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Nesting activity of the Loggerhead sea turtle, *Caretta caretta*, in Calabria during the 2016-2020 reproductive seasons

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Abstract

The nesting activity of the loggerhead turtle along the coast of Calabria during five reproductive seasons (2016–2020) is presented. From May to August, survey methods were conducted using traditional observations on foot and utilising additional innovative technologies, including electric fat bikes and drones. Monitoring was intensively focused on a key nesting area of approximately 40 km length located on the southernmost Ionian coast of Reggio Calabria, and, for only the 2020 season, the area was extended an additional 40 km northeast. In the five nesting seasons, 419 female emergences were recorded, 192 of which were classified as nests. Overall, 65.1% (n = 125) of nests were found along the area regularly monitored during all nesting seasons. The maximum nesting activity was observed in July, and the mean nesting success rate was 45.8%. The average number of eggs for all seasons was 92.2 (n = 163; SD: ± 21.1 ; range: 39–160). The mean hatching and emergence success rates were 70.8% and 62.5%, respectively. The mean incubation period was 49 days (n = 122; SD: ± 5.4 ; range: 42–82). Considering previous data from the same area, our study shows an actual increase in nesting activity, probably due to the implementation of an effective monitoring method that includes the use of new technologies, which made it possible to expand the regularly monitored area, and a greater awareness campaign in recent years. Regarding the reproductive parameters, our data are like those reported for other Mediterranean nesting grounds.

Keywords: Reproductive parameters; nesting data; sea turtle; conservation; Italy.

Introduction

The loggerhead turtle Caretta caretta is the most common sea turtle species in the Mediterranean (Casale & Margaritoulis, 2010). The species colonised the area during the Pleistocene through different colonising events. The oldest colonisation event occurred in Libya and the most recent occurred in Calabria, whose population has a unique Atlantic haplotype that is not found anywhere else in the Mediterranean (Clusa et al., 2013). Caretta caretta can potentially lay eggs throughout the Mediterranean, but approximately 96% of nests are found along the eastern basin, in Greece, Turkey, Cyprus, and Libya, and few nests have been recorded along the coasts of Egypt, Israel, Lebanon, Syria, Tunisia, and Italy, with occasional cases along the western basin of Spain, France, and their islands (Casale et al., 2018). All species of marine turtles are included in the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN), and loggerhead turtles (C. caretta) are listed globally as 'Vulnerable' (Casale & Tuker, 2017); however, the C. caretta Mediterranean subpopulation is classified as of the 'Least Concern' category (Casale, 2015). The latter is possibly due to several monitoring and conservation activities at nesting sites since the late 1970s and 1980 in the eastern Mediterranean basin (Greece, Turkey, and Cyprus) (Casale & Margaritoulis, 2010). Despite the classification in the low-risk category, many threats affect the nesting of sea turtles in the Mediterranean basin. On the mainland, the main threats occurring in the reproductive sites are from anthropogenic activities. Coastal development associated with recreational and tourist activities is a significant threat because the resulting habitat destruction is difficult to recover from. Furthermore, erosion and unlawful activities, such as driving on the beach and using heavy machinery for beach cleaning, contribute significantly to nest failure. Finally, climate change could become an important threat that may affect hatchling production, sex ratio, and cause habitat reduction.

In Italy, the attention towards verifying the presence of sea turtle nests by new public and private organisations has recently increased, and many projects are active in different regions for this purpose. However, many of these are inadequate, especially in terms of regularity, and this gap in information translates into an underestimation of the number of nests. Mingozzi *et al.* (2007) reported a national nesting overview that 30–40 nests are possibly laid in Italy annually. Overall, the distribution ranges from significant numbers on the southern coastline and islands (Calabria, Campania, Sicily) to single nesting records documented along the Tyrrhenian coast of Tuscany, Lazio, Sardinia, and from Abruzzo to Apulia along the Adriatic and Ionian coasts.

In particular, approximately 50-80% of the total nests documented countrywide lie along the Ionian coast of Calabria (n = 261 for the 2005-2014 period), recognised as the most important regular nesting ground of loggerhead turtles in Italy, and approximately 80% are concentrated along the "Costa dei Gelsomini" (Mingozzi *et al.*, 2007; 2016).

