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# Natura Croatica

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ČASOPIS HRVATSKOGA PRIRODOSLOVNOG MUZEJA

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## PROCEEDINGS FROM THE 6<sup>TH</sup> MEDITERRANEAN CONFERENCE ON MARINE TURTLES

16-19 October 2018, Poreč, Croatia



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PROCEEDINGS FROM THE 6<sup>TH</sup>  
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ON MARINE TURTLES

16-19 October 2018, Poreč, Croatia

**Guest Editor**

Bojan Lazar

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# PROCEEDINGS OF PAPERS, THE 6<sup>TH</sup> MEDITERRANEAN CONFERENCE ON MARINE TURTLES

**BOJAN LAZAR**

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This Supplement issue of *Natura Croatica*, international scientific journal of the Croatian Natural History Museum, contains selected, peer-reviewed collection of papers from the 6<sup>th</sup> Mediterranean Conference on Marine Turtles held at the Valamar Diamant Hotel Conference Center in Poreč, Croatia, between the 16<sup>th</sup> and 19<sup>th</sup> of October 2018.

The history of the Mediterranean Marine Turtle Conferences goes back to 1998, when the organisation of a Mediterranean conference on marine turtles was proposed by the Secretariat of the Convention on Migratory Species at the UNEP MAP-RAC/SPA meeting for the revision of the Action Plan for the Conservation of Mediterranean Marine Turtles in Arta, Greece. The initiative was endorsed at the RAC/SPA Meeting of Experts for Priority Actions (Tunis, February 1999), and the proposal was formally adopted by the Fourth Meeting of the National Focal Points for SPAs in the context of the Barcelona Convention (Tunis, April 1999). The Secretariat of the Bern Convention supported the idea, and eventually the three conventions, in collaboration with the IUCN/SSC Marine Turtles Specialist Group (MTSG), organised the First Mediterranean Conference on Marine Turtles in Rome in 2001.

The 6<sup>th</sup> Mediterranean Conference on Marine Turtles was organised by the Department of Biodiversity of the University of Primorska (Slovenia) in collaboration with the Blue World Institute of Marine Research and Conservation (Croatia), in the picturesque town of Poreč in Croatia, located on the coast of the northern Adriatic Sea - one of the most important feeding habitats for Mediterranean loggerhead turtles. The organisation was supported by UNEP MAP-RAC/SPA, Bern Convention, IUCN/SSC MTSG, and by the Croatian Natural History Museum – the institution that initiated the first studies on marine turtles in the Adriatic Sea 25 years ago.

Since the beginning, Mediterranean Marine Turtle Conferences had the same mission: to gather together those who share the common goal of the conservation of marine turtles and their environment, acting as a platform for enhancing collaboration, exchanging ideas, and sharing the state-of-the-art knowledge on sea turtle biology and conservation in the Mediterranean. The 6<sup>th</sup> Conference joined over 180 participants who presented 30 oral and 88 poster contributions assigned to five thematic sections: (i) Population biology and ecology, (ii) At-sea distribution and movements, (iii) Conservation and monitoring, (iv) Threats, and (v) Health and rehabilitation. The program also included six workshops: (i) Bio-logging for marine turtles; (ii) Using drones for marine research and conservation; (iii) Towards a strategy to mitigate illegal trade in

Mediterranean marine turtles; (iv) Whose biodiversity, whose values? Engaging with stakeholders to understand their perceptions; (v) Impact of marine litter on sea turtles, and (v) Marine turtle medicine and rehabilitation. Two plenary lectures entitled “Dynamic ocean management: a novel approach to interactions between people and oceanic pelagic organisms”, and “Introducing multi-species approach to conservation of marine megafauna beyond borders: the Adriatic case-study” were given by Dr. Larry B. Crowder (Hopkins Marine Station, Stanford University, USA) and Dr. Caterina M. Fortuna (National Institute for Environmental Protection and Research, Italy), respectively.

The scientific committee made awards to two students, Maissa Louhichi for the best student oral presentation and Jessica Ruff for the best student poster. Mr. Eladio Fernández-Galiano received the Lifetime Achievement Award for his accomplishments and dedication to the conservation of marine turtles in the Mediterranean.

This Supplement issue of *Natura Croatica*, funded by the Croatian Ministry of Science and Education, was intended to provide Conference participants with the possibility of publishing their results and making them available to the scientific community. On behalf of the Scientific Committee and the referees who reviewed all received submissions I hope that you will enjoy this special issue.

Bojan Lazar,

Guest-editor and president of the 6<sup>th</sup> Mediterranean Conference on Marine Turtles

# NORMAL ULTRASONOGRAPHIC FEATURES OF LOGGERHEAD (*CARETTA CARETTA*) EYES

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Mennonna, G., Lamagna, B., Affuso, A., Greco, A., Micieli, F., Costanza, D., Hochscheid, S. & Meomartino, L.: Normal ultrasonographic features of loggerhead (*Caretta caretta*) eyes. Nat. Croat., Vol. 29, Suppl. 1., 3-10, 2020, Zagreb.

The Loggerhead sea turtle (*Caretta caretta*) is widely distributed in the Mediterranean Sea but, like other sea turtles, it is considered a threatened species. Improving anatomical knowledge on normal aspects of the species is important for correct diagnosis and proper therapy and, therefore, for improving the chances of a complete recovery and a fast reintroduction into the wild for a greater number of sea turtle bycatch and other mishaps. For this reason, 15 Loggerhead turtles, with clinically healthy eyes, were submitted to ocular ultrasonographic (US) examinations. The US exam was quick, non-invasive, and simple to perform and permitted researchers to assess all the ocular features.

**Keywords:** Loggerhead, sea turtle, eye, ultrasound, biometric

Mennonna, G., Lamagna, B., Affuso, A., Greco, A., Micieli, F., Costanza, D., Hochscheid, S. & Meomartino, L.: Ultrazvučni nalaz normalnih značajki oka glavate želve (*Caretta caretta*). Nat. Croat., Vol. 29, Suppl. 1., 3-10, 2020, Zagreb.

Glavata želva (*Caretta caretta*) je široko rasprostranjena u Sredozemnom moru, no, kao i ostale vrste morskih kornjača, smatra se ugroženom vrstom. Bolje poznavanje anatomije vrste važno je za pravilnu dijagnostiku i terapiju, a time se povećavaju šanse potpunog izliječenja i brzog povratka u divljinu za veliki broj slučajno ulovljenih ili ozlijeđenih morskih kornjača. Iz tog razloga petnaest glavatih želvi podvrgnuto je ultrazvučnom pregledu očiju. Ultrazvučni pregled bio je brz, neinvazivan, jednostavan za provedbu i omogućio je znanstvenicima pregled svih očnih funkcija.

**Ključne riječi:** glavata želva, morska kornjača, oko, ultrazvuk, biometrija

## INTRODUCTION

The Loggerhead sea turtle (*Caretta caretta*) belongs to the Cheloniidae family. The distribution of this species is worldwide, included the Mediterranean Sea. In the Mediterranean Sea the nesting sites are mainly in the southern regions such as the coasts of Greece, Turkey and North Africa. In Italy, nesting occurs mainly along the coastlines of Sicily and Calabria (MINGOZZI *et al.*, 2007). Loggerhead turtles are also commonly encountered along the south-western coasts of Italy in the Tyrrhenian Sea, where ju-

veniles and adults utilize the rich local shallow habitats for foraging (HOCHSCHEID *et al.*, 2013; MAFFUCCI *et al.*, 2013).

These animals are threatened by multiple human activities: pollution of the seas, ingestion of fish-hooks and other foreign bodies (LAZAR & GRAČAN, 2011; SANTOS *et al.*, 2015), such as plastic bags that are confused with plankton, incidental catch in fisheries, propeller injuries, as well as, the destruction of nesting sites. The loggerhead sea turtle has been considered endangered for a long time (IUCN/SSC, 2002) but, thanks to the international efforts to protect the species, it has been reclassified as vulnerable (HOCHSCHEID *et al.*, 2018). In recent decades, the interest of the scientific community in these animals has increased. In order to improve the preservation and the treatments of these animals, the knowledge of the normal anatomy is mandatory and diagnostic imaging techniques represent a valuable means to reach this goal (VALENTE *et al.*, 2006; VALENTE, 2007; PEASE *et al.*, 2010; MENNONNA *et al.*, 2015; DE MAJO *et al.*, 2016; PEASE *et al.*, 2017). The sense organs, especially the sight, of these ancient animals concern biologists and veterinarians, particularly for their visual abilities both in water and out (NORTHMORE & GRANDA, 1991; OLIVER *et al.*, 2000; BARTOL *et al.*, 2002; MATHGER *et al.*, 2007; CROGNALE *et al.*, 2008). Often, turtles with ocular injuries are presented to veterinarians who work in rescue centers; therefore the knowledge of normal eye features is of a paramount importance.

Ultrasonography (US) has already been used to study the eye and to establish the normal biometrics in many species (PENNINCK *et al.*, 2001; HOLLINGSWORTH *et al.*, 2007; SPAULDING, 2008; LEHMKUHL *et al.*, 2010; DAR *et al.*, 2013; LAURIDSEN *et al.*, 2014; THAÍIS *et al.*, 2014; SOMMA *et al.*, 2015; RAPOSO *et al.*, 2017; MEOMARTINO *et al.*, 2018).

The aim of this study was to describe the normal ultrasonographic features and dimensions of the eyes in the Loggerhead sea turtle (*Caretta caretta*).

## MATERIALS AND METHODS

The ultrasonographic exams were performed on live Loggerhead sea turtles and the study was approved by the Italian Ministry of the Environment and the Protection of Natural and Marine Resources (protocol number 24471).

