



Nesting of *Caretta caretta* in Tuscany area (north-western Mediterranean Sea): insights of a recent colonisation phenomenon

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From 2013 to 2021 twenty-three *Caretta caretta* (Loggerhead turtle) nests were found along the coast of Tuscany (Italy, north-western Mediterranean Sea). Loggerhead nesting is a new event for this part of the Mediterranean Sea, which occurred only recently. Laid eggs were 2081 with 943 hatchlings, for an average hatching success of 45.31%, ranging from 0.1 to 95%, depending on the year and the site. After hatching, nest inspection was carried out and, when possible, eggs and hatchlings (dead and alive) were measured (e.g. curved carapace length with calipers in mm, body mass, with an electronic balance in grams) and standard environmental parameters at the nests were registered (e.g. nest chamber depth and width, grain size, sand temperature). We strongly recommend accurate monitoring and observations of the beaches along these coastlines to establish other nesting occurrences of loggerhead turtles in this region.

Keywords: loggerhead turtle, reproduction, clutch size, Tyrrhenian Sea

INTRODUCTION

Sea turtles are included in Directive 92/43/EEC "Habitat", implemented with DPR 357/97 and modifications, which provides specific rules on capturing, handling and detaining these animals. The inclusion of sea turtles in Annex IV of the Directive also provides the obligation on the part of the Member State to carry out monitoring and surveillance activities of their state of conservation, as well as their accidental capture or killing (Mo et al., 2013). From a conservation point of view, *Caretta caretta* is listed in Appendix 1 of CITES and in appendix II of Bern convention (1979). According to the IUCN it is Least Concern at Mediterranean subpopulation level (IUCN Red List; www.iucnredlist.org: Table 1, download 25 november 2020).

Nesting of marine turtles in the Mediterranean basin has been verified and monitored for decades. Main areas of nesting are in Greece, Turkey, Libya and Cyprus, but also reported in the western basin (Tomas et al., 2002), while feeding areas are the Tunisian continental platform, the Adriatic and Ionian seas, between the Balearic Islands and the Alboran Sea, the Egyptian continental platform and the Turkish coast (Casale et al., 2018). The species occurs

also in all the other marine areas of the Mediterranean, even if with lower frequency (Argano, 1992; Mingozzi et al., 2007; Casale et al., 2018). Available information regarding two breeding populations of marine turtles, the loggerhead turtle *Caretta caretta* and the green turtle *Chelonia mydas*, nesting in the Mediterranean has been greatly improved during the past two decades (Margaritoulis et al., 2003; Casale & Margaritoulis, 2010). Nevertheless, there are major knowledge gaps that still limit our capacity to accurately assess the conservation status of both species and predict the consequences of anthropogenic stressors, particularly fisheries bycatch and climate change.

In the Mediterranean Sea, loggerhead turtle *C. caretta* nesting sites are concentrated in the eastern and central basins, whereas the waters of the western basin host a large number of turtles for feeding activities in pelagic and coastal habitats (Marquez, 1990; Margaritoulis et al., 2003).

Sea turtle nesting in the western Mediterranean (i.e. southern Tyrrhenian Sea, as in northern Sicily, southern Calabria) has been reported since the early 1990s, but it was always referring to single sporadic events, widely scattered over the coasts bordering the basin. However,

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since the 2010s, reports of nesting activity have been increasing and, at least in some areas (i.e. northern Campania, Latium, Tuscany), new nesting sites have been reported (Mingozzi et al., 2007; Hochscheid et al., 2022).

Available data do not allow demonstrating historic nesting of the loggerhead on the Tuscany coasts. According to anecdotal information and from oral enquires and testimonies, it is thought that no one marine turtle has ever nested on the sandy coasts of this region in the last century. Also after widespread literature check, no substantiating evidence could be unearthed.

We aimed at describing the new *C. caretta* nesting phenomenon for the Tuscany area, as for nest geographical distribution, nest characteristics, clutch, egg and hatchling size, hatching success and incubation temperatures.

MATERIALS & METHODS

A probable loggerhead turtle hatching was registered in Tuscany for the first time on 3 October 2013 when 22 hatchlings were seen on the beach in Scarlino (Grosseto). On that occasion, no nest was found. Another loggerhead turtle nest was discovered accidentally on a beach in Giannella (Grosseto) on 6 September 2015, 50 km south from the 2013 nest. About 30 hatchlings were seen on the beach crawling seaward in the early morning. Later also the emergence point was discovered, where hatchlings tracks originated. It was monitored for some days and it was inspected on 10 September.

