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Caretta caretta Embryo Development and Nest Success Rate Study

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Abstract

This study investigates the nesting of *Caretta caretta* in the Maltese Islands (Central Mediterranean), focusing on embryo development, nest success rates, and the impact of anthropogenic activities. Ten Loggerhead turtle nests discovered between 2020 and 2023 were assessed. Clutch sizes (Mean 85 eggs and St.Dev. ± 14) and nest success rates (Mean 59% and St.Dev. ± 26.7) in these islands were found to be comparable to those in Sicily and Libya, but lower than those in Greece. Clutch sizes may be affected by anthropogenic disturbance where some female turtles seek different nest sites to lay their eggs. The variation in success rates may be attributed to various factors including temperature, heavy rainfall and anthropogenic disturbance. Unhatched embryos were often in the final stages of development when excavated 2 days after first emerging nestlings, underscoring the importance of waiting at least ten days after the first emergence of hatchlings before excavating nests to avoid disturbing potentially viable developing eggs. Morphometric comparative analysis of body-part ratios of remaining dead nestlings revealed various statistically significant differences among specimens from different nests derived from different parents, while no differences were observed among specimens from nests produced by the same parents, as discovered by separate genetic studies. This research highlights the critical need for increased turtle nest research and management to improve the reproductive success and conservation of *Caretta caretta*.

Keywords:

Loggerhead sea turtle, turtle nestling development, central mediterranean, maltese islands.

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Introduction

The Loggerhead Sea turtle (*Caretta caretta*) is listed as 'Vulnerable' in the IUCN Red List and of 'Least Concern' in the Mediterranean (Casale et al., 2018). As an Annex II species in the EU habitats directive, it requires conservation management. Major nesting sites in the Mediterranean are in Greece, Turkey, and Cyprus, with occasional nests in other countries including Malta (Camiñas-Hernández et al., 2020). Climate change, particularly global warming, affects marine turtles by altering their reproductive and life history traits, leading to shifts in nesting ranges and phenology (López García, 2021). Sea turtles are long-lived and slow-maturing species adapting slowly to the rapid anthropogenic changes (Patricio et al., 2021). It is therefore crucial that turtles are assisted by improving access to suitable sandy beaches for nesting for the species' survival. Female sea turtles require sandy beach habitat where the eggs may develop undisturbed for the whole duration of incubation (typically around 60 days) (Lyons et al., 2020). Nest site selection may range between safety and flooding or erosion risks when too close to the sea (Hays & Speakman, 1993) and desiccation and depredation risk if too far inland (Witherington & Bjorndal, 1991).

Clutch sizes (total number of eggs in a nest), vary by location and are influenced by environmental and anthropogenic factors that cause disturbance to the female sea turtle such as noise, sound and light pollution, and the presence of crowds and their activities on the beach at night when turtles usually come up to lay their eggs (Camiñas-Hernández et al., 2020). In Greece, the average clutch size is 116.5 eggs per nest, while in Libya it is 79.3 eggs per nest (Margaritoulis, 2005), while a separate study in Spain gave an average clutch size of 97 eggs per nest (Luna-Ortiz et al., 2024) and in Italy 92 eggs (Hochscheid et al., 2022). Nest success rates, which refer to the percentage of eggs that hatch, are influenced by various factors such as temperature, rainfall and anthropogenic disturbance. In Laganas Bay, Greece, the mean annual hatching success ranges from 61.7% to 80.2%, with an overall mean of 71.5% (Margaritoulis, 2005).

The Maltese Islands have been discovered to host Loggerhead sea turtle nests again since 2012, this was followed by various other nests found in sandy beaches of the Maltese archipelago in subsequent years including: 2016 (1 nest), 2018 (1 nest), 2020 (7 nests), 2021 (1 nest), 2022 (1 nest), 2023 (2 nests) and 2024 (8 nests). This shows an annual nesting trend since the 2020. These records also complement regional trends of increasing number of nests in the Central Mediterranean, such as in South-West Italy, South-East Sardinia, and North-West Tunisia (Hochscheid et al., 2022).

In the latest 2024 turtle nesting season, the first of eight nests was reported in mid-June at Mixquqa Bay (Golden Bay, Malta), followed by additional nests at Għajn Tuffieħa (Malta), Ir-Ramla l-Ħamra (Gozo), and Ġnejna Bay (Malta). The fifth nest was laid by the same turtle that laid the first nest of the season approximately a month earlier in Mixquqa Bay and the turtle that laid the 6th nest is the same one that laid the 8th nest (ERA, 2024a, 2024b, 2024c, 2024d, 2024e, 2024f, 2024g, 2024h). This was not the first time such an occurrence was recorded, as in 2020 genetic studies published by (Vella & Vella, 2023) have shown 2 egg-laying turtles had contributed to two nests each.