Thirty US of the eye were performed on fifteen Loggerhead sea turtles rescued from the south of the Tyrrhenian Sea and admitted at the Marine Turtle Research Centre (MTRC) of the Anton Dohrn Zoological Station in Portici (NA). All the turtles included were preventively submitted to a complete ophthalmic exam and had normal eyes. The straight length of the carapace (SCL) and the body weight (BW) were measured and recorded for all the turtles.

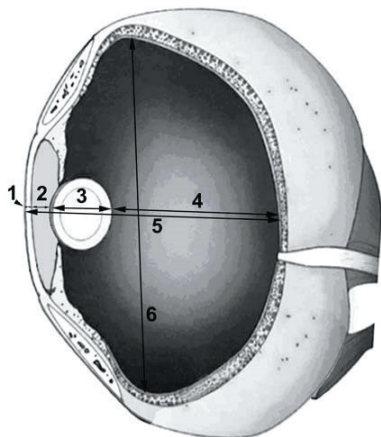
All the animals were manually gently restrained and sterile topical anesthetic (Novesin, oxybuprocaine hydrochloride 0.4%; Novartis Farma spa, Italy) was instilled before B-mode US. Twelve turtles were examined at the MTRC; the remaining three were examined at the Interdepartmental Center of Veterinary Radiology, during diagnostic investigations required for conditions not involving the eyes. Two ultrasound devices, equipped with a 12-MHz linear probe, were used: MyLab30® (Esaote, Florence, Italy) at the MTRC and Mylab Class C® (Esaote, Florence, Italy) at the Interdepartmental Center of Veterinary Radiology. The eye was coupled to the probe with a copious amount of sterile gel (Sterile Aquasonic 100; Parker La. Inc., Fairfield, NJ, USA) and the probe footprint was held in a horizontal and vertical position for the correspo-



nding US scans, until optimal images were obtained (Fig. 1). The ocular dimensions were measured using the US device software calipers. The following measurements were obtained for each eye: the polar axis (PA), the distance from the corneal surface to posterior wall of the retinal layer; the equatorial axis (EA), the maximal diameter perpendicular to the PA; the corneal thickness (CT), the distance between the two lines of the cornea; the anterior chamber depth (ACD), the distance from the posterior line of the cornea to the anterior capsule of the lens; the lens thickness (LT), the distance between the anterior and the posterior capsules; the vitreous chamber depth (VCD), the distance from the posterior capsule of the lens to the retinal surface (Fig. 2).



**Fig. 1.** The photo shows an ultrasonographic exam in progress: the turtle is gently restrained and the probe is placed on the right eye in a vertical position.



**Fig. 2.** Schematic drawing of the Logger-head eye with the measurements performed. Legend: 1 = corneal thickness; 2 = anterior chamber depth; 3 = lens thickness; 4 = vitreous chamber depth; 5 = polar axis; 6 = equatorial axis.

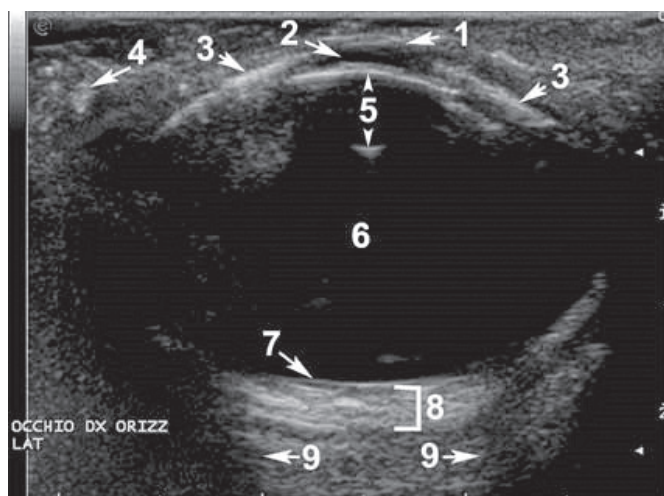
Data collected were analyzed for normality with the Shapiro-Wilk test and reported as Mean $\pm$ SD or Median (range) if normally or not normally distributed respectively. Differences between right and left eye in PA, EA, CT, ACD, LT, were analyzed with 2-sided Student's t-test or Mann Whitney test, according to their distribution. Correlation between SCL or BW and other variables were tested using Pearson's  $r$  or Spearman's  $r_s$  correlation coefficients, according to the distribution. Significance was set for  $P < 0.05$ . Data analysis was performed with dedicated software (JMP® 8.0.2, SAS Institute Inc., Cary, NC, USA).

## RESULTS

The sample was composed of 11 sub-adults (SCL < 65 cm) and 4 juveniles (SCL < 40 cm). Only 5 individuals were sexed as 2 males and 3 fe-

males. The mean SCL was 47.18 cm ( $\pm 10.22$ ; range 33.2-61.0 cm) and the mean BW was 18.79 kg ( $\pm 12.6$ ; range 6-32.7 kg).

The US exam of the eye was successfully performed on all the turtle but some difficulties were encountered because of the retraction reflex, particularly present in this species. Each exam lasted from 3 to 5 minutes. Starting from the eyelid, the US features of the eye were as described below: the cornea was formed by two hyperechoic parallel lines and showed a slight anterior convexity; the anterior chamber was thin and anechoic; the lens was formed by two hyperechoic convex lines, representing the anterior and posterior capsules, delimiting an anechoic parenchyma, and was relatively small compared to the eyeball; the scleral ossicle appeared as two hyperechoic lines at the level of the limbus followed by a shadowing artifact, obscuring the equatorial borders of the lens and, sometimes, of the eye; the vitreous chamber was anechoic; the retinal fundus was regular and no optic disc was visible; on some occasions, the cartilaginous portion of the posterior sclera was easily visible (Fig. 3).



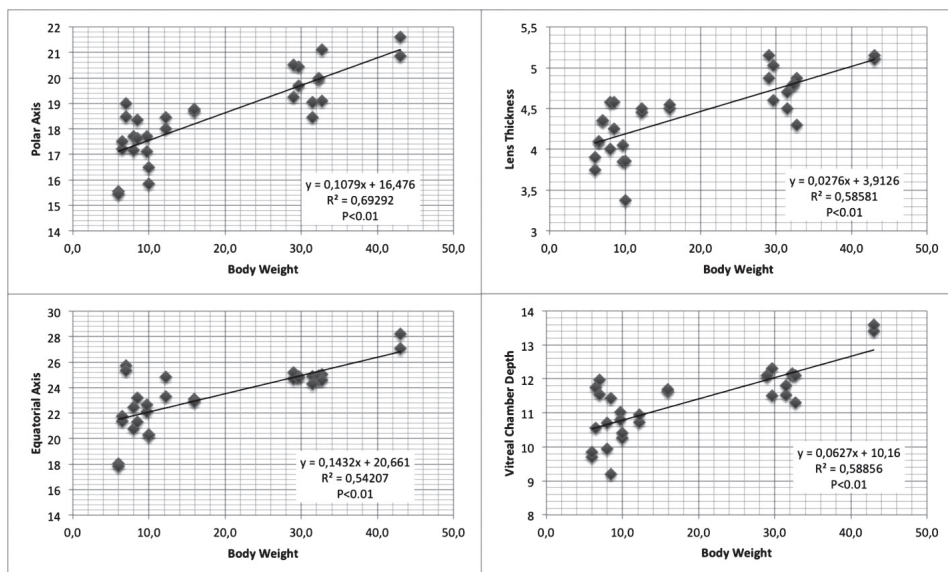
**Fig. 3.** Ultrasonographic image of the Loggerhead eye in the horizontal scan. Legend: 1 = cornea; 2 = anterior chamber; 3 = scleral ossicle; 4 = salt gland; 5 = anterior and posterior capsule of the lens; 6 = vitreous body; 7 = retinal layer; 8 = scleral cartilage; 9 = artifact shadowing from scleral ossicle.

Except for the CT, no statistical differences were found between right and left eye and the mean ( $\pm$ Dev.St.) are summarized in Tab. 1.

The SLC was significantly correlated only with VCD ( $p=0.01$ ) while BW correlated with PA ( $p<0.01$ ), EA ( $p<0.01$ ), LT ( $p=0.01$ ) and VCD ( $p<0.01$ ) (Fig. 4).

**Tab. 1.** - Mean ( $\pm$ Dev.St.) of the measurements performed, expressed in mm. \* Statistical difference between right and left eyes ( $P < 0.01$ ).

	Right Eye	Left Eye	Pooled Eyes
Polar Axis	18.41 ( $\pm 1.51$ )	18.60 ( $\pm 1.73$ )	18.50 ( $\pm 1.11$ )
Equatorial Axis	23.28 ( $\pm 2.40$ )	23.43 ( $\pm 2.51$ )	23.35 ( $\pm 2.41$ )
Corneal Thickness*	0.62 ( $\pm 0.12$ )	0.52 ( $\pm 0.06$ )	0.57 ( $\pm 0.11$ )
Anterior Chamber Depth	0.83 ( $\pm 0.16$ )	0.82 ( $\pm 0.18$ )	0.83 ( $\pm 0.16$ )
Lens Thickness	4.46 ( $\pm 0.50$ )	4.40 ( $\pm 0.41$ )	4.43 ( $\pm 0.45$ )
Vitreous Chamber Depth	11.41 ( $\pm 0.97$ )	11.26 ( $\pm 1.08$ )	11.34 ( $\pm 1.01$ )



**Fig. 4.** Linear correlation Body weight with Polar axis, Equatorial axis, Lens thickness, and Vitreous chamber depth.

## DISCUSSION

In the Loggerhead sea turtle the eye is placed within the skull (MADER *et al.*, 2017), dorsally and laterally; they have three keratinized eyelids, two mobile, dorsal and ventral, and a non-mobile medial lid, called a “secondary lid” (WYNEKEN, 2001). As in other animal species the ocular globe is formed by three layers that, starting from the outside to the inside, are the following: the sclera and, anteriorly, the cornea, formed by connective tissue; the uvea, the intermediate and vascularized layer; the retina, the inner sensory layer. In these animals there are two supporting structures, posteriorly the scleral cartilage and anteriorly the scleral ossicle (WYNEKEN, 2001; ARENCIBIA *et al.*, 2006; BRUDENALL *et al.*, 2008; JONES *et al.*, 2012).