Based on these sporadic events, from 2017 on, we carried out a standardised beach monitoring routine from early June to late August, along the several beaches located along the southern and central part of Tuscany coasts. We verified nesting attempts by checking the presence of loggerhead tracks on the sand, characterised by the typical 'U' shaped trace, as outcome and income from the sea. Depending on positive or negative results from inspection at the direction change of the turtle track, we assessed the false or actual nesting crawl. Due to the intensification of the nesting events during the recent years, beach monitoring (daily on foot or with fat bikes, and weekly with drones), was carried out on along the entire Tuscany coast, including the northern part, according to rules established by the authorisation of the Italian Ministry for Ecological Transition in derogation to DPR 357/97 (Mo et al., 2013).

As soon as a track or a turtle is detected along the beach, either during the night or early morning, the Sea Turtle Stranding Network (STSN) is in charge of verifying the presence of the nest. Location data (e.g. latitude and longitude, distance from the sea and the dune in m) were registered. We placed data logger (EasyLog USB EL-USB, www.lascarelectronics.com), with USB interface for configuration and download, under the sand at a depth of about 40 cm, close to the nest chamber, for continued registration of the temperature. The user-friendly control software window allowed setting the registration of temperature at regular intervals, every 30 minutes. The data could then be graphed, printed and exported to other applications for detailed analyses. We also registered

surface sand temperature using a digital thermometer (Hanna Checktemp; www.hanna.it), with a resolution of 0.1 °C and an accuracy of ± 0.2 °C. Sampling of sand for grain-size investigation was conducted near the nesting site. A 1000 g sand sample was collected within a plastic jar. The analysis was conducted following ISPRA guidelines (Romano et al., 2018). Three-dimensional classes were determined: gravel (> 2 mm), sand (< 2 mm > 0.063 mm), silt (< 0.063 mm) (Krumbein, 1934).

If the exact moment of the deposition is known, we consider a period for hatching between 45.6 to 70.0 days (6.4 to 10 weeks), as estimated for the Italian rookeries (Casale et al., 2018); 6-10 weeks of incubation, depending on the temperature, was reported also by Miller (1985).

Hatching is expected to occur during night-time, when an opposite temperature gradient is recorded: temperature under the sand is a little bit higher than on external sand (Matsuzawa et al., 2002). Therefore, from the 42nd day the nest will be surrounded by a fence against any possible disturbance or damage, and a corridor for hatchlings to reach the sea will be realised. From this moment, the nest has been watched and monitored by volunteers for 24 hours. During this period, we also registered superficial temperatures periodically during day and night. When a little subsidence does appear on the sand surface, it means that hatchlings are outside their eggs, provoking the sand to collapse among and around the clutch, and indicating they are close to emerging on the sand surface (pers. obs.). Within a maximum of 24 hours, the little turtles will be on the beach, crawling seaward. They perhaps will continue coming out from the sand, during the night, for the following two–three days. Each night, the time and number of hatchlings were recorded. No hatchlings were handled, nor disturbed during their crawling seaward. Only red light was used for observation, to avoid any stress or disturbance to the hatchlings.

We calculated incubation times (in days) for nesting events whose exact date of deposition and emersion were known (Ackerman, 1997; Matsuzawa et al., 2002; Miller et al., 2017; Watson & Lamont, 2022).

After three–four days from the last emergence of the last hatchling from the sand, the nest was inspected, digging it out to recover the hatched eggs, annotating the date of inspection. All the content of the nest was catalogued and the number of hatched and unhatched eggs, pipped eggs with dead hatchlings, and eggs with partially developed embryo and eggs with no discernible embryo were registered (Eckert et al., 1999). With a calliper, the diameter of unhatched eggs was measured in cm and their mass registered using a digital electronic scale with a precision of 0.1 g. In addition, the curved carapace length (cm) and total body mass (g) of dead and the still living hatchlings found in nest were recorded. Physical characteristics of the eggs chamber (e.g. depth and width) were measured.

For each nest, emersion success (ES percentage, number of emerged hatchlings/total number of eggs), and hatching success (HS percentage, hatched eggs/total number of eggs) were evaluated (Eckert et al., 1999).