This study investigated the nesting activity and egg clutches' remains of Logger head sea turtles in the Maltese islands during the 2020-2023 nesting seasons. It considered all 10 nests found during this 4-year period in five different nesting beaches as shown in 2 including: Ramla Bay (Gozo), Fajtata Bay (Malta), Mixquqa Bay (Malta), and Għadira Bay (Malta).

Materials and Methods

Dead turtle nestlings' remains after hatching events between 2020 to 2023 in the Maltese Islands were studied, after they were collected by the Environment Resources Authority (ERA) and made available for research through research permits. The nest remains collected by ERA were frozen after excavation.

Nest remains from 6 nests in 2020; 1 nest in 2021, 1 nest in 2022, and two nests in 2023 were thoroughly examined in what represents the first comprehensive study of *Caretta caretta* nest remains in the Maltese Islands. Refer to Table 1.

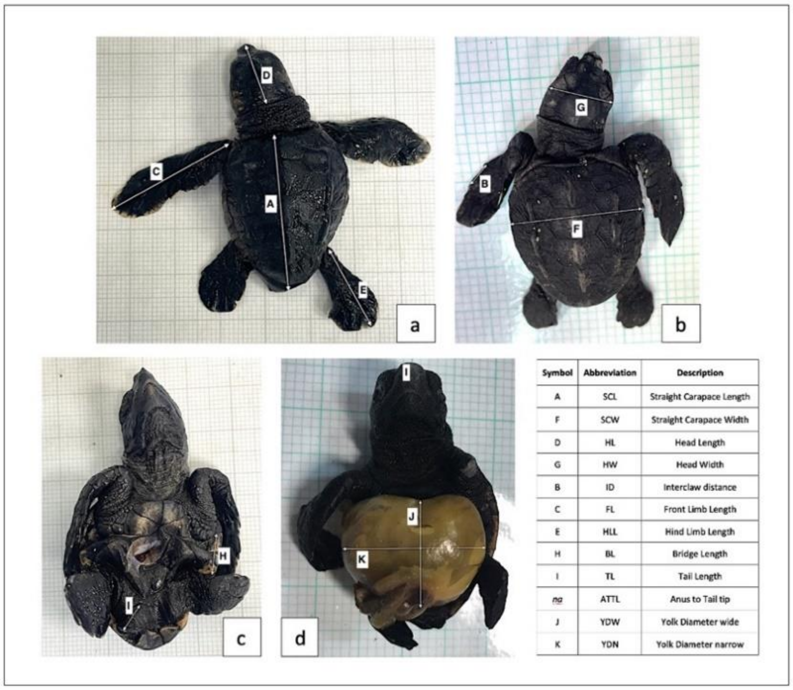
In the case of the 2022, the single nest found had its whole turtle clutch transferred to an artificial incubation unit under the care of ERA and an appointed NGO and its vet. This procedure did not produce any hatchlings and the remains of this clutch were forwarded to ERA after the NGO's vet undertook necropsies on the unhatched eggs. Due to these procedures, the specimens' remains suffered further deterioration prior to being made available for research, therefore only allowing for measurements of any identifiable anatomical features.

For each of the nests investigated, the first step was to establish the number of egg shells left behind from healthy hatched specimens and then work on unhatched eggs (unfertilised or fertilised and developing), partially hatched and hatched corpses in each of the nests. Considering this information, the success rate was measured as the number of successfully hatched individuals (number of empty shells found in the excavated nests) over the total number of eggs laid per nest (total number of unhatched eggs and eggshells in an excavated nest). Following this measure, the unhatched eggs were investigated in detail.

Each specimen egg was carefully cleaned to remove dirt or sand with a dry brush and weighed. Subsequently, the unhatched turtle specimen was extracted for analysis. The weight of both the specimen and its yolk were recorded in grams. In addition to the body weight, turtle size measurements of selected morphological features (Shown in Figure 1) were recorded in mm using calipers.

Embryological development research on sea turtles (including *Caretta caretta*) in the Mediterranean by (Kaska & Downie, 1999), shows the relationship between the size of morphological characteristics of an unhatched Logger head sea turtle to its embryonic developmental stage (Kaska & Downie, 1999). When considering this, one may estimate the stage of development reached by each of the dead unhatched turtles left in each of the nests investigated.