This study provides the normal ocular ultrasonographic and biometric features in the loggerhead sea turtle (*Caretta caretta*). Compared to mammalian species, the ocular globes in these reptiles have a spheroidal shape, with a polar axis shorter than equatorial one. They also present a slight corneal curvature, as well as a very thin anterior chamber and a relatively small lens.

The US examination was quite easy to perform on awake animals, however some difficulties resulted from the presence of the scleral ossicle and the retraction reflex, very pronounced in this species. The scleral ossicle, due to the shadowing artifact, did not permit the complete visualization of the equatorial border of the lens or, on some occasions, of the eyeball. In addition, sea turtles, like other species of reptiles, such as lizards and crocodiles, have two muscles, retractor oculi and protractor oculi, which insert on the sclera and determine the movement towards the inside and the outside

of the eye within the orbit. On the other hand, the absence of fur facilitates the transmission and the progression of the ultrasound beam.

Our results showed a good correlation between eye dimensions and body weight as already reported (RAPOSO *et al.*, 2017). Interestingly, the ocular dimensions in our sample were quite similar to those obtained in a previous study, in spite of the larger mean weight of those turtles (RAPOSO *et al.*, 2017). We speculate that the ocular dimensions correlate with body dimensions during growth but, in the subadult subjects, they attain their final dimensions.

Loggerhead sea turtles, like other species of sea turtles, can be affected by several eye diseases and many of those can make the anterior portions of the eye opaque (İŞLER *et al.*, 2014; MADER *et al.*, 2017), and prevent a normal ophthalmic examination. Conversely, ultrasonography allows the evaluation of the interior of the eye, even when it is obscured from direct visualization by any disease resulting in ocular opacity (MACKEY & MATOON, 2015). Therefore, the US exam has to be considered an important complementary technique for studying the eye in this species of reptiles.

The B-mode US features presented are useful for clinical evaluation and provide reliable information about the normal dimensions and intraocular anatomy of loggerhead eyes. Our results also showed that the ultrasonography of the eye is a quick, simple and safe exam to perform in Loggerhead sea turtles. The use of general purpose US devices equipped with relatively high-frequency probes (10-12 MHz) allows images of all the ocular structures to be obtained. Moreover, the US exam does not require sedation and it can be performed on conscious animals just after the instillation of eye anesthetic drops. In the authors' opinion, ultrasonography of the eye should become a routine in the examination of Loggerhead sea turtles.

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## SUMMARY

### Normal ultrasonographic features of loggerhead (*Caretta caretta*) eyes

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S. Hochscheid & L. Meomartino

Fifteen Loggerhead sea turtles admitted to the Marine Turtle Research Center of the Anton Dohrn Stazione Zoologica of Naples and with healthy eyes underwent ocular ultrasonography in order to assess the normal features and dimensions of the eyes. Polar axis (PA), equatorial axis (EA), corneal thickness (CT), anterior chamber depth (ACD), lens thickness (LT), and vitreous chamber depth (VCD) were measured. The sample was composed of 11 sub-adults and 4 juveniles with a mean SCL of 47.18 cm ( $\pm 10.22$ ; range 33.2–61.0 cm) and a mean BW of 18.79 kg ( $\pm 12.6$ ; range 6–32.7 kg). Compared to mammals, the ocular globes in these species have a spheroidal shape in which the PA is shorter than EA, a lesser pronounced convexity of the cornea and relatively small lens. Results show that ocular dimensions, in particular PA ( $p < 0.01$ ), EA ( $p < 0.01$ ), LT ( $p = 0.01$ ) and VCD ( $p < 0.01$ ) were correlated with body weight. In these animals several eye diseases are described, so knowing the normal features and dimensions of the eye allows a quicker diagnosis. Ultrasonographic exam performed using general purpose devices was demonstrated to be feasible in this species and must be considered particularly useful when the transparency of the anterior portion of the eye is lost.

# IS THE 2018 LOGGERHEAD NEST REALLY ANOTHER EXCEPTIONAL SEA TURTLE NESTING RECORD FOLLOWING THE 2012 AND 2016 PREVIOUS NESTING CASES IN MALTA?

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Mifsud, C., Attard V. & Demetropoulos, A.: Is the 2018 loggerhead nest really another exceptional sea turtle nesting record following the 2012 and 2016 previous nesting cases in Malta? Nat. Croat., Vol. 29, Suppl. 1., 11-21, 2020, Zagreb.

We report another nesting event by a loggerhead turtle in Ġnejna (Malta), in June 2018, with an extraordinary hatching rate of 99.1%. This follows the previously reported nesting in 2012 in the same bay, and the 2016 event in a nearby beach at Golden Bay, both beaches located in the NW of Malta. Before the 2012 record, scientifically recorded turtle nesting in Malta dated back some 100 years, although in one paper it is alleged that turtles may have been nesting in Malta up until some 50-60 years ago. Noting that loggerheads have an average remigration interval of 2 years, it is possible that the same turtle that nested in 2012 came back to Ġnejna to nest again in 2018. It is hoped that DNA analysis, which ideally follows at a later stage, will determine whether it was the same turtle.

Campaigns are currently ongoing to solicit greater reporting of nesting. Relocation of eggs is discussed. In this paper we also describe conservation measures that were set-up in this bay and in Golden Bay, including measures during hatching. Emergency conservation orders were issued in all the three nesting events, to protect the beaches in question from any major and potentially harmful activities. The sites were also surrounded and physically protected with a 24 hour monitoring scheme being set-up with the help of volunteers from Nature Trust Malta and government officials.

**Keywords:** *Caretta caretta*, sea turtle, nesting, Mediterranean, Malta, exceptional and sporadic nesting

Mifsud, C., Attard V. & Demetropoulos, A.: Je li gnijezdo glavate želve na Malti iz 2018. uistinu tek drugo izuzetno zabilježeno gniježđenje nakon prethodnih slučajeva iz 2012. i 2016.? Nat. Croat., Vol. 29, Suppl. 1., 11-21, 2020, Zagreb.

U radu donosimo podatke o novom gniježđenju glavate želve na plaži Ġnejna (Malta) u lipnju 2018., s izuzetnim omjerom izlegnutih kornjača od 99.1%. Taj događaj slijedi nakon prethodnih gniježđenja u istoj uvali 2012. i na obližnjoj plaži Golden Bay 2016.; obje plaže se nalaze na sjeverozapadu Malte. Prethodno podatku iz 2012., jedino znanstveno opažanje gniježđenja kornjača na Malti bilo je zabilježeno prije 100-tinjak godina, iako se u jednom radu spominje njihovo gniježđenje na Malti do prije 50-60 godina. S obzirom na interval prosječnog povratka glavate želve od dvije godine, moguće je da se ista kornjača, koja se gnijezdila 2012., vratila ponovno gnijezditi u uvalu Ġnejna 2018. godine. Nadamo se da će uslijediti DNA analiza kojom bi utvrdila radi li se o istoj kornjači.

\* The views expressed here do not necessarily reflect the views of the Environment Resource Authority

Trenutno se provode kampanje za poticanje boljeg izvještavanja o gniježđenju. U radu se raspravlja u premještanju jaja. Također opisujemo mjere zaštite poduzete u ovoj uvali i uvali Golden Bay, uključujući mjere tijekom gniježđenja. Hitne mjere zaštite poduzete su u sva tri gniježđenja da bi se dotične plaže zaštitile od potencijalno štetnih događaja. Lokacije su i fizički zaštićene i ograđene tijekom 24-satnog monitoringa, uz pomoć volontera iz Nature Trust Malta i upravnih tijela.

**Ključne riječi:** *Caretta caretta*, morske kornjače, gniježđenje, Sredozemlje, Malta, izuzetno i sporačno gniježđenje

## INTRODUCTION

Species conservation requires an understanding of the population, life histories, and behavioural patterns of the target species. Although the Mediterranean subpopulation of the Loggerhead turtle (*Caretta caretta*) has recently been categorised as 'Least Concern' (LC) on the IUCN Red List of Threatened Species 2015 (IUCN, 2015), this change in category from a previous threatened status is entirely conservation-dependent, because the 'current population is the result of decades of intense conservation programs, especially at nesting sites (CASALE & MARGARITOU, 2010) and the cessation of these programs would be followed by a population decrease'. Survival of this species thus depends primarily on implementation of conservation measures based on conservation priorities, like population ecology, biogeography, their reproductive biology and by curbing, as much as possible, threats (including anthropogenic ones) directly to the species and to their habitats, both on land (nesting) and at sea.

The main current threats to the Mediterranean subpopulation are represented by fishery bycatch and nesting habitat degradation due to coastal development (CASALE & MARGARITOU, 2010), as also outlined in the conservation and research priorities by Casale in 2018 (CASALE *et al.*, 2018).

The Mediterranean loggerhead population exhibits limited gene flow with that in the Atlantic (SHAMBLIN *et al.*, 2014; CARRERAS *et al.*, 2011) and thus represents a Regional Management Unit for conservation (WALLACE *et al.*, 2010). Annual adult female population nesting in the Mediterranean is estimated at 2,000 for *C. caretta* (GROOMBRIDGE, 1990) with over 7,200 nests per year in the whole Mediterranean (CASALE & MARGARITOU, 2010; CASALE *et al.*, 2018). Major nesting sites are found in Greece, Turkey, Libya (unquantified nesting) and Cyprus (BARAN & KASPAK, 1989; GROOMBRIDGE, 1990; KASPAK, 1995; BRODERICK & GODELY, 1996; KULLER, 1999; LAURENT *et al.*, 1999; MARGARITOU, 2000; CLARKE *et al.*, 2000; CASALE & MARGARITOU, 2010). Minor sites or scattered nesting occur in several other countries in the eastern basin, including Italy, Egypt, Lebanon, Israel, Syria and Tunisia (CASALE & MARGARITOU, 2010).