A strategic monitoring project aimed at protecting the small Calabrian nesting population, considering their unique and varying mitochondrial diversity (Garofalo *et al.*, 2009), was started in 2016 by the non-profit organisation "Caretta Calabria Conservation". This project was regularly carried out under the authorisation of the Italian Ministry for Environment, Nature Protection Directorate (Rome). The aim of this paper is to present the results of five nesting seasons (2016–2020) of *C. caretta* breeding in Calabria. We provide information on the nesting activity, temporal and spatial distribution of nests, clutch size, incubation duration, hatching, and emergence success.

Materials and Methods

The study area includes the entire region of Calabria in the southernmost part of the Italian Peninsula and consists of approximately 800 km of coastline. Along the Ionian coast, there are mainly wide sandy beaches, small, eroded sections, and rocky cliffs near the headland, while the Tyrrhenian coast is mostly rocky with small sandy sections. For the years 2016–2020, considering the largest extension of potentially suitable coastlines, intensive beach monitoring was mainly focused on a regular nesting area from Capo D'Armi (37.951515° N; 15.684434° E) to Capo Bruzzano (38.038950° N; 16.144913° E) (approximately 40 km length of sandy beach) in the southernmost part of the peninsula. For 2020, the survey was extended for another 40 km lenght to the northeast up to the beaches of Marina di Gioiosa (38.303000° N; 16.342428° E). In other potentially suitable areas, monitoring was occasionally performed during all seasons. Patrolling began in late May and lasted until mid-August. The survey methods were conducted using traditional observations on foot, but also utilised more innovative technologies, including electric fat bikes and drones (2019 and 2020). Every season, the beach surveys and collection of data were performed by an expert team assisted by volunteers, and the same transect was patrolled every 2 days, usually in the early morning (6:00–9:00 a.m.) or in the afternoon (6:00–8:00 p.m.). The drone, piloted by personnel experienced in the recognition of female emergence tracks, was mainly used for relatively long and/or difficult-to-access transects. The monitoring was conducted almost exclusively at the first light of day, also using polarised lenses to improve the contrasts of light and shadow, thus facilitating the identification of flipper-prints. The use of the drone allowed us to monitor more kilometres and increase the frequency of checks. The only limitation of the instrument is linked to weather variations, as in the case of wind or rain, it was not possible to use it, and the survey was always assisted by patrolling on foot or fatbike. All emergence tracks discovered were catalogued according to the method of Schroeder and Murphy (1999), marked on a map (Google Earth) and georeferenced. Only the tracks that resulted in nests were subsequently dug carefully to locate the egg chambers. The goal of this method is to allow an investigator to evaluate the distance from the sea and then intervene with relocation for nests at risk of inundation, to affix an anti-predation metal grating (1 m × 1 m; mesh size 10 cm) and protective fences when the nesting beach is heavily anthropogenic. The percentage of nesting success was calculated as $(N/(N + T) \times 100)$, where N is the number of successful nesting attempts, and T is the number of unsuccessful nesting attempts. The duration of the nesting season was calculated by the dates of the first and last emergence, and of the first and last nests of the season. If the nests were laid at a sufficient distance from the shoreline (≥ 15 m), the point was georeferenced, and triangulation was performed using two fixed points on the dune. Eighty-five nests were secured by relocation (Wyneken et al., 1988) because they were at risk of inundation (laid less than 15 m) or in a few cases placed in an unfavourable substrate. The value of 15 m was chosen arbitrarily and considered the highest debris line left by the tide and the slope of the beach. This was not an absolute value for which each individual case was evaluated at the time of discovery. Both relocated and natural nests were monitored daily throughout the incubation period. Hatched nests were excavated at least 3 days after the last hatchling emergence. Excavation was undertaken manually, and the nest contents were sorted and counted. Clutch size was the number of eggs laid in a nest; it was determined at the time of oviposition, during relocation, or could also be estimated after emergence by counting eggshells (Miller, 1999) and other nest contents: unhatched eggs, hatchlings (dead or alive), and dead/live pipped eggs (dead/live hatchlings that had only broken eggs). When estimating clutch size, we evaluated the error associated with the estimate of the number of hatched eggs from fragments found in the nest after hatching using the method reported by Ceriani et al. (2021). Hatching success was defined as the percentage of eggs that produced hatchlings, including hatchlings that were unable to leave the nest (excluding pipped eggs). Incubation duration was defined as the elapsed period (in days) from egg laying until the emergence of the first hatchlings.