The body measures of dead turtles remaining in each nest, refer to Figure 1, allowed for the investigation of embryonic developmental stage similarities or differences between unhatched turtles from different nests laid on Maltese beaches in the 2020-2023 nesting seasons. This research also assesses the effectiveness of nest care and management that may affect nest success rates and conservation measures.



Symbol	Abbreviation	Description
A	SCL	Straight Carapace Length
F	SCW	Straight Carapace Width
D	HL	Head Length
G	HW	Head Width
B	ID	Interclaw distance
C	FL	Front Limb Length
E	HLL	Hind Limb Length
H	BL	Bridge Length
I	TL	Tail Length
na	ATTL	Anus to Tail tip
J	YDW	Yolk Diameter wide
K	YDN	Yolk Diameter narrow

Figure 1. Morphological features measured for dead turtle embryos and hatchlings (photos by adriana vella and isaak koroma, conservation biology research group-um)

Comparative analyses were undertaken after calculating body parts' proportions, to make these measures independent of the total size and the developmental stage at which the nestling ceased to develop. Four different body proportions were studied: 1) Straight Carapace Length (SCL) vs Straight Carapace Width (SCW); 2) Straight Carapace Length vs Fore Flipper Length (FFL); 3) Straight Carapace Length vs Hind Flipper Length (HFL); 4) Straight Carapace Length (SCL) vs Head length (HL).

For each of the nests, the proportions obtained were used to determine whether any distinctions or similarities in phenotypic characteristics emerged between nestlings from different nests. This was carried out to investigate whether there is a significant difference between the morphological proportions of turtles from different nests found in Malta and Gozo.

Four different body size proportions were studied for embryonic, or hatchling turtles sampled as indicated earlier. The ratios were calculated for each turtle studied. A dataset was prepared for each of the nests and tested for normality by a Shapiro-Wilk test. Following a positive test for normality, a series of unpaired two-tailed T-test were then performed to test whether there is a significant difference between the nests being compared or not. This type of T-test was chosen as one is comparing the means of two independent groups (Kim, 2015).

The hatching success of the Loggerhead turtle nests considered was measured as the number of empty shells divided by the total clutch size as determined during nest excavation by ERA and through examining of these nest remains in the laboratory during this study.

Figure 2. Map of discovered nests between the 2020 and 2023 nesting seasons. 1. Ramla Bay; 2. Għadira Bay; 3. Golden (Mixquqa) Bay; 4. Ġnejna Bay; 5. Fajtata Bay (Vella & Vella, 2023-Figure 1)

Figure 2 shows the 5 beaches in which nests were discovered between the 2020 and 2023. In 2020, nests were laid on each of the beaches shown on the map except beach 4. On the other hand, for 2021 and 2022, nests were only laid at Ramla Bay (Gozo). In the 2023 nesting season, one nest was laid on beach 1 and another on beach 4.

Results

The following results consider data collected from dead turtle nestlings between 2020 and 2023, in the Maltese Islands.

When considering all nests studied, the total percentage number of hatched (empty shells) nestlings composed 54%, dead unhatched embryos within their shells composed 38%, dead partially hatched embryos (still partly covered with their eggshell) composed 5%, and dead hatched corpses (out of their egg shells) composed 3% of the total clutches.

When each of the nest's data was investigated Table 1, the data was used to measure each nest's hatching success rate. Differences may be due to both anthropogenic disturbance and environmental factors (Margaritoulis, 2005). The average clutch size was found to be of 85 eggs (St.Dev ± 14) for the 10 turtle nests considered.

Table 1. Information on the nests laid in malta and gozo (2020 - 2023 nesting seasons)

NEST No.	1	2	3	4	5	6	7	8	9	10
Condition of Eggs	Mixquqa 09.10.20	Ghadira 25.09.20	Ghadira 09.10.20	Fajtata 03.09.20	Gnejna 18.08.23	Ramla 04.08.20	Ramla 09.09.20	Ramla 07.09.21	Ramla 19.08.22	Ramla 14.08.23
Hatched Eggs	76	16	70	29	17	82	57	75	0	32
Unhatched Eggs	11	61	12	28	31	19	40	8	70	0
Partial Hatch Eggs	5	2	4	20	0	0	1	7	0	0
Hatchling Corpse	0	0	0	20	0	1	0	0	0	0
Total No of Eggs	92	79	86	97	48	102	98	90	70	88
Success rate (%)	83	20	81	30	35	80	58	83	0	36

The Ramla Bay clutch of 70 eggs laid on the 19th of July 2022 was excavated on the 19th of August 2022 and taken to an artificial inncubation centre under the care of an NGO but none of these eggs finalised their development.