It is interesting to note that initially, Italy was not known to host any major nesting sites (ARGANO & BALDARI, 1983; ARGANO *et al.*, 1992) but a recent review reported many recent nesting reports (MINGOZZI *et al.*, 2007; BENTIVEGNA *et al.*, 2008; SENEGAS *et al.*, 2008). In Sicily, there are potential coasts for sea turtle nesting, and nests have occasionally been reported there by tourists or local people (MINGOZZI *et al.*, 2007). This is quite noteworthy considering the close proximity of Sicily to Malta. In 2011, Casale reported 11 nests, which in comparison to past records was a relatively high number possibly, due to the 'awareness campaign carried out in 2011 to solicit such reports'. This also inferred that Sicily (CASALE, 2011) and possibly other minor islets nearby may host a much higher nesting activity than previously thought. It is also crucial to note that nesting events in the central and western Mediterranean (TOMÁS *et al.*, 2002; DELAUGERRE & CESARINI 2004; BENTIVEGNA

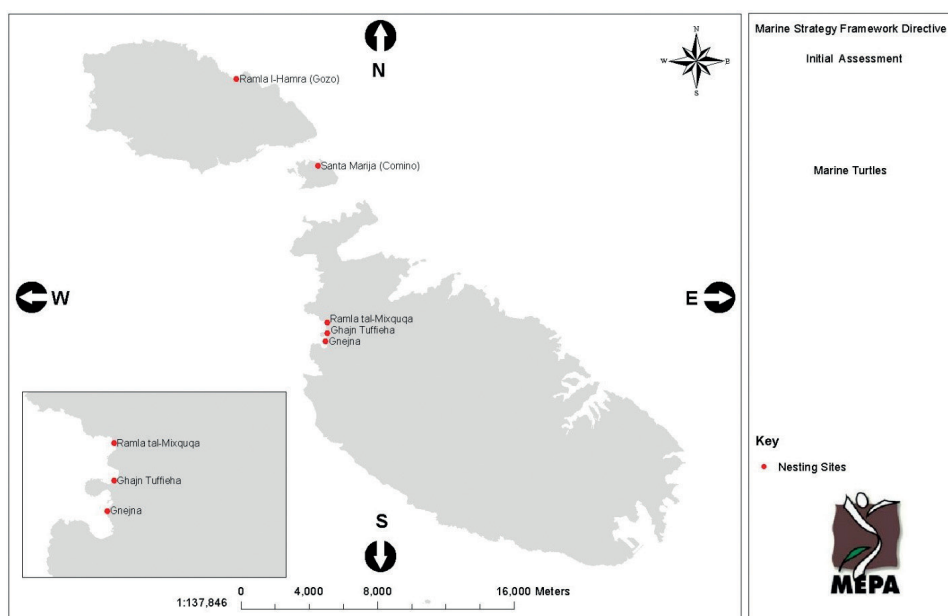


*et al.*, 2008; SENEGAS *et al.*, 2008; TOMÁS *et al.*, 2008; BENTIVEGNA *et al.*, 2010; CARRERAS *et al.*, 2018) have been increasing greatly in the last decade.

## MATERIALS AND METHODS

The main aim of this work was to acquire important clues for better conservation, on local nesting events (MIFSUD *et al.*, 2007), all of which had been reported to authorities or NGOs by people on the beaches in question. Since this was quite an exceptional phenomenon in the Maltese Island (MIFSUD, 2010), the best conservation measures possible were set up and further awareness-raising with the public was carried out to stimulate the population to make such reports.

Both Ġnejna bay and Golden Bay (Fig. 1) are important beaches in Malta, with large numbers of tourists and locals visiting daily. Ġnejna is 228.2 metres long and about 50 metres wide at its widest point (MSFD Initial Assessment Report on Habitats; based on the 2008 aerial photos). Golden Bay is 317 metres long and 82 m wide (MSFD Initial Assessment Report on Habitats; based on the 2008 aerial photos). These beaches are amongst the very few sandy beaches present in the Maltese Islands. Sandy (particle size 0.063–2mm), shingle (particle size 2–256 mm) or mixed sand and shingle shores are



**Fig. 1.** Location of the sporadic recent and historic recorded nesting of *Caretta caretta* – extracted from the Malta Environment and Planning Authority (2013). MSFD Initial Assessment - Reptiles. Retrieved from <https://era.org.mt/en/Documents/MSFD-InitialAssessment- MarineTurtles.pdf>

**Ad-hoc nesting** events reported from Malta for 2012, 2016 and 2018 in Ġnejna, Ramla Tal-Mixquqa and Ġnejna respectively. Before the 2012 record, scientifically recorded turtle nestings in Malta were sporadic dating back some 100 years, though one paper carried hearsay reports about turtles that may have been nesting up till some 50–60 years ago in Ghajn Tuffieha (see map). Other sporadic nesting beaches historically recorded were from Gozo (Ramla l-Hamra) and from Comino (Santa Marija), as marked in the above map.

restricted to only small pockets along the Maltese coast occupying circa 2.5% (AXIAK & SAMMUT, 2002) of the Maltese coastline or an estimated 6.5km.

On the side of Ġnejna Beach, there is a small kiosk and the owner also has a number of other commercial beach/sea related activities, including renting of kayaks, paddle boats and speed boats. Overlooking Golden Bay, there is a large five-star hotel and a small part of the beach is also privately operated by this hotel. A large kiosk is present on the hill above the beach and a number of other operators and kiosks are present near or on the beach with activities related to hiring of paddle boats, deck chairs and umbrellas. Golden Bay forms part of Il-Majjistral Nature and History Park and both bays are Natura 2000 protected areas.

These nesting events were reported by a person or persons on the beach. For the 2012 (MIFSUD *et al.*, 2015) and 2016, the report received was during the actual oviposition, when there were several barbecues lit on the beach. This is very common practice on Maltese beaches during summer evenings. For the 2018 case, the person in question reported such nesting to NTM (Nature Trust Malta-an e-NGO) the next day. Date of nesting was conventionally assigned as the day after the night when the clutch was laid i.e. 21<sup>st</sup> June for 2012, 2<sup>nd</sup> August for 2016 and 25<sup>th</sup> June for 2018 (see Tab. 1). Following such reports, a number of Government officials from the Environment Authority as well as staff from Nature Trust Malta, visited the area immediately – for 2012 and 2018, it was done early the next day after nesting and for 2016, officials were present during or right after the actual nesting. In 2012, the nest was some 5 meters (or less) away from the sea on the lowest part of the beach, easily subject to covering by waves, hence it was decided to relocate the eggs, into a dug-out egg chamber some 60-100 m away from the original nest. For the 2016 and 2018, no relocation was necessary, as the nest was more than 8-10 meters away from the water's edge. The decision for the 2018 event was quite difficult in view of the previous prognostications of the effect of the clay on the 2012 nest. However, following the advice of NTM, which had 'gently' dug the nest to confirm its presence; it was decided not to relocate, since the area where the nest was, did not seem to contain clay.

In 2012 (MIFSUD *et al.*, 2015), the relocation was done as per standard procedures and the number of eggs was recorded. The artificial nest was dug out as closely as possible, in terms of depth, diameter and shape (flask shaped), to the natural nest.

Close collaborations were established in all cases with the commissioner of the Police and with the Administrative Law enforcement officials. Press releases were immediately drafted and issued through the help of the National Affairs Office (and PR office) of the Environment Authority and by NTM.

In all the nesting cases on the day of or a few days after nesting, an Emergency Conservation Order (ECO), banning barbecues, and other harmful activities like the use of cars in the adjacent area, were issued. A large area of about 4m x 4m around the nesting was cordoned off with green shallow mesh plastic in each case. In these ECOs, the use of caravans and camping was prohibited in the area adjacent to the cordoned-off area. In Ġnejna, vehicles were forbidden to get closer than 20 m to the nest and in Golden Bay such a prohibition was not necessary as vehicles do not normally drive on the sandy beach itself. Large catering and other activities were also banned close to the cordoned off area. Nearer to the hatching time, the southern part of the mesh was opened to give free access to the hatchlings, in case of unnoticed emergence.

A 24 hour surveillance was set up in all the nesting cases, with government officials also from the department of Works Division, and with Volunteers from NTM. In these nesting events, the Maltese officials immediately contacted the regional and international experts on the subject in view of the lack of direct experience on nesting in the Maltese coast and their advice was followed. An aluminium cage was placed around the nest in every case, for the further protection of the nest as per the advice from the Cypriot author of this paper. '*Marine Turtles Encounter Guidelines*' (short versions and long version) were written as well as 'what one should do if one encounters a nesting turtle coming up the beach...again'. The latter was done in case of the eventuality of multiple nesting by the same turtle. These guidelines were issued on the Authority's website and the leaflets were appended to the cordoning mesh and to other beaches.

In 2012, a local notice to mariners was also issued to pre-advise that when the eggs hatched (issued 3 weeks prior to the expected hatching date), the maritime traffic and fishing in the bay would be prohibited during the hatching time frame. Signage with this information and details of a call line for emergencies, were also appended in the area prior to the stipulated date of hatching. For the 2012, however, since the nest had failed, these regulations were then not eventually put into force.

In the ECO of 2016 and 2018, the above-mentioned clauses re maritime traffic, were incorporated in the actual ECO. All the lights (for all nesting cases) in the adjacent areas overlooking the beach, were either turned off and/or had red filters put on them. Warning signs were put up all around the beach (stipulating activities not permitted during that time) and a number of FAQ and other information posters were also appended all around the cordoning mesh.

