microhabitat types such as open sand, forest border, and forest in Guinea-Bissau, West Africa (Patricio et al., 2018). Kamel and Mrosovsky (2005) reported that repeatability behavior in nest site selection in hawksbill sea turtles is inherited and may show potential for further evolution. In loggerhead sea turtles, females showed high variability in nest site selection, but larger females (>93 cm curved carapace length) showed higher repeatability in distance from vegetation on Boa Vista Island, Cabo Verde (Martins et al., 2022). In addition, Nordmoe et al. (2004) stated that it is more consistent to hypothesize that leatherback sea turtles nest close to the location of the previous nest rather than being fidelity based on a specific location on the beach.

Repeatability, which measures the proportion of total variation that is due to differences between individuals, is important for selection (Falconer and Mackay, 1996). Moreover, Boake (1989) asserts that it also reveals individual consistency. High and statistically significant repeatability in a behavioral trait indicates the potential for a genetic basis (Dohm, 2002). This highlights the importance of understanding behavioral repeatability in sea turtle nest site selection. In this case, repeatability can provide insight into whether factors such as distance to the sea and vegetation, temperature, and nest depth are important. These factors, which may affect the population's future and have a significant impact on nest site selection, are either genetically determined or random. As a result, this situation can provide guidance for future conservation and monitoring programs for the sea turtle population. Studies on the Samandağ green turtle population have reported that nests are exposed to flooding (Sönmez and Yalçın Özdilek, 2013; Sönmez et al., 2024), nests close to vegetation are at high risk of predation (Sönmez 2018), and female biased hatchlings are produced (Yalçın Özdilek et al., 2016). Nest site repeatability is known to exist in green turtles (Patricio et al., 2018; Heredero Saura et al., 2022). However, this has not been confirmed for the Mediterranean green turtle population. The aim of this study was to investigate the repeatability of nest site preference in terms of spatial distribution (distance to sea and vegetation, horizontal distance, and nest depth) in Samandağ green turtles.

Material and Methods

The study was conducted at Samandağ beach (36°07'N, 35°55'E) in the eastern Mediterranean during the 2013 nesting season. The beach was divided into 3 sections: Çevlik (5.5 km), Şeyh-hızır (4.1 km), and Meydan (4.4 km) sub-sections (Figure 1). Çevlik Harbor in the north was defined as the reference (zero) point for determining the horizontal distribution of nests. The study was conducted only in the Şeyh-Hızır sub-section due to very high nest density (Sönmez et al., 2024).

The data were collected within the framework of the collaboration protocol signed between Hatay Directorate of Nature Protection and National Parks of the Ministry of Agriculture and Forestry and Samandağ Environmental Protection and Tourism Association under the title "Research and conservation of sea turtle (*Chelonia mydas* and *Caretta caretta*) populations on Samandağ beach in Hatay " during the nesting season of 2013. Five people monitored the beach at night to observe the nesting female turtles. After nest camouflage was completed, turtles were tagged with metal tags. The tags were placed on the posterior margin of the left anterior flipper, as suggested by Balazs (1999). After the turtles returned to the sea safely, the distance from the sea and vegetation of the nest were measured (the vertical distance from the egg chamber to the tide line and vegetation). Also, the distance to the reference point of the nest was recorded with GPS (± 5 m, GARMIN). During night patrols throughout the nesting season, when a previously tagged female was found nesting (another nest belonging to the same female), the same measurements as above were recorded.

All nests were monitored during incubation and protected against predators with cages. A week after the first hatchling emerged, the nests were excavated and the remains were examined. During nest excavation, the number of eggshells, dead embryos, and nest depth were recorded. Of these records, only nest depth was used for repeatability analysis. The vertical distance from the sand surface to the bottom of each nest was measured using a flexible tape measure to determine the nest depth.

Nonparametric Wilcoxon Signed Rank Test was used to compare the distance to the sea and vegetation, nest depth, and horizontal distance of nests belonging to the same female (Salleh et al., 2021). The repeatability measure in Gaussian data with the linear mixed-effects model (LMM) was used to assess whether there were within-individual preferences in nest site selection (Nakagawa and Schielzeth, 2010; Patricio et al., 2018). Linear mixed models directly estimate the variances,

which are both necessary and sufficient for calculating repeatability. The predominant method for estimating unbiased variance components in linear mixed models is restricted maximum likelihood (Nakagawa and Schielzeth, 2010). Therefore, repeatability analysis for Gaussian data, i.e., distance to sea and vegetation, distance to reference point (horizontal distance), and nest depth rtpR package in R, was performed using the Linear Mixing Model (Nakagawa and Schielzeth, 2010; Patricio et al., 2018). The results of the repeatability analysis are presented along with the R value, standard error (SE), and 95% confidence interval (CI) values.