Tables 2 (a-b). show the different averages and standard deviations for each anatomical feature measured condusive to the estimation of the respective developmental stages for turtle embryos remaining in each nest.

Tables 2 (a & b). Average and standard deviation for body measures of dead specimens in nests after hatching period Malta (a) and Gozo (b). Locations: 1. Mixquqa 26.98.20; 2. Ghadira 25.09.20; 3. Ghadira 09.10.20; 4. Fajtata 03.09.20; 5. Gnejna 18.08.23; 6. Ramla 04.08.20; 7. Ramla 09.09.20; 8. Ramla 07.09.21; 9. Ramla 19.08.22; 10. Ramla 14.08.23.

Malta Nest

		Turtle nestlings average measures (mm) & Standard deviation for each nest											**Mean Dev. Stage**
Nest		**SCL**	**CCL**	**SCW**	**HL**	**HW**	**BL**	**FL**	**HIL**	**ID**	**TL**	**TL - AT**	
1	AVG (N-5)	32.0	43.2	27.0	17.6	12.5	10.7	27.9	17.4	7.5	5.8	1.9	27.0
	STDV	4.0	5.2	2.7	0.7	1.1	0.8	3.0	1.6	0.6	1.3	1.8	
2	AVG (N-47)	38.2	46.9	29.9	19.1	14.4	11.8	33.4	20.4	8.6	8.1	3.5	30.0
	STDV	1.3	1.8	1.2	1.1	1.1	0.7	0.9	0.8	0.7	0.7	0.4	
3	AVG (N-5)	36.7	49.4	30.3	19.4	14.7	11.3	31.9	20.2	8.3	7.8	3.5	30.0
	STDV	0.8	1.1	2.8	0.6	0.3	0.5	0.9	1.3	0.3	1.4	0.4	
4	AVG (N-3)	39.8	43.7	28.5	18.6	14.9	12.0	28.8	18.4	7.8	9.0	3.7	31.0
	STDV	0.4	2.1	1.8	0.2	0.4	0.9	2.0	1.2	0.3	0.5	0.4	
5	N1	35.0	na	26.4	17.7	13.4	11.8	23.5	20.2	8.2	7.0	3.7	28.0

Gozo Nests

		Turtle nestlings average measures (mm) & Standard deviation for each nest											**Mean Dev. Stage**
Nest		**SCL**	**CCL**	**SCW**	**HL**	**HW**	**BL**	**FL**	**HIL**	**ID**	**TL**	**TL - AT**	
6	N1	36.6	na	28.5	18.0	14.0	13.0	26.0	17.0	8.0	10.2	2.8	29.0
7	AVG (N-6)	35.0	48.3	28.4	18.9	13.3	12.5	29.8	19.4	7.9	6.0	3.0	29.0
	STDV	2.8	2.3	2.8	1.3	1.2	1.9	3.2	1.6	1.1	1.6	0.8	
8	AVG (N-7)	33.7	36.7	27.1	17.5	11.8	10.6	27.4	18.8	7.5	7.7	1.7	28.0
	STDV	5.2	9.5	3.9	3.1	1.8	2.3	5.6	4.6	1.6	3.5	0.5	
9	AVG	30.4	na	18.6	na	na	na	18.3	14.4	na	na	na	27.0
	STDV	5.2	na	na	na	na	na	4.5	na	na	na	na	
10	AVG (N-8)	35.3	45.9	26.6	19.7	13.7	10.1	31.1	20.0	9.4	8.2	4.2	28.0
	STDV	1.4	3.1	2.5	1.1	2.1	1.4	1.5	0.5	1.2	1.2	1.1	

The measures in Tables 2a & b shed light on the developmental stages at which the left-over turtle embryos after hatching had stopped developing when collected from their respective nests. In examining all the nests' remains, the measured individuals were beyond the 26th developmental stage (Kaska & Downie, 1999) and thus in their advanced stages of embryological development. Furthermore, in the Fajtata nest, measurements included hatchling corpses, resulting in data representative of fully developed hatchlings, consistent with the literature (Kaska & Downie, 1999).