In all cases, on a number of occasions, sand bags had to be used to lessen the effect of waves coming up near the nest. In the 2018 nesting, since it had been noted how rain water would behave following the 2012 nest and storm, measures were taken to mitigate this by placing sand bags to help divert the currents during heavy rain. This worked well as there were a number of small storms and sand bags helped to divert the water flow.

A protocol was also drafted for the eventuality of hatching, in order to control the people potentially attending the hatching event. Another protocol (both in Maltese and in English) was also drafted for the surveillance personnel. Security officials and volunteers were advised to check the nest every 20 minutes even during the night when the hatching time was getting closer. For Ġnġena, an agreement had also been made with the nearby five star hotel to switch off the lights facing the bay when hatching was noted to have started. A number of radio and TV programmes were also attended by key members and all the conservation measures were featured on the evening news. A number of media releases were also issued to alert about the government notices.

In 2012, in the last 2 weeks prior to the stipulated hatching the then Maritime Authority was notified to remove the swim zone (which has a line and a number of floating buoys), since it was understood that this might interfere with the swimming hatchlings. In 2016 and 2018, weights were positioned on various points on the swim zone line, for the same reason.

## RESULTS

For the 2012 nest on the 79<sup>th</sup> day (7<sup>th</sup> September) we excavated the nest (Tab. 1); the top layer (some 5 eggs) had eggs with fully developed embryos inside (at the latest stages of development). 1 embryo of these 5, had a yolk sac which was undergoing absorption, implying the embryos died some 2-3 days prior to hatching. This embryo was about 5 cm long. Eggs beneath were less developed showing mid-stages of development whilst the lowest layers of eggs had embryos in either the initial stages of development or with no discernible embryo at all. 7 embryos were quite big (late stages of development) between 4.2 to 4.8 cm and about 18 embryos were between 3.0 to 4.0, 4 embryos were between 2.4 - 2.8 and 8 embryos between 1.5 - 2.2 cm. Some (approximately 23) were at very early stages, and approximately 8 eggs seemed to have no discernible embryo at all.

When the chamber was dug out, it was noticed that the lowest layer was very wet and the bottom of the nest had either embedded water and/or been inundated with rain water in the previous weeks and the nest had not drained. A lot of blue clay material at this level was also found in the nest.

For the 2016 nest (Tab. 1), hatching happened on the 26<sup>th</sup> September with 56 days of incubation (nesting on 2<sup>nd</sup> August). Exhumation was done on the 29<sup>th</sup> September. After analysis of the remnant egg shells and all nest contents, it was discovered that 79 (max 80) eggs had hatched (hatching was most probably in one episode on the 26<sup>th</sup> September) and 13 eggs remained unhatched, with 1 hatchling which had not made it out of the nest. It was thus calculated that the initial nesting clutch was of 93 or 94 eggs. The success rate was thus about 86 %. Out of the 13 eggs, 7 were opened for examination. 2 had no discernible embryo (not even at early stages as not even an embryonic disc was observed), 1 was at the very initial stage of development, 3 were at the middle stage of development and 1 was at late stage of development (possibly a few days before it would have hatched).

For the 2018 nest (Tab. 1), hatching occurred on the 22<sup>nd</sup> August after 59 days of incubation. The nest was exhumed on the 28<sup>th</sup> August. It was discovered that the nest had 112 eggs of which only 1 had not hatched. Thus in 2018, 99.1 % hatching success was expected.

**Tab. 1.** Depiction of the recent sporadic nests from 2012-2018 with details on nest parameters

Year	Name of location	Nesting	Number of eggs	Hatching date	Incubation days	Exhumation date	Fertility	Hatchling success
2012	Gnejna Bay (eggs relocated within 24hrs)	21 <sup>st</sup> June	79	n/a	n/a	7 <sup>th</sup> September	7 embryos @ late stages of development & ~ 18 embryos were mid stages, 12, mid but smaller, 8 mid and smaller embryos and 23 were at very early stages. 8 eggs seemed to have no discernible embryo.	0 %
2016	Golden Bay- ir-Ramla Tal-Mixquqa	2 <sup>nd</sup> August	93/94	26 <sup>th</sup> September	56	29 <sup>th</sup> September	13 eggs unhatched; 1 hatchling died in nest; 7 opened for examination: 2 no discernible embryo; 1 very initial stage of development, 3 were @ mid stage & 1 was at late stage of development	86 %
2018	Gnejna	25 <sup>th</sup> June	112	22 <sup>nd</sup> August	59	28 <sup>th</sup> August	Only 1 egg did not hatch	99.1 %

rienced. As in the 2016 case, it appeared that all hatchlings emerged on the same night and for 2018, all had hatched in one very quick wave of emergence, as the event did not take more than about 15 to 20 mins, according to the volunteers who were on site.

## DISCUSSION

As previously discussed, it seemed probable that the huge amount of clay present in the nest of the 2012, had led to the failing of this nest. This was evident from the high clay content which was subsequently found in the nest after exhumation and the fact that the underside of the nest was found inundated by seawater and/or the ineffective draining of the heavy rainfall of the last weeks before the expected hatching time. This water or wetness was probably the cause of the death of embryos in the bottom layer at the early stage whilst the upper ones got lost at a much later stage (since the upper embryos were either middle stage or fully formed). These embryos might have got damaged, due to the high demand on the air that the developing embryos were placing on the already limited air in the nest chamber. The waves and rain had inundated the nest on several occasions in the end of August and beginning of September, despite the sandbags placed around the nest. The latest 'torrential' rains in the area had been recorded between the 2-3 Sept, though it is speculated that at this stage most of the embryos might have already been dead with the exception of possibly the top-most 2.

In 2016, most of the eggs hatched, with an 86 % success rate, quite comparable to the good hatching success in Cyprus and Greece (and unlike in Sicily), though there are even higher success rates in certain areas (Demetropoulos, pers. comm). The good results were probably due to the good sand present in Golden Bay and since the rain experienced in the previous few weeks was not torrential and anyway the 'good' porous sand provided very good water drainage, all combined with the good conservation measures established during the incubation time.

In 2018, the exceptional record of 99.1 % was quite an astonishing result. Though the initial decision was quite challenging, in view of the 2012 failed nest, the immediate area around the nest seemed to lack clay particles and the nest was not too near the sea, hence the decision was made not to relocate and keep the nest in its natural place.

According to MARGARITOUΛIS & REES (2008), from a study ongoing since 1982, reproductively older turtles produce more nests (multiple nesters) and lay more eggs than the reproductively younger turtles. It can thus possibly be postulated that the Maltese case, can be a case of neophytes that may have started nesting for the first time in Malta in 2012 with an inadequate initial place (this initial nest was too near the sea and the area was completely inundated with sea water the next day) and which nest had to be relocated. The initial nest only had 79 eggs. In 2016, 93/94 eggs were deposited, whilst in 2018, 112 eggs were laid. The hatching success also increased over time. In all cases, no other nesting was found in the same year, although these can be cases of missed nesting or non-observed nesting in the Maltese Islands, which would then possibly have succumbed in view of sand compaction by mechanical removal of the *Posidonia* banquettes which is a common practice in summer. Nesting events in the central and western Mediterranean (TOMÁS *et al.*, 2002; DELAUGERRE & CESARINI, 2004; BENTIVEGNA *et al.*, 2008; SENEGAS *et al.*, 2008; TOMÁS *et al.*, 2008; CARRERAS *et al.*, 2018) have been increasing during

the last decade, and seem to be associated with ongoing colonisation, which is favoured by global warming. This recurrent nesting in Malta also points in this direction.

It is important to note that as postulated by CARRERAS *et al.* (2018), colonisation of appropriate new habitats is crucial for species survival at the evolutionary scale under changing environmental conditions. MAFUCCI *et al.* (2016) further state that the loggerhead turtle has begun to nest steadily beyond the northern edge of the species' range in the Mediterranean basin.

The hypothesis that it was the same turtle that was involved in all the Maltese recent nesting cases reported can only be proven through DNA analysis of these eggs and embryos. Analysis from all these nesting episodes will need to be followed up to check the rookery provenience of the mother and if it was the same turtle in all these nesting cases. Similar studies to those undertaken by CARRERAS *et al.* (2018) would need to be undertaken to prove this point.

The huge efforts to increase public awareness because of the overall goal of sea turtle protection, which is crucial to the survival of the species, was seen to be imperative in all the cases of these nesting events. The outreach program in which the local people, tourists and the general public were targeted was created and done throughout all the nesting periods and afterwards. Turtle conservation ultimately depends largely on awareness of the issues by the public. We cannot guarantee conservation but we are sure it goes a long way.

Relocation of Sea turtle nests to protect them remains a commonly used strategy around the world (BLANCK & SAWYER, 1981; WYNEEKEN *et al.*, 1988) and in 2012 no other option was available, because of the proximity of the nest to the sea. However, in 2018 this was not the case, and despite the failed hatching in 2012, the right decision was made to keep the nest in its original position.