Results

In the five nesting seasons (2016-2020), a total of approximately 10,000 km of beach was patrolled, of which

approximately 8,000 km covered the main area from Capo D'Armi to Capo Bruzzano on the Ionian coast. During this period, 419 female emergences were recorded, 192 of which were classified as nests. The annual number of emergences ranged from 55 in 2017 to 155 in 2020. The annual number of nests ranged from 20 in 2017 to 66 in 2020 (Table 1). Twelve nests were found along the coast from Capo d'Armi to North Tyrrhenian and 180 along the Ionian coast (Table 2, Fig. 1). Overall, 65.1% (n = 125) of nests were found along the area monitored regularly in all nesting seasons with a mean of 25 nests/year (range: 12–36) and a density of 0.63 nest/km (range: 0.30–0.90). The lowest number of nests in the main study area was observed in 2017, while the highest number of nests was observed in 2020 (Table 2). Attempts started during

Table 1. Number of emergences found during the last 5 years along Calabrian coasts obtained from occasional and methodical surveys (N.S.: nesting success).

Years	Attempts	Nests	Emergences	N.S. (%)
2016	44	42	86	48.8
2017	35	20	55	36.4
2018	20	36	56	64.3
2019	39	28	67	41.8
2020	89	66	155	42.6
	227	192	419	45.8

Table 2. Number of nests found during the last 5 years along the Calabrian coasts subjected to different sampling efforts. Bold data was obtained from intensive surveys.

		Ionian coast		Low Ionian to Tyrrhenian coast	
Years	C. D'Armi to C. Bruzzano	C. Bruzzano to Marina Gioiosa	Marina Gioiosa to North Ionio	C. D 'Armi to North Tyrrhenian	Overall
2016	26	1	12	3	42
2017	12	0	7	2	20
2018	27	4	3	2	36
2019	24	3	0	1	28
2020	36	22	4	4	66
Overall	125	30	25	12	192

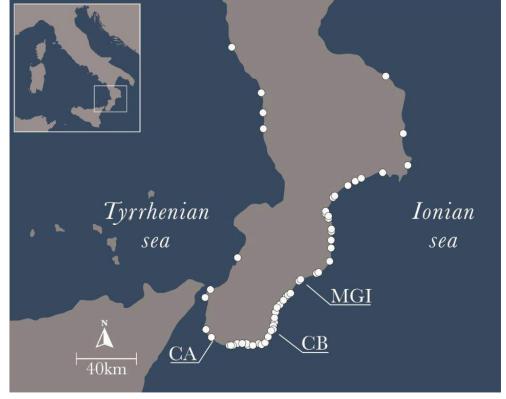


Fig. 1: Nest distribution of loggerhead turtle along the Calabrian coasts for the 2016-2020 reproductive seasons. Each white dot corresponds to a single nest. (CA: Capo d'Armi; CB: Capo Bruzzano; MGI: Marina di Gioiosa Ionica).

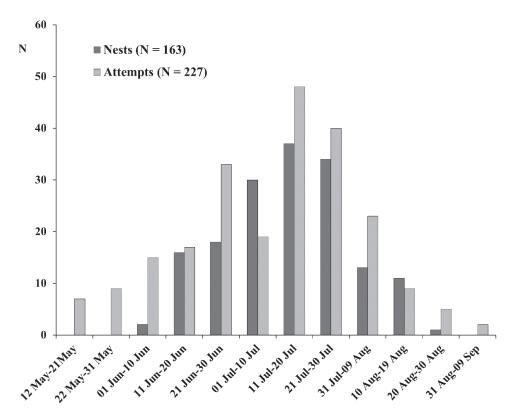


Fig. 2: Temporal distribution of emergences in Calabria.

the second half of May and ended at the beginning of September; nesting started at the beginning of June and lasted until the end of August. In both cases, maximum activity was observed in July (Fig. 2).

The earliest attempt occurred in the second half of May, while the first nest was recorded in the first half of June for all seasons. For the 2017 and 2019 nesting seasons, the last attempt coincided with the last nest, while for the seasons 2016 and 2018, the last attempt occurred 2 days after the last nest and for 2020, the last attempt occurred 13 days after the last nest; this is an "aberrant" case. The mean duration of a nesting season for 2016-2020 was 75.6 days for the attempts and 64.4 days for the nests (Table 3).