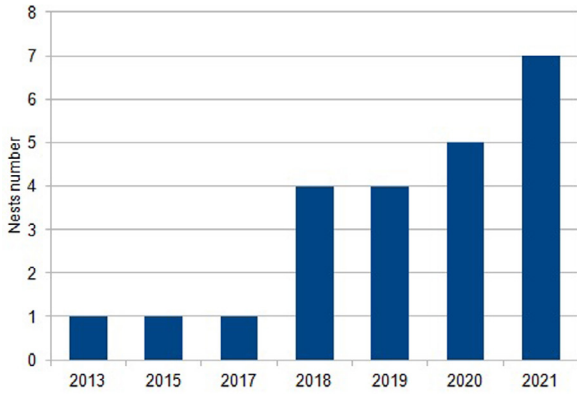


Figure 1. Number of nests per year

In most of the nesting events, we collected samples to perform several analysis: bacteriological, virological, pollutants and contamination on unhatched eggs; others on morphology (Zuffi et al., 2023) and genetic (Tolve et al., 2023) were conducted on dead embryos and pipped. Development of whole genome sequencing techniques now allows the characterisation of whole mtDNA genome sequences, which can provide a much more comprehensive scenario of loggerheads' phylogeographic history.

Considering chemical contamination among possible threats to hatching success, toxicological investigations were conducted on biological material sampled from four nests laid along the Tuscan coast during summer 2019. Egg contents, embryos, and embryonal annexes were analysed to evaluate the presence of organochlorine compounds such as DDTs, 30 of 209 congeners of PCBs and HCB using Marsili & Focardi (1996) analytical method (Ceciarini et al., pers. obs.).

All the operations were carried out within the Cetaceans, Elasmobranchs and Sea Turtle Stranding Network co-ordinated by Tuscany Observatory for Biodiversity and following ISPRA Guides Lines (Mo et al., 2013).

RESULTS

During the period 2013–2021 along Tuscany coasts, we detected 23 *C. caretta* nests. Despite having been a sporadic and limited phenomenon (only one nest per year in 2013, 2015, 2017, no nests in 2014 and 2016), from 2018 nesting events established in Tuscany with an increased number of nests in each year (Fig. 1). All the data recorded are summarised in Table 1. Geographical distribution of the beaches chosen by turtles along the Tuscany coast indicates a preference for the southern part and the Elba Island (Fig. 2). From 2016 on, we also registered 29 attempts of nesting with an extension toward the north of the region (Fig. 3). Nine out of the 23 nests, potentially in danger because of the short distance from the shoreline, were relocated on the same beach or the nearest beach with similar characteristics at a greater distance from the maximum known line of storm surge. Distance from shoreline was registered for 15 nests and it ranged from 7.7 m to 24.2 m (mean 14.5 ± 5.5 m). The relocated nests were initially located at 7.7 m and 15 m from the water.

Grain size analysis performed for ten nests showed that sand class ranges from $< 2\text{mm}$ to $> 0.063\text{ mm}$ were predominant, with 67% of occurrence. Temperature profile recorded for seven nests are shown in Figure 4. Temperature for eggs incubation ranged from 26.8 to 32.5 °C. Temperature has no normal distribution (Kolmogorov Smirnov test = 17.360, $P < 0.0001$), with significant differences among nests (Kruskall-Wallis = 4602.809, 6 df, $P < 0.0001$) (Fig. 4). Pair-wise comparisons underlined that only Santa Lucia1 2018, Rimigliano 2018, and Cecina 2019 nests were similar (Santa Lucia1_2018 vs Rimigliano_2018 $P = 0.458$; Santa Lucia1_2018 vs Cecina_2019 $P = 0.997$; Rimigliano_2018 vs Cecina_2019 $P = 0.520$).

We showed nest temperatures and incubation period, calculated for seven nests (according to Watson & Lamont, 2022) in Table 2a. Incubation period varied from a minimum of 47 days in 2019 to a maximum of

Table 1. Data of the Tuscany nests of *Caretta caretta* (NA = not available; measures of hatchlings and eggs were presented as mean ± 1 SD)