Table 3. The significant differences for ‘SCL vs SCW’ proportional ratios between nests

SCL vs SCW	P Value
Mixquqa 26.98.20 x Għadira 25.09.20	<0.01
Mixquqa 26.98.20 x Fajtata 03.09.20	0.04
Għadira 25.09.20 x Fajtata 03.09.20	<0.01
Ramla 07.09.21 x Fajtata 03.09.20	0.03

Table 4. The significant differences for ‘SCL vs HL’ proportional ratios between nests

SCL vs HL	P Value
Mixquqa 26.98.20 x Għadira 25.09.20A	<0.01
Mixquqa 26.98.20 x Fajtata 03.09.20	0.05
Għadira 25.09.20 x Ramla 09.09.20	0.01
Għadira 09.10.20 x Fajtata 03.09.20	0.02
Fajtata 03.09.20 x Ramla 09.09.20	0.02
Ramla 18.08.23 x Għadira 25.09.20	<0.01
Ramla 18.08.23 x Fajtata 03.09.20	<0.01
Ramla 18.08.23 x Ramla 04.08.20	0.01

Table 5. The significant differences for ‘SCL vs FL’ proportional ratios between nests

SCL vs FL	P Value
Mixquqa 26.98.20 x Fajtata 03.09.20	0.01
Għadira 25.09.20 x Fajtata 03.09.20	<0.01
Għadira 09.10.20 x Fajtata 03.09.20	<0.01
Fajtata 03.09.20 x Ramla 09.09.20	0.02
Ramla 18.08.23 x Fajtata 03.09.20	<0.01

Table 6. The significant differences for ‘SCL vs HIL’ proportional ratios between nests

SCL vs HIL	P Value
Għadira 25.09.20 x Fajtata 03.09.20	<0.01
Fajtata 03.09.20 x Ramla 09.09.20	0.01
Ramla 18.08.23 x Għadira 25.09.20	0.02
Ramla 18.08.23 x Fajtata 03.09.20	<0.01

When considering the results shown in Tables 3 to 6 collectively, certain trends showing significant differences in body size proportions of dead hatchlings from different nests is apparent. Hatchlings from the Fajtata nest demonstrate significant differences in more than two morphological ratios when compared with the hatchlings’ body proportions from the other four different nests. Specifically, in the comparisons between the hatchlings of Fajtata 03.09.20 vs. Mixquqa 26.98.20, Fajtata 03.09.20 vs. Għadira 25.09.20, and Fajtata 03.09.20 vs. Ramla 18.08.23, significant differences emerged for three out of the four proportions analyzed. The hatchlings from Għadira 25.09.20 have shown significant differences in more than two morphological ratios with the embryo specimens of three nests: Għadira 25.09.20 vs. Mixquqa 26.98.20, Għadira 25.09.20 vs. Fajtata 03.09.20, and Għadira 25.09.20 vs. Ramla 18.08.23. Using two-tailed T tests, significant phenotypic differences in nestlings’ anatomical proportions between clutches were detected even at this early stage of life.

Discussion

An increase in *Caretta caretta* nesting activity has been recorded in the Maltese Islands since 2012, with 2020 yielding the largest number of detected nests in a single nesting season during the period of study (2020-2023). Five different nesting beaches as shown in Figure 2 have been used by Loggerhead sea turtle, these including: Ramla Bay (Gozo), Fajtata Bay (Malta), Mixquqa Bay (Malta), and Għadira Bay (Malta). More recently in

Summer of 2024, 8 nests have been reported by (ERA, 2024a-h) but nest success rates and remains have not been investigated in detail yet. This however indicates a consistent increasing presence of nesting females using Maltese beaches.

Clutch size is either determined at the time of egg-laying or during the inspection of nest contents after hatchlings emergence is completed. The clutch sizes of each of the nests studied Table 1 were found to vary. Though positive correlation between clutch sizes and the female body size has been reported, with larger females having larger clutch sizes and smaller females having smaller clutch (SPA/RAC-UNEP/MAP, 2021), this aspect was not assessed due to the lack of data availability on egg laying female sizes and the small number of such females till now. However, there may be other parameters that affect variability in clutch sizes.

When considering the nests studied here, the average clutch size was of 85 eggs (St. Dev. ± 14, n=10). This is also very similar to results from the neighboring island of Sicily, where the study found an average clutch size of 84.7 eggs (Prato et al., 2022). The clutch size recorded in the Maltese Islands seems to be less than those of Greek main nesting beaches and Spanish nests laid between 2016 and 2019 (Luna-Ortiz et al., 2024), however, it is higher than those reported on Kuriat island in Tunisia and in Libya which are similar to most *Caretta caretta* nest sizes in the Central Mediterranean region (SPA/RAC-UNEP/MAP, 2021). In comparison however, when considering clutch sizes per nest it is also relevant to consider cases of multiple clutching by the same female turtle, as shown by (Vella & Vella, 2023), finding that some females laid their eggs in different beaches.