The statistical analyses were performed with rptR (Stoffel et al., 2017), ggplot2 (Wickham, 2016), and ggstatsplot (Patil, 2021) packages in R (R Core Team 2020 and R Studio Team 2020).

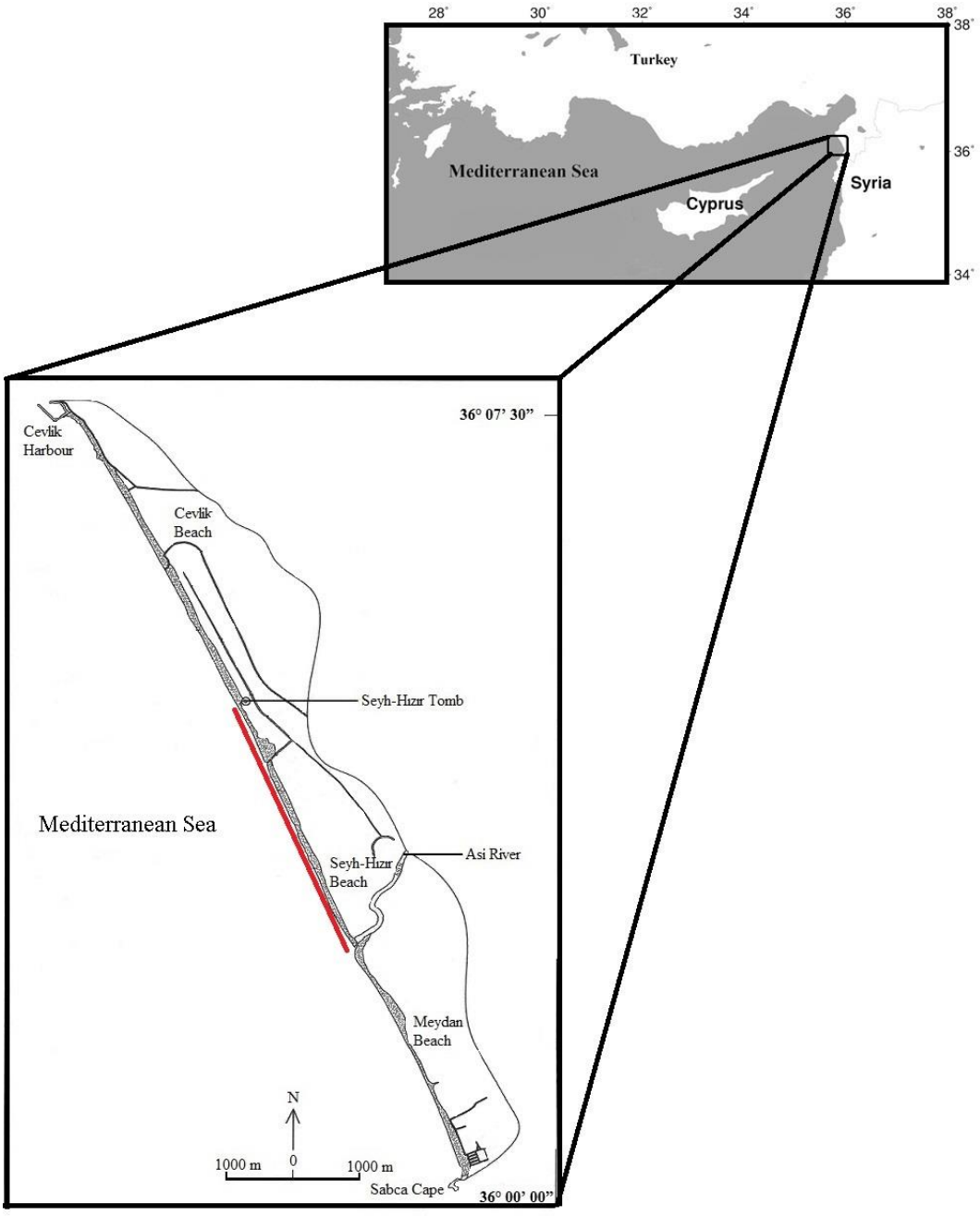

Figure 1. General view of Samandağ beach (red line shows the Şeyh-Hızır sub-section where tagging was done for repeatability analysis)

Results

A total of 1175 green turtle nests were recorded during the 2013 nesting season. 91 turtles were tagged; 36 of these turtles were observed during their second and third nests, and 55 turtles were observed only during their first nest. Since only two turtles were observed during their third nest, all data from the third nest of these two turtles were excluded from all statistical analyses. Thus, 72 nests (first and second nests) of 36 turtles were analyzed for repeatability. The median box plots of the distance to the sea and vegetation, horizontal distance, and nest depth for the first and second nests are shown in Figure 2. There was no significant difference between the distance to the sea and vegetation, the horizontal distance, and the nest depth of the first and second nests of the tagged females (see Table 1 for details).