	2013	2015	2017	2018	2019	2020	2021	Total
Number of nests	1	1	1	4	4	5	7	23
Number of eggs	NA	72	118	372	375	479	665	2081
Number of hatchlings	NA	60	99	55	164	131*	68**	378
Hatchling success (%)	NA	87.5	87.3	46.5	47	25.4	12.0	
Emergence success (%)	NA	83.33	83.90	13.39	44.96	31.00	80.00**	
Hatchlings measured	NA	3	4	17	3		4	31
Hatchlings carapace curve length (cm)	NA	3.6 ± 0.06	4.6 ± 0.31	4 ± 0.57	3.7 ± 0.25		4.1 ± 0.20	30
Hatchlings mass (g)	NA	15.3 ± 0.58	15.6 ± 1.21	13.3 ± 1.60	14.2 ± 3.27		14.8 ± 0.06	24
Number of eggs measured	NA	9	14	197	204	125	13	569
Egg diameter (cm)	NA	3.6 ± 0.07	3.4 ± 0.60	3.4 ± 0.21	3.7 ± 0.19	3.9 ± 0.27	3.6 ± 0.17	535
Egg mass (g)	NA	25.4 ± 3.36	22.1 ± 6.80	23.2 ± 2.60	22.5 ± 4.73	18.9 ± 3.12	19.6 ± 5.89	544

*partial, only for 3 nests **partial, only for 1 nest

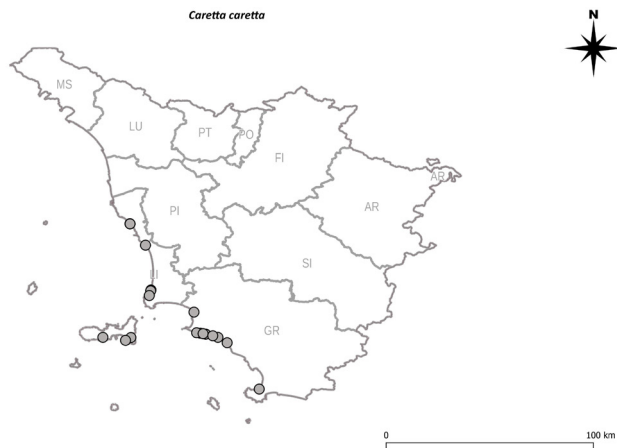


Figure 2. Geographical distribution of *Caretta caretta* nesting in Tuscany (and provinces) during the period 2013–2021

74 days in 2020, with a mean duration of 59 days; the nesting period ranged from 10 June to 6 October (Table 2b). As to the nests from which we collected temperature data ($n = 7$), we applied the algorithms by Ackerman (1997), Matsuzawa et al. (2002), Miller et al. (2017) and Watson & Lamont (2022) to calculate the expected date of emergence (Table 2b), verifying the suitability of the used algorithms.

No hatching was recorded in six nests. Hatchings success (HS) averaged 51% (range 0% to 95%) and emergence success (ES) averaged 40% (range 0–84%). It was not possible to access to the material contained inside the eggs chamber during or after the inspection procedure for six out of the seven nests laid in 2021.

We had a total of 2081 eggs laid and 943 hatchlings. The number of eggs laid ranged from 64 to 127 with a mean value of 95 eggs. For the ten studied nest chambers, eggs features were as follows. Diameter of eggs was on average 3.59 ± 0.27 cm ($n = 410$, from 3.43 ± 0.15 cm for Santa Lucia1 nest to 4.11 ± 0.21 cm for Rimigliano nest; min range 2.4 cm, max 4.9 cm). Egg size was significantly different among nests (ANOVA, $F = 56.299$, 9 df, $P < 0.0001$). Egg mass was 22.7 ± 4.04 g ($n = 419$, from 19.61 ± 5.89 g for Morcone nest to 31.42 ± 2.58 g for Capoliveri nest; min range 18 g, max 35 g). Egg mass was significantly different among nests (ANOVA, $F = 6.333$, 9 df, $P < 0.0001$).

Hatchling carapace curve length (CCL) was 4.05 ± 0.52 cm ($n = 31$, 21 alive and 10 dead; min 3.4 cm, max 6 cm) and mass was 12.35 ± 4.61 g ($n = 24$, min 9.9 g, max 17.8 g). On the dead specimens and on the term embryos found inside the eggs, 34 samples were taken for genetic analyses and 102 for insights about development stage (Miller, 1999; Zuffi et al., 2023).