The Maltese turtle nests studied, revealed hatching success rates ranging between 83% in Ramla Bay 2021 to 20% in the first nest laid in Għadira Bay on 25.09.20 after a natural incubation period. Of the 10 nests considered, four had similar high success rates, whilst the other six nests had a lower success rate. When considering the local hatching success rates, shown in Table 1, the average was found to be 59% (St. Dev. ± 26.7, n=9) (excluding Ramla 2022 artificially incubated nest where all embryos died), there are similarities to other studies, such as a study by (Prato et al., 2022) in Sicily, where the average nest success rate was reported to be 63% (Prato et al., 2022).

In many cases, undisturbed nests demonstrate a high hatching success rate, conversely, nests that experience disturbances caused by humans or animal predators tend to exhibit a lower success rate of 25% or even lower (Sea World, 2023). Human disturbance may affect the nest in a direct manner both during the process of egg laying if crowds are present on the beach at night or gather around the egg laying turtle or after a nest has been laid if it is not buffered against human trampling and any mechanical beach cleaning operations (Zhang et al., 2006; Sun, 2024).

The Maltese turtle nest success rates are lower to other important nesting sites in the Mediterranean, for instance, the Alagidi Beach (in Cyprus) turtle nest success rates averaged at 79.1% (Broderick & Godley, 1996), the Zakynthos (in Greece) turtle nest success rates averaged between 66.6 and 68.9% (Margaritoulis et al., 2011) and in Calabria (in Italy) turtle nest success rates averaged at 86.0% in a study by (Prato et al., 2022).

Table 1 shows that there is a difference between success rates that were laid in the same bay, by the same female turtle during the same nesting season. Specifically, in Għadira Bay, the 25th of September 2020 hatching of a clutch of 79 eggs had a success rate of 20.3% while on the 9th of October 2020 hatching of another clutch of 86 eggs by the same turtle female had a success rate of 81.4%. To understand such a discrepancy, one must consider multiple factors. When it comes to anthropogenic activity, both nests should have had minimal impacts as the nests were immediately located once laid, and conservation measures were put into action by ERA officials and an NGO (Borg, 2020). Hence, the difference in the nests' success rates could have been due to environmental factors (Margaritoulis, 2005). When taking these two Għadira 2020 nests as an example, both endured a thunderstorm on the 13-14th of September 2020. However, this storm may have affected the nests differently due to their respective embryonic developmental stages of incubation. In this case, the 25.09.20 nest the turtle embryos were in their later stages, with just 8 days to the first hatching, opposed to the later laid clutch that was at an earlier developmental stage. Further research on clutch vulnerability to adverse weather conditions according to the developmental stage is crucial for improvement of nest management.

For this reason nests' protection should be undertaken with careful record keeping of all nest-related environmental parameters measurable without disturbing the nest. This may also include the depth of the nest after hatching.

In another case of multiple clutching by the same turtle, the study by (Vella & Vella, 2023) has shown that the female laid her eggs in different beaches, the first nest was laid in Mixquqa Bay (Malta) whilst the second nest was laid in Ramla Bay (Gozo). Both nests had a very high success rate, suggesting that both nests had suitable conditions while the interruption of egg laying itself may have been caused by anthropogenic disturbance during egg laying in crowded beaches and coastal developed areas with light and other forms of disturbance and pollution. Such disturbance in Maltese turtle nesting beaches may have led to the Ramla Bay 2022 clutch (later incubated artificially) being laid too close to the shoreline and in risk of inundation, which nesting Loggerhead sea turtles usually avoid, trying to lay their eggs further inland with mean distances between 20m to 40m from the sea (Hays & Speakman, 1993; Lyons et al., 2020). However apart from human disturbance, climate change may also impact turtle nesting habitat through sea level rise that may narrow sandy beaches hindering turtles from finding enough distance from the shoreline for safe development of their clutch (Anand et al., 2024). This is another important consideration for Maltese sandy beaches that needs to be assessed for their sustainable suitability for turtle nesting in the face of a predicted sea level rise of over 2m by 2100 (Bamber et al., 2019; Lyons et al., 2020).

Loggerhead sea turtle nests in the Maltese Islands are excavated by local ERA officers 2 to 3 days after the first hatchlings emergence is recorded. However, a study carried out in Sicily in 2011 by (Casale et al., 2013), showed that hatchlings do not always emerge simultaneously, and emergence may take up to 10 days (Casale et al., 2013). When taking this into consideration, side by side with the measured unhatched Għadira 25.09.2020 turtles' morphological features it was found that most of the unhatched embryos were at the final 30th developmental stage. Therefore, the low nest success rate in this case might also be due to a premature excavation of the remaining turtle eggs from the nest. To avoid this, it is recommended that after the first hatchlings come out the nest should remain protected and undisturbed for another 10 days. These results and recommendation may help managers improve their conservation measures.