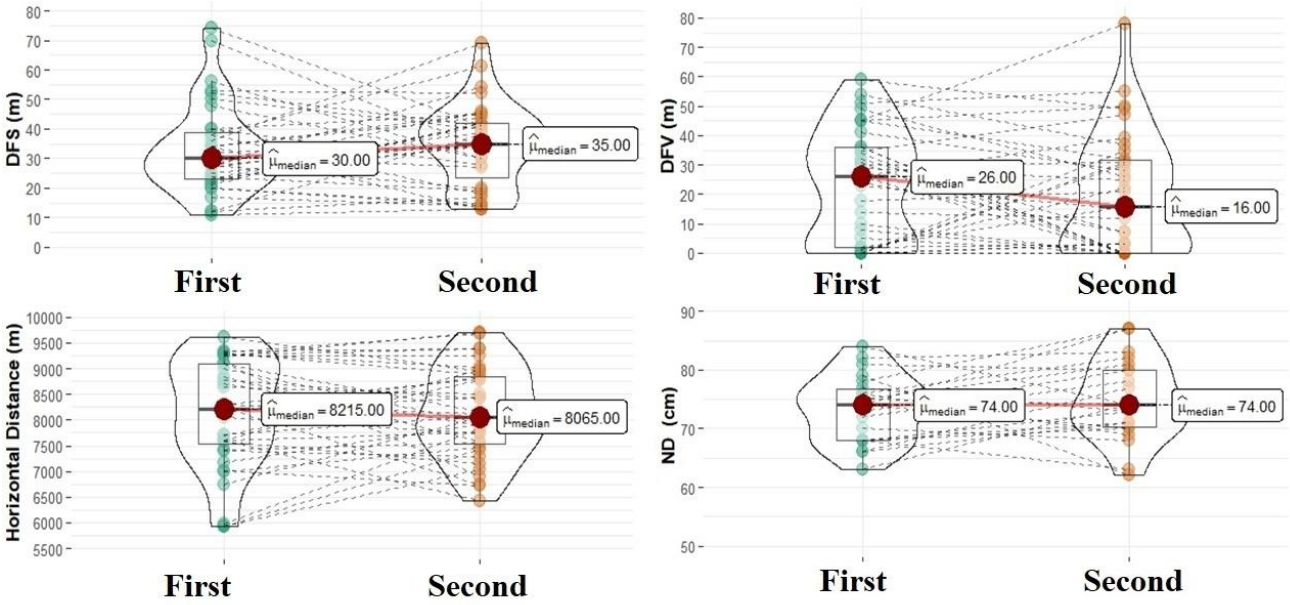

Figure 2. Box plots and medians of the first and second nests in terms of distance to sea, distance to vegetation, horizontal distance and nests depth (DFS: distance from sea, DFV: distance from vegetation and ND: nest depth)

Table 1. The Wilcoxon signed-rank test and repeatability measure of the two consecutive nests of green turtle females (DFS: distance from sea, DFV: distance from vegetation and ND: nest depth)

	Wilcoxon Signed Rank Test			Repeatability Measure			
	n	Wilcoxon	p	R	SE	p	95% CI
DFS	35	280	0.57	0.323	0.147	0.0301*	0.002 – 0.592
DFV	35	339	0.30	0.515	0.129	0.0006*	0.223 - 0.720
Horizontal	36	324	0.89	0.584	0.113	0.000723*	0.327 – 0.758
ND	22	89	0.23	0.304	0.176	0.0875	0 – 0.627

Individuals at their nest site had the highest repeatability for horizontal distance (see Table 1 and Figure 3a), followed by distance to vegetation (see Table 1 and Figure 3b), and distance to the sea (see Table 1 and Figure 3c). In contrast, nest depth showed no significant repeatability between individuals (see Table 1 and Figure 3d). In the multiple assessment (distance to sea + distance to vegetation + horizontal distance), when nest depth was excluded (due to no significant repeatability), the analyses showed a high repeatability within individuals at the nest location (R: 0.547, SE: 0.13, $p<0.001$, and CI: 0.238–0.758).