We computed the number of eggs, hatching, and emergence success for 163 out of 192 nests because disorientated hatchlings were found in 29 nests, therefore, it was impossible to find the egg chamber. The average

number of eggs for all seasons was 92.2 (n = 163; SD: \pm 21.1; range: 39–160). The error associated with the method used to determine the clutch size was 14%; of 85 nests evaluated, 72 were without error. The mean percentages of hatching and emergence success were 70.8% and 62.5%, respectively (Table 4).

In the five nesting seasons, 14,590 eggs (n = 163) were laid, 71% of which hatched, 25% of which remained unhatched, and 4% of which were dead pipped. Furthermore, 2% of the hatchlings died inside the nest (Table 5).

The mean incubation period (mean IP) was calculated using the nests for which the data of laying and emergence of hatchlings were fully obtained (n = 122). The mean IP was 49 days (SD: \pm 5.4), with a minimum of 42 days recorded in 2018 and 2020 and a maximum of 82 days in 2016. The mean IP for each year was between 47.3 and 52 days (Table 6).

Table 3. First and last dates for attempts and nests in Calabria for the years 2016-2020 and duration of nesting seasons.

					Duration of nesting season (days) from		
Years	First attempt	First nest	Last attempt	Last Nest	First to last attempt	First to last nest	
2016	5 Jun	06 Jun	25 Aug	23 Aug	81	78	
2017	28 May	04 Jun	07 Aug	07 Aug	71	64	
2018	14 Jun	14 Jun	12 Aug	10 Aug	59	57	
2019	12 Jun	12 Jun	10 Aug	10 Aug	59	59	
2020	16 May	13 Jun	01 Sept	19 Aug	108	64	

Table 4. Mean clutch size (± S.D.; range) and the mean percentage of hatching and emergence success in nests found in Calabria during five years.

	Clutch size						
Years	n	Mean	± S.D.	Range	HS(%)	ES(%)	
2016	33	91	19.9	46-136	72.3	66.6	
2017	16	105	22.8	72-160	72.7	64.9	
2018	28	88	20.3	57-137	73.0	59.5	
2019	26	93	23.2	69-147	73.4	63.4	
2020	60	84	18.7	39-118	67.4	60.5	
Overall	163	92.2	21.1	39-160	70.8	62.5	

Table 5. Number of total eggs, hatched eggs, unhatched eggs, dead pipped and hatchlings that died inside the nest (HatchDIn) for five years (2016-2020).

Years	n	Total N Eggs	Hatched Eggs	Unhatched Eggs	Dead Pipped	HatchDIn
2016	33	3000	2169	723	108	44
2017	16	1680	1219	288	167	43
2018	28	2466	1788	543	135	35
2019	26	2415	1780	551	84	39
2020	60	5029	3391	1509	129	45
Overall	163	14590	10347	3614	623	206

Table 6. Mean incubation period recorded annually (±S.D.)

Years	n	Mean IP (days)	± S.D.	Range
2016	27	52	8.3	44-82
2017	15	47.3	2.1	44-52
2018	24	47.9	4.1	42-55
2019	24	47.3	2.5	44-53
2020	32	50.1	4.6	42-66

Discussion

Nesting activity

The Mediterranean coast hosts an average of approximately 7000 nests/yr concentrated in 52 major nesting sites located mainly along the East Basin (Casale *et al.*, 2018). In Italy, due to the lack of adequate monitoring in potentially suitable areas, the phenomenon of nesting is considered marginal and underestimated. Currently, the only area to be regularly monitored in the last 20 years is situated along the Ionian coast of Calabria. Previous data included 36 nests from the 1988–2004 period (Mingozzi *et al.*, 2007) and 261 nests with a density of 12 to 27 nests per year for the 2005–2014 period (Mingo-

zzi et al., 2016). Compared to the latter study, our study showed an increase in nesting activity: 180 nests over 5 years (2016-2020), with a minimum of 18 for 2017 and a maximum of 62 for 2020. This is probably due to the use of an effective monitoring method that included the use of new technologies (fat bikes and drones), making it possible to expand the regularly monitored area and providing a greater awareness campaign in recent years. This finding is particularly evident in the 2020 reproductive season for Capo Bruzzano-Marina di Gioiosa. The survey activity is aimed at identifying and protecting the largest possible number of nests, and ensuring the entry into the sea of the largest number of hatchlings. Without a regular monitoring program, many nests could be destroyed due to recreational and tourism activities related to coastal development and unlawful practices (e.g., use of heavy beach cleaning machinery, driving on the beach).