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## SUMMARY

### **Is the 2018 loggerhead nest really another exceptional sea turtle nesting record following the 2012 and 2016 previous nesting cases in Malta?**

C. Mifsud, V. Attard & A. Demetropoulos

We report another nesting event by a loggerhead turtle in Ġnejna (Malta), which happened on the 25<sup>th</sup> June 2018, with an extraordinary hatching rate of 99.1% on the 22<sup>nd</sup> August 2018. This follows the previously reported nesting in 2012 in the same bay, and the 2016 event in a nearby beach at Golden Bay (ir-Ramla Tal-Mixquqa), both beaches located in the NW of Malta. Before the 2012 record, scientifically recorded turtle nesting in Malta had only been reported by Despott, dating back some 100 years, although in one paper, it is alleged that turtles may have been nesting in Malta up until some 50-60 years ago. Noting that loggerheads have an average remigration interval of 2 years, it is possible that the same turtle that nested in 2012 came back to Ġnejna to nest again in 2018. Even the 2016 nest in Golden Bay, may have been by the same turtle, since the beaches are in close proximity. Any 'missed' nesting in 2014, may have been due to nesting elsewhere, noting that Malta only has 2.5 % of its beaches that are sandy, or it may have been the case of an unobserved nesting resulting later in a failed nest due to sand compression from mechanical beach cleaning. It is hoped that DNA analysis, which ideally follows at a later stage, will determine whether it was the same turtle. Campaigns are currently ongoing to solicit greater reporting of nesting through more meticulous monitoring for sporadic nesting prior to beach cleaning which is carried out daily in summer in the early mornings. Following the unsuccessful hatching of the 2012 nesting, it was suggested that the high amounts of clay material in this sandy beach, together with the huge rainfall event during the last phase of the nest, may have contributed to the failed nest. It had been previously suggested that Ġnejna beach may not be optimal for development of the turtle's embryos, resulting in either low emergence success or none at all. Despite this, in 2018 a decision was taken not to relocate the eggs in Ġnejna to Golden Bay, where a high hatching emergence had been sustained in 2016. The decision not to relocate was based on the fact that the nest was not close to the waterline, as the 2012 nest and that digging in the nest area to confirm nesting showed that clay seemed not to be present in this small zone. In 2012, relocation was done in the same beach in the 12 hour period after nesting. In this paper we also describe the conservation measures that were set-up in this bay and in Golden Bay, including measures during hatching. Emergency conservation orders were issued in all the three nesting events, to protect the beaches in question from any major and potentially harmful activities. The sites were also surrounded and physically protected with a 24 hour monitoring scheme being set-up with the help of volunteers from Nature Trust Malta and government officials.



# NESTING OF THE LOGGERHEAD TURTLE (*CARETTA CARETTA*) IN THE SOUTHEAST ADRIATIC CONFIRMED

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Pirol, V. & Haxhiu, I.: Nesting of the loggerhead turtle (*Caretta caretta*) in the southeast Adriatic confirmed. Nat. Croat., Vol. 29, Suppl. 1., 23-30, 2020, Zagreb.

The nesting of sea turtles in Eastern Adriatic has not been previously reported, even though the possible nesting of the loggerhead turtle in Albania has been hypothesized. Data for nesting activities have been collected since 2002 along the Albanian coastline. Anecdotal evidence assembled over the years has provided important information regarding the possibility of the nesting of the loggerhead turtle in Albania. This study confirms the nesting of the loggerhead turtle in Albania. Although sporadic, it takes place along the entire Adriatic coast of Albania. This information shifts the border of nesting known so far from the northeast Ionian (Greece) to the southeast Adriatic Sea (Albania).

**Key words:** loggerhead, nesting, Albania, southeast Adriatic

Pirol, V. & Haxhiu, I.: Potvrđeno gniježđenje glavate želve (*Caretta caretta*) u jugoistočnom Jadranu. Nat. Croat., Vol. 29, Suppl. 1., 23-30, 2020, Zagreb.

Gniježđenje morskih kornjača u istočnom Jadranu dosad nije bilo potvrđeno, iako se pretpostavljalo da postoje moguća gniježdišta glavate želve u Albaniji. Podaci o gniježđenju duž albanske obale prikupljaju se od 2002. godine. Povremena opažanja prikupljena tijekom godina pružaju važne informacije o mogućim gniježdištima glavate želve u Albaniji. Ovaj rad potvrđuje gniježđenje glavate želve u Albaniji. Iako povremeno, odvija se duž cijele albanske obale Jadrana. Ta činjenica pomiče dosad poznatu granicu gniježđenja od najbližih sjeveroistočnih jonskih obala prema jugoistočnom Jadranu (Albanija).

**Ključne riječi:** glavata želva, gniježđenje, Albanija, jugoistočni Jadran

## INTRODUCTION

The loggerhead turtle (*Caretta caretta*) is a common and the most abundant sea turtle specimen found in the Mediterranean (GROOMBRIDGE, 1994). Nesting sites are found mainly along the coastline of the eastern Mediterranean basin, with the highest number of nests reported in Greece, Turkey, and Cyprus (MARGARITOU LIS *et al.*, 2003a; CASALE & MARGARITOU LIS, 2010; CASALE *et al.*, 2018).

Nesting areas of loggerheads in the Ionian have been reported and surveyed since 1977 on Zakynthos Island, Greece (MARGARITOU LIS, 1982), while nesting was found to have occurred almost up to the northern boundaries of Greece, on Corfu Island (MAR-

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GARITOU LIS *et al.*, 2003a; MARGARITOU LIS & PANAGOPOULOU, 2010). Nesting on Corfu island, which is evaluated by MARGARITOU LIS (2000) as “moderate” ( $20 < x < 100$  nest/season) seems to be the northernmost nesting distribution for the east coast of the Ionian, even though beaches like Kroz, Borsh and Palase have been classified as potential nesting sites in the Ionian coast of Albania (HAXHIU, 2010).

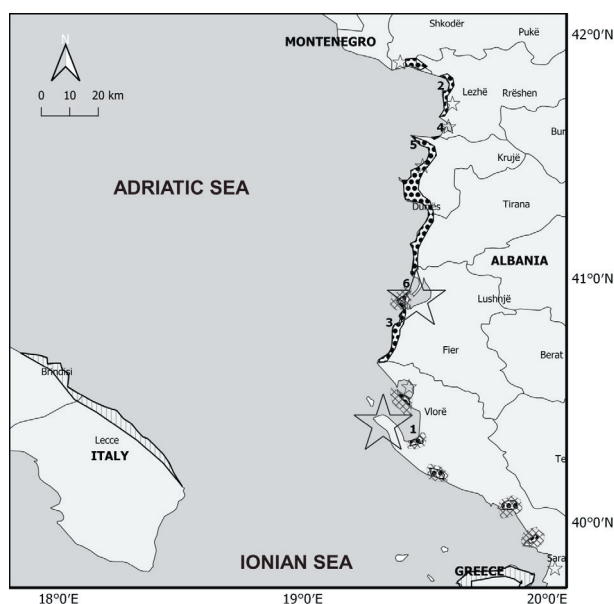
Nesting in the Adriatic is occasional, with a few nests reported along the south Italian coast (BASSO, 1996; MINGOZZI *et al.*, 2007, BENTIVEGNA *et al.*, 2010), but with no proper nesting sites (CASALE, 2010). Nesting of the loggerhead turtle in the eastern part of the Adriatic has not been reported previously, even though through the presence of small sized turtles has been reported and, with anecdotal reports of adult females (PIROLI & HAXHIU, in press), nesting on the Adriatic coast of Albania (HAXHIU, 2010), and Croatia has been hypothesized (LAZAR *et al.*, 2000; LAZAR, 2010). HAXHIU (2010) proposed the Adriatic coastline of Orikum, Narte, beaches near the mouth of Semani and Shkumbini Rivers, and Lalzi Bay as possible nesting sites in Albania, and reports the presence of matured eggs found in the oviduct of a turtle killed for culinary purposes in Orikum in 2004. The anecdotal reports, the continuous presence of female loggerheads in Albanian waters (PIROLI, 2011; WHITE *et al.*, 2011, 2012, 2013; PIROLI & HAXHIU, 2012, 2013; HAXHIU & PIROLI, 2016, 2018) and the new haplotypes found by mitochondrial DNA analysis in turtles foraging at Drini Bay (YILMAZ *et al.*, 2011) were an important stimulus to the search for nesting activities in Albania.

## METHODS AND MATERIALS

Surveys for possible nesting activities in Albania have been conducted since 2002. They covered data collection along the sandy beaches of the Adriatic and the Ionian coastline (Fig. 1). These surveys took place during the nesting season in the Mediterranean - May to September- and were organized according to the minimum data standards for nesting beach monitoring guidelines (SWOT, 2011) combined with questionnaires for fishermen and the local community. The survey team has been watching for tracks of females or hatchlings left in the sand through early morning beach patrols (5am-10am), before the beach was crowded, and then proceeded with meetings and interviews. When possible (when there was any elevation) beaches were observed with binoculars to cover larger areas, and key events were documented through photographs and by using the necessary equipment for measuring distance, diameter, temperature etc. Questionnaires were prepared and were aimed at collecting as much information as possible concerning possible nesting occurrences and the genuineness of them. To increase the accuracy of data the nesting surveys were combined with awareness campaigns under various projects implemented in both Adriatic and Ionian coastline.

## RESULTS

The evidence collected from these surveys provided important information for the possibility of the nesting of the loggerhead turtle in Albania. Hatchlings were reported to have been seen by a fisherman crawling toward the sea at Rana e Hedhun (Drini bay) in August 2007, while at Semani beach turtles were seen by fishermen and citizens moving toward the sand dunes during the night in July 2010 (Fig. 1). The genuineness of these two anecdotal reports, and many others collected over the years, still could not be evaluated as no photograph or any other form of documentation of any of these possible nesting events exists.



**Fig. 1.** Beaches evaluated by this study as suitable for nesting with black dots ellipse, Nesting sites proposed by HАХИИ (2010) with a grey mesh ellipse, Protected beaches with a star, Nearest nesting sites in Italy and Greece with grey stripes ellipse; 1- Eggs found in a killed turtle in 2004 (HАХИИ, 2010), 2- Hatchling emerging reported by a fisherman in 2007, 3- Nesting females reported nesting in 2010, 4- Eggs found in a tank in 2010 (PIROLI, 2011), 5- Hatchlings emerging on a small beach in 2017, and 6- Nest found in 2018. (Photo by Google maps modified by V. Pirolì).