The genetic analyses conducted has revealed two different mitochondrial haplotypes: one is CC-A2.1, which is predominantly Mediterranean strain, but also Atlantic CC-A3.1, which is a newly identified strain. HCB, DDTs and PCBs were present in all samples analysed. Even though the mean values of the contaminant classes were different in each nest, all samples had the same concentration

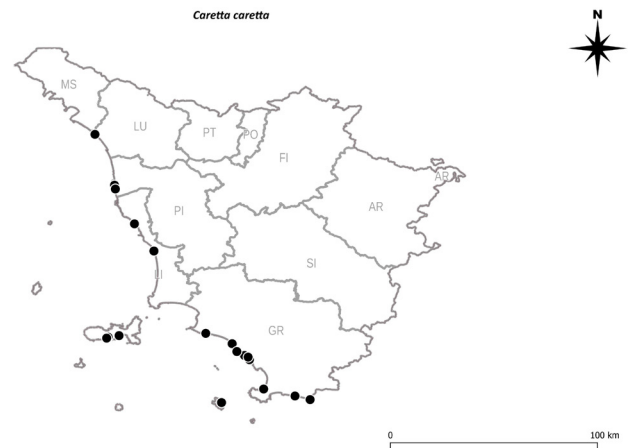


Figure 3. Geographical distribution of *Caretta caretta* nesting attempts in Tuscany area (and provinces) during the period 2016–2021

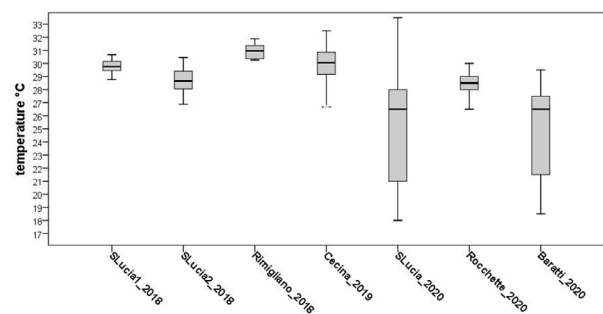


Figure 4. Temperatures values (median, interquartiles and extremes) in the studied nests

ranking: PCBs > DDTs > HCB. Even if these xenobiotic contaminants are strongly banned and regulated by several laws, it is important to investigate their presence in the marine biota, mostly because they can potentially affect with adverse effects living organisms, due to their persistence in marine environments. Nevertheless nowadays, a direct correlation between contaminant levels and hatchling success does not exist (Ceciarini et al., pers. obs.).

DISCUSSION & CONCLUSION

The Cetaceans, Elasmobranchs and Sea Turtle Stranding Network (CESTSN) in Tuscany (Italy, north-western Mediterranean) has been operating since 1980. More recently, it was implemented thanks to the former Tuscany Observatory for Cetacean, now Tuscan Observatory for Biodiversity (Osservatorio Toscano Biodiversità, OTB) sensu art. 11 L.R. 30/2015.

This experience of the Tuscany region represents a real contribution to the international effort for the conservation of the sea and its resources. This region has created a co-ordinated and synergic network among its members, which are ARPAT (Environmental Protection Agency, Tuscany Region), IZSLT (Experimental Zooprophyllactic Institute, Latium and Tuscany) universities, research centres, museums, aquaria,

Table 2a. Average nest temperature (nest T), standard deviation (SD), range (minimum and maximum) values, and the average nest temperature within the 5–50 incubation days range (Watson & Lamont, 2022) (* = data for the 5–50 days interval not available)

Nest id	Year	Nest T (°C)	SD	Range °C	Average T, 5-50 day (°C)
Slucia1	2018	29.27	0.66	27.37-33.22	*
Slucia2	2018	28.69	0.86	26.9-30.5	*
Rimigliano	2018	30.59	1.26	27.1-31.9	*
Cecina	2019	29.83	1.46	25.3-32.5	29.96
Slucia	2020	25.35	3.59	18.0-33.5	27.07
Rocchette	2020	28.14	1.33	23.0-33.0	28.70
Baratti	2020	25.08	3.41	18.5-29.5	27.10

environmental associations and fishermen. In Tuscany, the presence of the three species of sea turtle (*Caretta caretta*, *Chelonia mydas*, *Dermochelys coriacea*) is reported and a great effort has been made during past and recent years to recover stranded and by-caught animals, dead or alive (Meschini, 1998; Meschini et al., 1998; 2000; 2006). Our data integrate a much recent assessment of nesting expansion of *C. caretta* in north-western Mediterranean waters (Hochscheid et al., 2022). They support the speculation that this region may have once hosted a nesting population of loggerhead turtles. These records are some of the very rare nesting occurrences in the western Mediterranean (Tomàs et al., 2002; Gonzalez-Paredes et al., 2021; Hochscheid et al., 2022). Among the Italian records of this study, they represent the northernmost-recorded nesting area in the Mediterranean until 2021 (Delaguerre & Cesarini, 2004; Sénégas et al., 2008; Hochscheid et al., 2022). Our records of nesting attempts, many of them failed because of human disturbance during the turtle activity on the beach, should be considered as a recent and new