Nesting success: potential factors that influence the site selection

The start and end of the nesting season are highly cyclical and are related to favourable environmental conditions. Several studies have shown that the sea temperature near nesting sites provides a stimulus for nesting (Sato *et al.*, 1998; Weishampel *et al.*, 2004; Mazaris *et al.*, 2009). Overall, the start date is comparable to the dates recorded from Greece (see Margaritoulis, 2005). Interannual fluc-

Table 7. Reproductive parameters of sea turtle Caretta caretta in Mediterranean Sea, modified from Casale et al. (2018).

	Clutch size Mean ± SD or range of means	IP (days) Mean ± SD or range of means	HS (%) Mean ± SD or range of means	References
Italy				
Calabria	92.2 ± 21.1	49 ± 5.4	70.8	Present study
Calabria	99 ± 5.85	46	86	Mingozzi et al. (2007)
Sicilia	97.9 ± 12.5	70	27.4	Casale et al. (2012)
Linosa		47		Corti et al. (2011)
Lampedusa		67	81.5	Prazzi et al. (2010)
Cyprus				
Algadi	70 ± 21.7	$48 \pm 2.9\ 51.8$	79.1 ± 20.8	Godley et al. (2001)
Northern Karpaz		51.8		Ilgaz & Baran (2001)
Egypt	64	48.1–53.5		Campbell et al. (2001)
Greece				
Bay of Chania	110.3-117.1	53.3-54.3		Margaritoulis et al. (2003)
Bay of Messara	108.1			Margaritoulis et al. (2003)
Kefalonia	99.8-120.4	54.9		Margaritoulis et al. (2003)
S. Kyparissia Bay	105.2-126.8	48.1–53.9		Margaritoulis et al. (2003)
Lakonikos Bay	107.1-126	52.1-59.3		Margaritoulis et al. (2003)
Rethimo	102.0-124.6-190	51.7-55.2		Margaritoulis et al. (2003)
Zakinthos	116.5	51.3-69.8	71.5	Margaritoulis et al. (2005)
Israel	82	54		Silberstein & Dmiel, (1991
Lebanon	72.7			Newbury et al. (2002)
Libya	33–105	47	33.3–95.9	Margaritoulis et al. (2003)
Spain	97	41.2		Tomás et al. (2002)
Tunisia	87.85	64		Bradai & Jribi (2010)
Turkey				
Dalyan	72.3–79.7	52.3	60.4	Türkozan & Ylmaz (2008)
Dalaman	79	49.3	79.7	Kaska <i>et al</i> . (2010)
Fethiye	80.7	56	59	Türkozan (2000)
Patara	68.20 ± 20.75	50	44.05	Olgun et al. (2016)
Kizilot	78.5–79.7	49.8–59.6	63.5	Türkozan (2000)
Anamur	76.4	49.9		Uçar et al. (2012)
GoksuDeltasi	71	53	20.1	Durmuş et al. (2011)
Akyatan	71.8 ± 5.3	50.8 ± 3.4	53.9	Yilmaz et al. (2015)

tuations in nesting efforts are common in Mediterranean loggerhead populations, and these have been recorded in some studies (e.g., Broderick & Godley, 1996; Turkozan, 2000; Margaritoulis & Rees, 2001). The fluctuations are probably the result of the specific reproductive characteristics of sea turtles; most females do not nest every season and show irregular remigration patterns, while a portion of females nest several times within the same season (Dodd, 1988). However, nesting success provides a measure of the difficulties encountered by turtles during nesting procedures. In the present study, the mean value of this parameter was 45.8% (range: 36.4–64.3), similar to what has been reported in the past for the same

area (43.6%; Mingozzi *et al.*, 2007) and from other major Mediterranean rookeries (38.6%; range: 28.6–57.6; Margaritoulis & Rees, 2001; 25.7% Margaritoulis, 2005; Turkey 26.5%, Durmuş *et al.*, 2011; 32.34% Olgun *et al.*, 2016). Many factors could influence nesting success, and the identification of possible cues driving nest site selection has received considerable attention (Miller, 1997). Results from a series of studies have identified several physical and chemical parameters associated with nest site location (e.g., width, slope, and vegetation cover of the beach, salinity, sand particle size, pH, organic content, conductivity, water content, and sand temperature) (Mortimer, 1995; Garmestani *et al.*, 2000). For example,

in Greece, a study conducted on the largest colony of Zakynthos Island identified that, among a series of (both biotic and abiotic) factors, beach width and the objects present at the back of the beach were significantly correlated with nesting behaviour (Mazaris *et al.*, 2006). In the study area, comprising from Capo D'Armi to Capo Bruzzano, coastal erosion and dynamism of the beaches are probably the factors that could most influence the nesting attempts. Coastal erosion leads to a reduction of the beach (especially of the width) with the consequent reduction of the favourable micro-environmental variations that characterise the wide beaches. The high dynamism mainly brings about a change in the texture of the sand which changes the degree of humidity, thus influencing the construction of the egg chamber.