The first undoubted evidence of the possibility of the nesting of the loggerhead turtle in Albania that we have documented consisted of some eggs found in a tank on July 30<sup>th</sup>, 2010 (PIROLI, 2011). A female loggerhead turtle (AL0274; CCL= 70 cm) found bycaught in set-nets (stavnik) at Drini bay (Fig. 1) was kept overnight by the fishermen in a tank at Patok area to be examined the next morning by the research team. These eggs, laid under stress, were fully matured (Fig. 2), but could not be reallocated for incubation as they had been immersed in water for hours. These eggs would possibly have been laid somewhere along the Albanian coastline since the nearest nesting beaches are in Greece, or Italy at a distance of at least 130 km (Fig. 1).

In 2017 pictures of some loggerhead turtle hatchlings were provided to the survey team (Fig. 3). About fifteen hatchlings were reported to have been found moving around at a small beach at Kepi i Rodonit on August 26<sup>th</sup> at about midnight. The area – a narrow, white sandy beach about 60m long, about 13m at its widest- was checked upon the reporting and even excavated to find the location of the egg chamber or any other evidence. No results were found, but 4 weeks had passed since the emerging of the hatchlings. The area was a beach highly disturbed by tourists, and the exact location of the nest was not reported. Based on the photographs provided, the hatchlings were of the loggerhead turtle (*Caretta caretta*). According to the emerging date the nest was evaluated to have been laid sometime in the end of May or beginning of June. This area is just few kilometers from the place where the turtle laying the eggs in the tank was bycaught (Fig. 1) and where the bycatch of adult female turtles has been recorded each year.



Fig. 2. Eggs found in a tank at Drini Bay, 2010. (Photo by I. Haxhiu)



Fig. 3. Hatchlings found at Kepi i Rodonit, 2017. (Photo by A. Ligaci)

On June 19, 2018 some tracks were reported to have been found at Divjaka beach (Fig. 1). The tracks, 69cm wide, were of a loggerhead turtle. By following the movement of the tracks and using the stick method, the egg chamber was located and eggs were found (Fig. 4). The nest was 7.5m from the water line, and 2m from the high tide. The nest was laid in a grey small granule beach with temperature measured next to the nest at a depth of 5 cm reaching about 39°C at 2pm, while at a depth of it decreases to 33°C. This temperature is a suitable temperature for incubation, meanwhile the water temperature on the same day at a depth of 0 m was 28°C.

Beach examination for possible nesting (Tab. 1) was conducted along both the Ionian and the Adriatic coastline and several beaches along the Adriatic coast were evaluated as suitable for nesting (Fig. 1). Beach evaluation was based on the elevation, granule size, presence of dunes and vegetation, disturbance and sand temperature. The most probable beaches for loggerhead nesting are: 1- In Drini Bay: some parts of the area known as Rana e Hedhun, Kune-Vain-Tale and some small beaches at the northern part of Kepi i Rodonit, while Godull area, even if it provides the best conditions otherwise,





Fig. 4. The nest found at Divjaka beach, 2018. (Photo by V. Piroli)

is highly polluted with plastic items and we think this diminishes the possibility of nesting; 2- Some small beaches of the southern side at Kepi i Rodonit and Lalzi Bay; 3- The beaches of the Durrës area are suitable for nesting but this area remains one of the most disturbed by tourism which might diminish the nesting possibility; 4- The entire area between the deltas of the Shkumbini e Vjosa River; 5- In Vlora bay: Nartë and Orikum; 6- Ionian sea: some small areas at Palase, Borsh and Krorëz.

## CONCLUSIONS

This evidence definitely confirms that the nesting of loggerhead turtle in Albania does happen, even if only sporadically. This new finding shifts the border of nesting known so far from the northeast Ionian (Greece) to the southeast Adriatic (Albania). The knowledge about the presence of the juvenile and adult loggerhead turtles at Drini Bay needs to be supplemented with information on movement patterns and genetic, to find any possible link with the new haplotypes found in loggerheads of this area (YILMAZ *et al.*, 2011). We think that hatchlings found at Kepi i Rodonit, the eggs laid in a tank in Patok, and the significant presence of the female turtles at Drini Bay (PIROLI & HAXHIU, in press) are evidence that Drini Bay is not just a development and foraging area, but an interesting habitat for female loggerhead turtles at the same time.

Nesting in Albania seem to happen as early as the end of May and the beginning of June so further studies regarding findings of the abundance and recurrence of nesting of the loggerhead turtle in Albania need to start as early as May.

Nesting, based on this evidence and the results of beach evaluations, seems to happen sporadically along almost the entire Adriatic coastline, from Drini Bay to Vlora Bay and possibly in some small areas along the Ionian coastline. Further research is needed to understand the situation better, especially on collecting data regarding hatching success, finding any genotypic variation, hatchling sex ratio, threats, and especially with all the changes foreseen within the global warming/climate change scenario,

**Tab. 1.** Beach evaluation for loggerhead turtle nesting possibility in Adriatic and Ionian coastline of Albania.

No	Name of the beach	Appropriate for nesting			Presence of	
		Elevation	Granule size	Sand temperature	Dunes & Vegetation	Human disturbance (1 no disturbance-5 high)
1	Velipojë	✓	✓	✓	✓	3
2	Rana e Hedhun	✓	✓	✓	✓	1
3	Shëngjin	✓	✓	✓	✓	5
4	Kune	✓	✓	✓	✓	3
5	Vain	✓	✓	✓	✓	2
6	Tale	✓	✓	✓	✓	3
7	Godull	✓	✓	✓	✓	1
8	Kepi i Rodonit north beaches	✓	✓	✓	✓	2
9	Kepi i Rodonit south beaches	✓	✓	✓	✓	2
10	Shën Pjetër	✓	✓	✓	✓	4
11	Rrushkull	✓	✓	✓	✓	2
12	Porto Romano	✓	✓	✓	✓	5
13	Sektor Rinia	✓	✓	✓	✓	5
14	Kallm	✓	✓	✓	✓	5
15	Currila	✓	✓	✓	✓	5
16	Vollga	✓	✓	✓	✓	5
17	Plepa	✓	✓	✓	✓	5
18	Golem	✓	✓	✓	✓	5
19	Shkëmbi i Kavajës	✓	✓	✓	✓	5
20	Karpen	✓	✓	✓	✓	2
21	Domen	✓	✓	✓	✓	2
22	Spille	✓	✓	✓	✓	2
23	Divjakë	✓	✓	✓	✓	3
24	Seman	✓	✓	✓	✓	4
25	Darzezë e Re	✓	✓	✓	✓	4
26	Pishë Poro	✓	✓	✓	✓	1
27	Zvërnec			✓	✓	3
28	Nartë	✓	✓	✓	✓	4
29	Vlorë	✓	✓	✓		5
30	Radhimë			✓		5
31	Orikum	✓	✓	✓	✓	5
32	Gadishulli i Karaburunit			✓		1
33	Palasë	✓		✓		5
34	Dhërmi	✓		✓		5
35	Gjipe	✓		✓		5
36	Jalë	✓		✓		5
37	Himarë	✓	✓	✓	✓	5
38	Porto Palermo			✓		5
39	Qeparo	✓		✓	✓	5
40	Borsh	✓		✓	✓	5
41	Lukovë	✓		✓		5
42	Sarandë			✓		5
43	Ksamil			✓		5



which might cause an increase of nesting in Albania in the future. From all the beaches evaluated as suitable for egg incubation only Divjakë beach has some degree of protection as it is a recreational site within the territory of the Divjake-Karavasta National Park. In the event of any increase of nesting events in the future and/or the finding of areas with continuous nesting events, there will be a great need for mitigation and protection of the area.

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# OCEANIC GIANTS IN THE MEDITERRANEAN: FIRST MITOCHONDRIAL ANALYSIS OF LEATHERBACK TURTLES (*DERMOCHELYS CORIACEA*) IN THE ADRIATIC AND TYRRHENIAN SEAS

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**Garofalo, L., Lorenzini, R., Marchiori, E., Poppi, L., Giglio, S., Madeo, E., Mizzan, L. & Novarini, N.: Oceanic giants in the Mediterranean: first mitochondrial analysis of leatherback turtles (*Dermochelys coriacea*) in the Adriatic and Tyrrhenian seas. Nat. Croat., Vol. 29, Suppl. 1., 31-36, 2020, Zagreb.**

The leatherback turtle *Dermochelys coriacea* (Vandelli, 1761) is an occasional trophic visitor of the Mediterranean basin. So far, very few individuals have been genetically analysed from this sea and none from Italy. We sequenced a mitochondrial DNA fragment of three specimens of *D. coriacea* found in recent years along the North-Adriatic and Tyrrhenian shores. They were all females approaching adult stage. Causes of death were attributable to the main threats for sea turtles in Mediterranean waters, all related to human activities (collisions with boats, entanglement in fishing nets and plastic debris ingestion). Two different mitochondrial haplotypes were observed, with the two North-Adriatic turtles sharing the same one. Compared to known *Dermochelys* sequences and previous genetic characterization of rookeries, these results suggest that the most probable origin of at least two of the three leatherbacks was the western Atlantic.