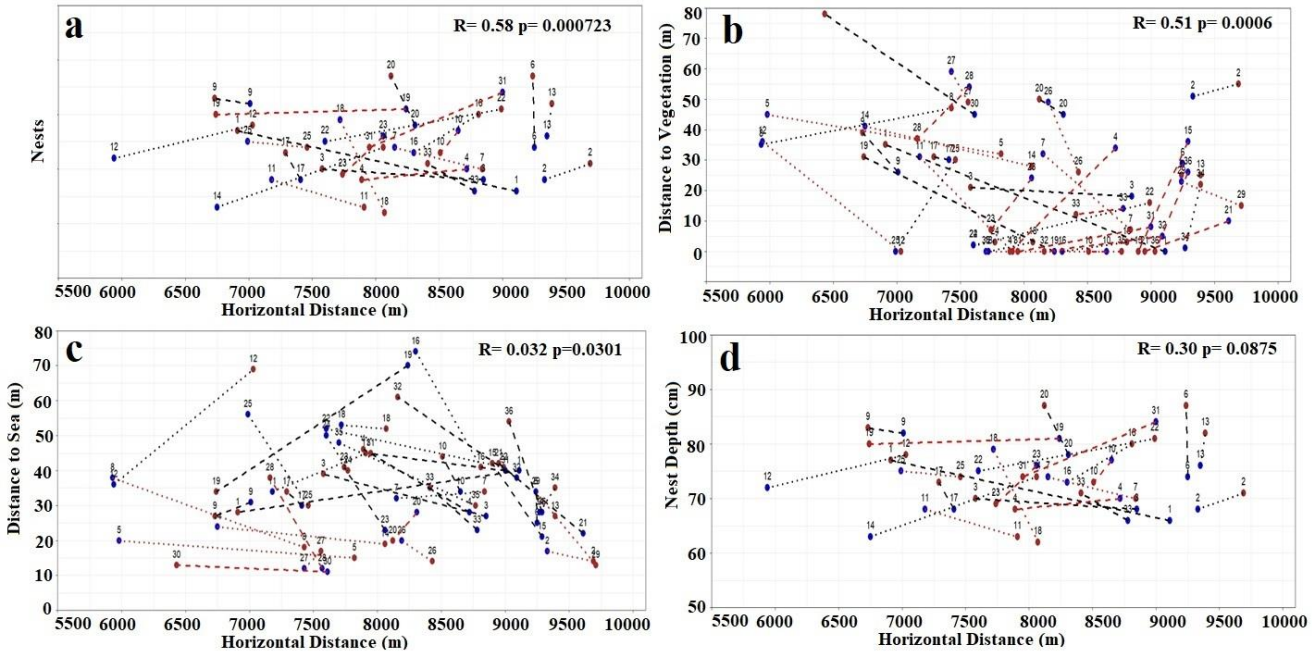

Figure 3. Graphical representation of individual locations between two consecutive nests of green turtle females and repeatability for horizontal distance (a), distance to vegetation (b), distance to sea (c), and nest depth (d) (blue dot indicates the first nest and red dot indicates the second nest; dotted line indicates positive change; and dashed line indicates negative change).

Discussion

Nest site selection, defined as the placement of nests in areas that differ from random sites (Wilson, 1998), has been proposed to provide developing embryos with a higher probability of survival at selected sites than at unselected sites (Schwarzkopf and Brooks, 1987). While all species of sea turtles follow a similar nesting procedure, there is considerable variation in the selection of nesting sites among different species and populations. For example, loggerhead turtles in Cabo Verde preferred to nest in the middle of the beach and avoided nesting close to both the shoreline and the vegetation line (Martins et al., 2022). Furthermore, it was reported that leatherback turtles lay predominantly in open sand and green turtles in vegetated areas in Suriname (Whitmore and Dutton, 1985). Additionally, Hart et al. (2014) reported that olive ridley turtles in Mexico prefer to nest on open beaches from the berm to the vegetation line. Green turtles nest mostly in areas with vegetation, while loggerhead turtles nest mostly in areas without vegetation on Akyatan beach, Türkiye (Türkozan et al., 2011). Similarly, green turtle nests are often reported to be in vegetation at the back of the beach, where the risk of flooding is lowest in the Chagos Archipelago, Indian Ocean (Stokes et al., 2024).