Moreover, the current development activities and increased utilisation of coastlines have come together with new threats for coastal biodiversity (Lotze *et al.*, 2006; Coll *et al.*, 2012) and for the organisms which temporarily use coastal habitats (e.g., marine mammals, seabirds, estuarine fish, and sea turtles) for resting, foraging, or reproduction (Dimitriadis *et al.*, 2018). The presence of artificial night lighting, bathing establishments, and anthropogenic features present on the beach near small urban settlements for the entire breeding season could discourage females in emergence. However, now this is a hypothesis and further years of monitoring are required to arrive at a measure of the degree of impact that human activities have on the study area.

Reproductive parameters

We calculated that the mean clutch size in Calabria was 92.2 eggs while the mean incubation period and the mean hatching success rates were 49 days and 70.8%, respectively, over five seasons. We include a summary table for the reproductive parameters (Table 7) recorded in other Mediterranean nesting areas and reported by Casale *et al.* (2018) (Supplementary Table S15).

The clutch size from our study is like that reported in previous studies for the same area and for other Italian nests. The variation in clutch size is the result of body size differences, and the loggerheads nesting in Greece are larger than those nesting colonies in Turkey and Cyprus (see Margaritoulis, 2005). The same considerations can be made for females nesting in Calabria, whose dimensions (mean Curved Carapace Lenght = 75.0 cm, from six nesting females) are comparable to the females nesting in Cyprus and Turkey (see Table S17 of the Supplementary in Casale et al., 2018). In this study, the mean incubation periods for each year were between 47.3 and 52 days, with a minimum of 42 days and a maximum of 82 days. Incubation duration is directly proportional to nest temperature, which is a parameter subject to seasonal variations, latitudinal variation, sand colour, and episodic events such as rain and depth of eggs. Substantial differences in incubation period exist among the other nesting beaches in Italy, particularly between the duration of incubation recorded in Sicily (including Lampedusa) and that in Calabria, while small differences are evident with the rest of the major Mediterranean sites.

Hatching success differed seasonally, but no trend was detected. Only for the 2020 reproductive season, we recorded a particularly low value, considering the average of the other years. This could be because of cyclone "Ianos" which in September affected the coast causing the flooding of some nests and consequently arresting embryonic development. Another important factor that can influence the number of hatchlings that reach the sea is of night lighting. For the nests found during the monitoring, this problem was solved by shielding the nest with nets and continuously checking the nests during the nights of emergence. For undetected nests, this represents the most severe threat encountered in the area. In these cases, the hatchlings are often found to be disoriented, dehydrated, or crushed by cars.

Conclusion

In Calabria, the high coastal extension with beaches potentially suitable for nesting, especially on the Ionian coast, and the number of nests found in 2020, resulting from an increase in the monitoring effort, suggest that the nesting activity is certainly becoming much more important. A study on climatic niche models showed that our area has good climate suitability which will most likely become more suitable in the future (Almpanidou *et al.*, 2016). Therefore, to further understand the phenomenon, it would be desirable to continue and extend the monitoring to larger sectors and to detect the factors that could influence the success of nesting, such as beach characteristics, the impact of temperature, and weather conditions. However, the effort, in terms of both personnel involved and economic resources, is not insignificant.

In addition, it is also known that small populations are particularly relevant in the conservation of species, as they provide an important contribution to overall genetic diversity (Eckert *et al.*, 2008). Furthermore, conservation measures focusing on the improvement of hatchling recruitment success can be very beneficial for the turtle population (Mazaris *et al.*, 2009b). Finally, the consideration that low abundance sites have good recovery potential that may drive long-term population increase (Mazaris *et al.*, 2017) is particularly encouraging for our conservation work of finding and protecting nests over large areas. We believe that the attention for this minor reproductive site should be maintained and strengthened.

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