On the other hand, this is not the case for the low success rate for the Fajtata Bay nest, as the nest was discovered late and after hatchlings started to emerge, therefore unprotected. When excavated, many recently hatched turtles were found dead in the nest, with only one living hatchling that died moments later. The nest was in the central area of this very crowded bay, and nestlings were unknowingly heavily trampled upon by beachgoers (Borg, 2020).

In assessing nest success, it is crucial to consider the numerous factors that interact to influence turtle egg hatching outcomes. For the Maltese Islands, climatic conditions are predominantly favourable during the nesting season, characterized by limited precipitation, with only a few instances of rain or adverse weather scattered across three months—specifically, one rainy day in July and two in both September and October. Temperature fluctuations during nesting periods for nests studied had a mean maximum temperature of 31°C

(St.Dev. ± 1.8, n=6) and a mean minimum temperature of 23°C (St.Dev. ± 2.3, n=6). Moreover, a trend towards rising local air temperatures is observable from one year to the next. The eastern Mediterranean is known to be the warmest region of this sea. However, it is interesting to understand if due to certain phenological shifts, countries such as Malta, situated in the central Mediterranean region may see earlier nesting similar to that experienced in the east. The first nest for the 2020 season was laid in May, like major nesting beaches in the Eastern Mediterranean (Patel et al., 2016), however, long-term studies may help corroborate this scenario.

Another factor that is known to greatly effect Loggerhead sea turtle nests are pathogens. The growth of microorganisms, such as fungi and bacteria, has the potential to reduce the hatching success and cause embryological death. Among microorganisms, fungal infections by the genus Fusarium are considered one of the main causes of globally declining turtle populations, which is also known as Sea Turtle Egg Fusariosis (STEF). Infected individuals show pink discoloration and incomplete development (Pietroluongo et al., 2023). Sea Turtle Egg Fusariosis (STEF), is a global fungal disease, impacting egg viability and inducing embryo mortality in nests of the Loggerhead sea turtle. This pathogen represents a significant risk to sea turtle populations in the Mediterranean, with confirmed occurrences in adjacent areas like Italy. In fact, evaluations conducted along the Tuscan coast during the summer of 2020, revealed that eggs of *Caretta caretta* exhibited symptoms consistent with STEF infection (Risoli et al., 2023). This emerging pathogenic fungal infection, that may affect plant, invertebrate, vertebrate and human health as well (Zhang et al., 2006), have been reported to be originating from disturbed soils or urban particles reaching beaches and the sea with run offs (Gleason et al., 2020). Floating plant tissues and plastics with fungi growing on them may also be carried to egg-laying beaches, apart from being ingested by free swimming sea turtles (Duncan et al., 2019). This points toward improving environmental monitoring and management to mitigate the rise of such deleterious conditions affecting turtle nest success and species survival (Dülger & Dülger, 2022; McGough et al., 2015).

In the 2022 nesting season, the nest was laid (Ramla Bay, Gozo) was taken to an artificial incubation unit, through a joint effort by ERA and an NGO due to fears of nest inundation (Zammit, 2022). However, this incubation effort yielded no hatchlings. The unhatched turtle eggs of this 2022 Ramla Bay nest, which had undertaken necropsy dissections by the managing NGO's vet, were subsequently forwarded for this research. These specimens were found to be extremely dehydrated and heavily dismantled with many eggs found to have missing embryos. Therefore, research on these artificially incubated failed turtle egg embryos could only be undertaken using any measurable anatomical features. Among these remains the lengths of the front limb of 16 specimens could be measured, and the measurements suggest that these hatchlings stopped developing between their 26th to 29th developmental stage. Such results would seem to contrast with media reports indicating that the hatchlings stopped developing before the transfer to artificial incubation that took place on the 19th of August 2022 (Zammit, 2022). As the date of laying of eggs was known it would mean that the eggs' transfer to artifical incubation occurred at the 24th developmental stage, which is equivalent to day 28-32 of the natural incubation period (Kaska & Downie, 1999). So apart from noting that certain studies show a negative correlation between nest success and relocation, especially when the relocation takes place after day 12 of incubation (Ahles & Milton, 2015), it should also be noted that conditions in the artificial incubation in this case may have deteriorated the chances of survival of this clutch of turtle eggs. Details of the aritificial incubation were not available to this study.