The present study found that green turtles in Samandağ exhibit intra-individual consistency and high repeatability in their nest site selection. This suggests that green turtles selected areas close to the vegetation line (just in front of the vegetation rather than in the vegetation zone) but far from the sea as nesting sites. Previous studies have reported nest site repeatability for leatherback turtles in French Guiana (Kamel and Mrosovsky, 2004), hawksbill turtles in Guadaloupe (Kamel and Mrosovsky, 2005), green turtles in Guinea-Bissau (Patricio et al., 2018), and eastern Pacific green turtles in northwestern Costa Rica (Heredero Saura et al., 2022). The presence of a high degree of repeatability may represent a process constancy in the nest site selection among sea turtles (Heredero Saura et al., 2022). Although constancy may be advantageous for turtles that encounter ideal conditions for egg development, it can also be disadvantageous when conditions turn unsuitable (Bowen and Karl, 2007). Bowen and Karl (2007) proposed that there may be mixed strategies within a sea turtle population, with some individuals choosing to scatter their nests, while others prefer to nest in a restricted area. The green turtle population at Cabuyal beach in northwestern Costa Rica exhibits this mixed nesting strategy, with some individuals nesting at relatively large distances from each other (Heredero Saura et al., 2022). Thus, long-distance nests may benefit from changing unfavorable conditions due to the dynamic structure of the beach. In fact, poor nest site repeatability has sometimes been observed in some populations in response to local beach dynamics (Pfaller et al., 2022).

Studies have reported that green turtles mostly prefer vegetated areas for nest site selection (Whitmore and Dutton, 1985; Türkozan et al., 2011; Heredero Saura et al., 2022; Stokes et al., 2024). In contrast, Patricio et al. (2018) found high repeatability in green turtles and reported that 67% of nests were located in open sand, with the remainder in forests and forest edges. Nesting farther from

the sea may reduce the risk of inundation (Sönmez and Yalçın Özdilek, 2013; Stokes et al., 2024) and thus tend to increase hatching success (Patricio et al., 2018; Martins et al., 2022). In addition to increasing hatching success by reducing the risk of flooding, nest site selection in vegetated areas (under trees rather than in grassy areas) with high repeatability may result in cooler temperatures and thus less female bias (Patricio et al., 2018; Heredero Saura et al., 2022). However, nests in forest edges and habitats had lower hatching success than those in open sand (Patricio et al., 2018). While the behavior of repeatability in terms of distance from the sea may be aimed at reducing the risk of flooding on Samandağ' beach, proximity to vegetation lines may increase the likelihood of predation. It has been reported that the probability of predation is higher in nests close to vegetation on Samandağ beach (Sönmez, 2018).

Samandağ green turtles showed high repeatability in horizontal distance in addition to distance to the sea and vegetation. The median of the horizontal distances between the first and second nest of females show that there is only a 150 m difference between two consecutive nests (see Figure 2). This shows that females tend to nest in the same area of the beach with high repeatability.

The most obvious limitation of the study is its single year nature, which limits the generalizability of the results to more than one year, and the sample size limitation with only 36 turtles observed for their second nest. The repeatability of nest sites between years has been demonstrated for hawksbill sea turtles in Guadeloupe (Kamel and Mrosovsky, 2006). Furthermore, the fact that the study was conducted on only one section of the beach (i.e., Şeyh Hızır) is an important limitation. If similar studies had been conducted on the entire beach or even on nearby beaches, different results might have been obtained. This is because a green turtle nested in both Snoubar (Syria) (about 100 km from Samandağ beach) and Samandağ beach during the same nesting season (Sönmez et al., 2017).

Another limiting factor is that the study did not consider external factors that may influence nest site selection, such as predation pressure or human activities. In fact, the entire Şeyh-Hızır subsection where the study was conducted has a low anthropization frequency (Sönmez et al., 2024). The low frequency of anthropization can be considered as an important factor in nest site selection. Similarly, Siqueira-Silva et al. (2020) reported a higher frequency of anthropization in areas with fewer nests in northeastern Brazil. Furthermore, on Kenyan beaches where human activity is intense, it has been associated with high organic matter content, which significantly affects the number of green turtle nests on the beach (Obare et al., 2019).

In conclusion, green turtles nesting on Samandağ beach showed high repeatability in nest site selection, especially in terms of distance to vegetation and horizontal distance. However, the results of the present study were conducted during a single nesting season and only in a sub-section of the beach. It may be recommended that future studies investigate the repeatability of nest site selection across years and across the entire nesting beach. This will test whether similar results occur in different nesting seasons and across the entire nesting beach.

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Conflict of Interest

The author has no relevant financial or non-financial interests to disclose.

Author Contributions

Bektaş Sönmez (BS) conceived the ideas and designed methodology and analysed the data, and wrote of the manuscript.

Ethics Approval

No ethics committee approval is required for this study.

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