behavioural pattern shown by loggerhead turtles in this part of the Tyrrhenian Sea. This behaviour corroborates the recorded nesting activities. Further observations of beaches are strongly recommended to establish other nesting occurrences of loggerhead turtles in this region. Reports on occasional nests in the western Mediterranean appear to have become more frequent recently and although the available data are still too few, a possible connection to global warming should not be excluded: a rise in temperature of the western basin can modify known sea turtle behaviours.

Different incubation temperatures among nests suggest that, at least from this dataset and considering the pivotal temperature for sex determination (e.g. 29.3 °C, Mrosovsky et al., 2002; 28.7 °C, Ackerman, 1997), that some nests could have produced more males than females and some others more females than males.

Comparing the observed vs the expected incubation period (Table 2b), it is evident there are discrepancies among the several estimation algorithms.

Incubation period varies among populations and with beach latitude. For the Tuscany nests, the range was between 47 and 74 days, and fell within that reported by Casale et al. (2018) for Italian seas (45.6–70 days). However, these authors report a hatchling emergence success ranging between 24.7% and 86%, while our data represent higher maximum values and the lowest minimum value (see Hochscheid et al., 2022).

Diameter of eggs of 3.6 cm was a little bit smaller than reported in literature for Mediterranean basin (Turkey: 3.9 cm, Marquez, 1990) and elsewhere (4.5 cm, Marquez, 1990; 4.1 cm, Miller, 1997). Egg weight measurements reported in literature are less frequent than those regarding diameter, and available data outside the Mediterranean area range from 26.2 to 46.8 g (Marquez, 1990; Miller, 1997), higher than our mean value of 22.7 g. This could be related to a smaller reproductive female size in this geographical area. Size and weight of hatchlings are considered to be correlated directly with the size of the eggs. In Turkey, straight carapace length (SCL) of 3.99 mm (from 3.7 to 4.2 cm, n = 50) have been reported (Marquez, 1990); for Atlantic and Pacific Oceans

Table 2b. Deposition date, emergence date, incubation period and the expected incubation period, according to main references (with % estimated error vs observed incubation days, where possible), among the studied nests

Nest id	Year	Deposition date	Emergence date	Incubation period (days)	Expected incubation	Expected incubation	Expected incubation	Expected incubation
					Ackerman, 1997	Watson & Lamont, 2022	Matsuzawa et al., 2002	Miller et al., 2017
Slucia1	2018	28 July	23 September	57	58.7 (2.98)	/	54.8 (3.86)	51 (10.52)
Slucia2	2018	9 August	----	/	62	/	57.6	54
Rimigliano	2018	10 June	28 July	49	54.2 (10.6)	/	49.2 (0.4)	48 (2.04)
Cecina	2019	25 June	----	/	52	64.5	52.4	50
Slucia	2020	15 August	----	/	/	70.9	82.5	83
Rocchette	2020	25 July	6 October	74	65 (12.16)	67.3 (9.05)	60.9 (17.70)	60 (18.91)
Baratti	2020	11 August	----	/	/	70.8	85.3	84

total mass range was 18.8–21.2 g (Marquez, 1990; Miller, 1997). Our data show slightly bigger hatchlings (4.05 cm CCL) with a lower mass (12.3 g).

Mean number of laid eggs was 95 (range of 64–127 eggs) for our geographical area, while Marquez (1990), for the Mediterranean basin, reported 83 for Greece and 93 for Turkey. Casale et al. (2018) reported 97.9–99 eggs as range of means for Italy; for the Mediterranean basin, the minimum registered was 64.3 eggs and the maximum 126.8. For oceans, Miller (1997) and Marquez (1990) reported 106.5 and 112.4 eggs respectively.

In conclusion, this study relates to a new nesting area of loggerhead turtle *C. caretta*, and shows how important it is to investigate this marine reptile and its nesting activity in areas with little information, to undertake appropriate conservation management measures in the near future.

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