At the population level, relocation may have adverse effects while inundated clutches, despite having a lower success rate, may still produce live turtles even if with a sex bias, with more male offspring compared to non-inundated clutches (Sea World, 2023). Based on this, it is recommended to only relocate turtle clutches soon after egg-laying when conditions for nonrelocation would yield clear close to zero hatchlings.

Experienced and knowledgeable conservation researchers may assist with such considerations and therefore involved in the evaluation of turtle conservation measures to take.

When comparing the average success rate of 2020, 59% (St.Dev. ± 27.8, n=6), and the 2 nests of 2023, 36% and 35% respectively, a decrease in nest success rate is noticed. Both nests were identified immediately, and a perimeter was set up to safeguard the nests, furthermore, both nests experienced no adverse weather conditions. Therefore, great caution and attention for various environmental paraments need to be kept under check, also considering the potential effects of pathogenic infections and environmental vectors of these pathogens affecting health and growth of the nestlings. For example, nesting beaches with high presence of clay and silt have shown to impact hatching success for Loggerhead Sea turtles (Marco et al., 2017). Future research on turtle nest sediment compositon and microbiology may be useful to expand on the environmental variable potentially affecting nest success rates. The results obtained in this study highlight the need to improve future management of Loggerhead Sea turtle nests in greater detail and expertise to achieve effective conservation results for this species during this critical life cycle phase.

As shown in Tables 3 to 6, this study investigated the variance in proportional body measures between nestlings of different nests. After running a series of T-tests, on SCL vs SCW, SCL vs FL, and SCL vs HL, and SCL vs HIL; proportional ratios having significant differences were found. This analysis is particularly interesting when taking into consideration the study by (Vella & Vella, 2023) which identified nests belonging to the same female in the same nesting season. Għadira 25.09.20 and Għadira 09.10.20 nests both belong to the same female and male reproducers and showed no significant differences in any of the phenotypic body ratios being investigated. The 2020 Mixquqa Bay clutch and Ramla Bay '02.08.20' clutch also both belonged to the same female and male reproducers (Vella & Vella, 2023), however, due to only one hatchling available from the Ramla Bay nest, such comparisons of morphological features could not be undertaken. Overall, these preliminary results seem to point toward phenotypic difference or similarity relating to genetics of the parents involved allowing for significant morphological diversity or similarity linked to the genotypic diversity or similarity between clutches respectively.

Conclusion

To protect sea turtle nesting sites, local authorities and NGOs should conduct daily beach monitoring during nesting season to identify and safeguard nests immediately. Comprehensive reports on nesting sites, including data recorded such as: coordinates, depth, and nest temperatures, weather conditions during the whole nesting period are essential for better analysis of nesting trends and conditions in different beaches in the Maltese Islands. Sustained conservation research involving scientific investigations on all the stages of the species' life history is essential to effectively plan and manage activities on nesting beaches and out at sea that do not hinder normal Logger head sea turtle behaviours and allow best survival chances starting from the nesting and hatching phases. Establishing large buffer zones around nests can minimize human disturbance. Nests should be excavated for inspection of clutch remains after 10 days from the first hatching to ensure that the nest is not excavated prematurely, sacrificing any late hatchlings. This is a recommendation that comes after discovering that there were several turtle eggs with embryos at the final stages of development when excavated and frozen by the environment authority. This increased timeframe allows the minimizing of disturbance to unhatched eggs so that more of the hatchlings may emerge naturally. Timely excavation improves hatching success and turtles' viability. Enforcing regulations in Natura 2000 sites, including those not dedicated to marine turtle conservation, is crucial for preserving biodiversity and the good status of the marine environment. Vessel traffic and anchorage in sandy bay areas known to be used by turtles should be reduced and monitored to allow undisturbed passage of turtles trying to reach the beaches to lay their eggs during the critical period of egg laying. Public awareness campaigns can educate and engage communities in conservation efforts to understand environmental regulations and why they are essential for the survival of vulnerable species and biodiversity. Reduced noise and light pollution on beaches used by turtles for nesting and regular beach clean-ups undertaken manually by volunteers and public, may enhance the number and health of turtles coming to lay their eggs. These recommendations target significant improvements in the conservation of sea turtles during a critical stage of their life cycle.

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IK and AV both contributed to laboratory work and to writing this publication. IK undertook analyses to produce the tables of results.

Data Availability Statement

All relevant data are made available in this publication.

Conflicts/Competing of Interests

Authors have no conflicts or competing interests.

Compliance with Ethical Standards

This research work used dead specimens that were collected by the Environment Protection Authority of Malta and forwarded for research under relevant permitting and with the approval of University of Malta institutional research ethics committee.

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