**Key words:** leatherback turtle, mtDNA, origin, central Mediterranean, human threats

**Garofalo, L., Lorenzini, R., Marchiori, E., Poppi, L., Giglio, S., Madeo, E., Mizzan, L. & Novarini, N.: Oceanski divovi u Sredozemlju: prva mitohondrijalna analiza sedmoprugih usminjača (*Dermochelys coriacea*) iz Jadranskog i Tirenskog mora. Nat. Croat., Vol. 29, Suppl. 1., 31-36, 2020, Zagreb.**

Sedmopruga usminjača *Dermochelys coriacea* (Vandelli, 1761) povremeni je posjetitelj Mediterana. Dosad je genetički analizirano vrlo malo tih jedinki, pritom nijedna iz Italije. Sekvencionirali smo fragment mtDNA tri primjerka *D. coriacea* pronađenih u posljednje vrijeme na obalama sjevernog Jadrana i Tirenskog mora. Sve su bile subadultne ženke. Uzorci smrti pripisani su glavnim prijetnjama morskim kornjačama u Mediteranu, a sve su povezane s ljudskom aktivnošću (sudari s plovilima, zapetljavanje

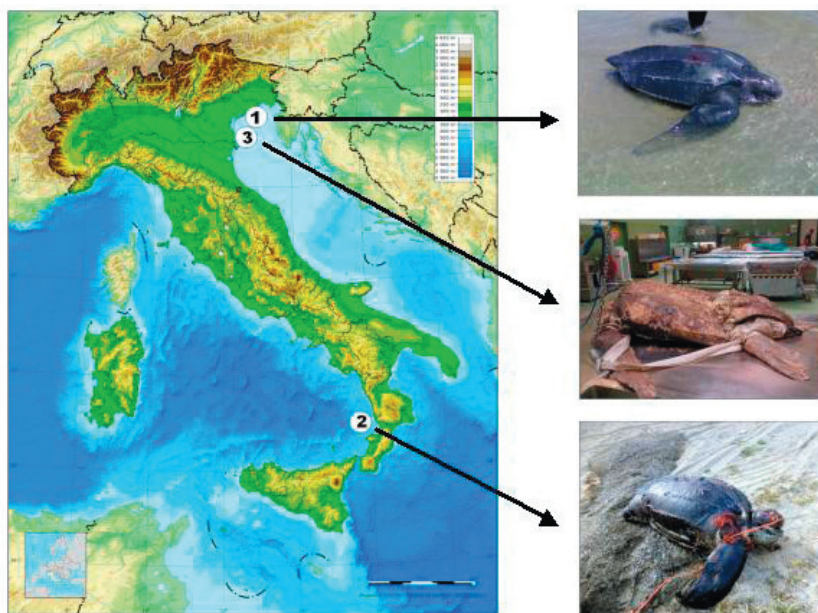
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u ribarske mreže i gutanje plastičnog otpada). Zabilježena su dva mitohondrijalna haplotipa, pri čemu su dvije sjevernojadranske kornjače imale isti haplotip. U usporedbi s poznatim *Dermochelys* sekvencama i ranijim genetskim istraživanjima gnijezdišta, ovi rezultati sugeriraju da je najvjerojatnije porijeklo bar dviju od tri usminjača bio zapadni Atlantik.

**Ključne riječi:** sedmopruga usminjača, mtDNA, porijeklo, centralni Mediteran, humane ugroze

## INTRODUCTION

The leatherback turtle *Dermochelys coriacea* (Vandelli, 1761) is distributed worldwide at tropical and subtropical latitudes. It is reported in Mediterranean and Italian waters occasionally during feeding migrations (CASALE *et al.*, 2003), but no nesting within the basin has ever been confirmed by direct observations. The species is globally listed as Vulnerable in the IUCN Red List of Threatened Species (A2b,d: [www.iucnredlist.org](http://www.iucnredlist.org)), although threats and trends of abundance vary considerably in different populations. In order to assess population structure (DUTTON *et al.*, 2013) and to allow the identification of source rookeries for stranded or incidentally caught animals (PROSDOCIMI *et al.*, 2014; RODEN *et al.*, 2017; VARGAS *et al.*, 2019), western and eastern Atlantic colonies were genetically characterised for a variable fragment of the mitochondrial DNA (mtDNA). Very few individuals found in the Mediterranean have been previously genotyped (VELLA & VELLA, 2016; RODEN *et al.*, 2017), and none from Italian waters, which are a central crossroad in migration routes within the basin and an important foraging area for Mediterranean and Atlantic sea turtles (GAROFALO *et al.*, 2013a; 2013b). To trace the origin of three individuals from Italy [two found along the North-Adriatic coast (NOVARINI *et al.*, 2010, 2017) and one bycaught in a fishing net in the Tyrrhenian waters off Calabria, Fig.1], we sequenced mtDNA control region fragment, the same marker analysed previously for the nesting colonies in the western and eastern Atlantic (DUTTON *et al.*, 2013).



**Fig. 1.** Recovery sites of the turtles analysed. Photo credits: 1) C. Rigato; 2) M.A.R.E. Calabria; 3) UniPD.

## MATERIALS AND METHODS

Information about the three leatherback turtles is summarized in Tab. 1. Size and sex of the animals, as well as causes of death, were determined during necropsy, although the 2014 specimen had missing gonads due to the bad state of the carapace and was therefore sexed from external morphology (NOVARINI *et al.*, 2017). Muscle samples (25 mg) were collected and frozen. DNA was extracted with the QIAamp Mini Kit (QIAGEN). A mtDNA control region fragment of 763 bp encompassing the tRNA<sup>Pro</sup> and the D-loop was amplified and sequenced using the primer pair LCM15382/H950G (ABREU-GROBOIS *et al.*, 2006), following the protocols as in (MARCHIORI *et al.*, 2017). These primers are used to amplify the same DNA segment of different sea turtle species: *Caretta caretta*, *Chelonia mydas* and *D. coriacea*. Sequences were analysed using the DNA Sequencing Analysis Software version 5.1 (Applied Biosystems). The multiple alignment program included in the package Vector NTI version 9.1 (Invitrogen) was used to align the sequences. Our sequences were deposited and compared with those available from the same species in GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). Haplotypes were classified according to the nomenclature used in DUTTON *et al.* (2013).

**Tab. 1.** Field data, necroscopic results and mtDNA haplotypes for the turtles.

ID	Date of recovery	Site of recovery	Cause of death	CCL (cm)	Sex	mtDNA haplotype	GenBank Acc. N°
1	02.06.2009	Lido di Venezia (VE)	Bacterial gastroenteritis	138	F	Dc3.1	MK674798
2	11.10.2013	Lamezia Terme (CZ)	Asphyxia in fishing nets	143	F	Dc1.1	MK674797
3	03.10.2014	Chioggia (VE)	Boat strike	147	F	Dc3.1	MK674798

## RESULTS

The sequenced leatherback turtles (Tab. 1) were all females, at threshold sizes (STEWART *et al.*, 2007) between subadult and adult stages (mean CCL:  $142.7 \pm 4.5$  cm). All causes of death appeared to be related to the main threats for sea turtles in the Mediterranean waters: collisions with boats, entanglement in fishing nets and plastic debris ingestion, which in sample 1 may have caused severe bacterial gastroenteritis (POPPI *et al.*, 2012). Two different haplotypes were found: Dc1.1 and Dc3.1 (sequences deposited in GenBank, accession numbers: MK674797 and MK674798, respectively). The latter haplotype (Dc3.1) was shared by the two North-Adriatic turtles.

## DISCUSSION AND CONCLUSIONS

DUTTON *et al.* (2013) described seven and nine distinct Management Units (MUs) for *Dermochelys coriacea* based on mtDNA and nuclear microsatellite data, respectively, from known leatherback nesting colonies of the western and eastern Atlantic. Ten haplotypes were found, of which Dc1.1 was the most common and was present in all rookeries at frequencies ranging from 39% (Brazil) to 94% (Florida). The second most common haplotype in the same colonies was Dc3.1, which was found in all western Atlantic (Caribbean) populations and in Gabon (Africa) at frequencies ranging from



3% (U.S. Virgin Islands) to 61% (Brazil). Haplotypes Dc1.1 and Dc3.1, separated by five mutations, are central in the two-star-shaped phylogroup network of D-loop haplotypes recovered by DUTTON *et al.* (2013). While haplotype Dc1.1 is frequent in both eastern and western Atlantic colonies (so it is not a good “source-tag”), haplotype Dc3.1 is widespread in western Atlantic colonies and rare in the eastern ones. The presence of Dc3.1 in two of our three turtles may thus be indicative of their western Atlantic origin, even though they cannot be ascribed to a specific colony.

Further analysis using nDNA markers (microsatellites) would allow more precise assignment of leatherbacks to the nesting population of origin (STEWART *et al.*, 2016; RODEN *et al.*, 2017), while more extensive sampling is needed to better assess patterns of connectivity between Mediterranean foraging areas and Atlantic nesting populations. Our three female *D. coriacea* were large sub-adults, and they all appear to have died as a result of human activities off the Italian coasts. Therefore, anthropogenic impacts in the central Mediterranean area may negatively affect not only the Mediterranean populations of loggerhead and green turtles (GAROFALO *et al.*, 2013a; 2013b), but also Atlantic leatherbacks, just before recruitment to their reproductive populations.

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## SUMMARY

### Oceanic giants in the Mediterranean: first mitochondrial analysis of leatherback turtles (*Dermochelys coriacea*) in the Adriatic and Tyrrhenian seas

L. Garofalo, R. Lorenzini, E. Marchiori, L. Poppi, S. Giglio, E. Madeo, L. Mizzan  
& N. Novarini

The leatherback turtle *Dermochelys coriacea* (Vandelli, 1761) is sighted in Mediterranean and Italian waters occasionally during feeding migrations, although no nesting within the basin has ever been confirmed by direct observations. Very few individuals have been previously genetically analysed from this sea and none from Italy, which is a central crossroads in Mediterranean migration routes and an important foraging area for several marine animals. Three individuals of *D. coriacea* found in recent years (2009-2014), two stranded along North-Adriatic shores and one bycaught in fishing nets in the Tyrrhenian waters off Calabria, were genetically analysed. They were sub-adult females (mean curved carapace length:  $142.7 \times 4.5$  cm). Causes of death for the three animals were attributable to the main threats for sea turtles in Mediterranean waters, all directly and indirectly related to human activities (collisions with boats, entanglement in fishing nets and plastic debris ingestion). A mitochondrial DNA fragment encompassing the control region was amplified and sequenced for each individual. Two different haplotypes were observed, with the two North-Adriatic turtles sharing

the same haplotype. The two haplotypes cannot be ascribed to a single colony, due to their common presence in most of the Atlantic rookeries previously characterized. However, the leatherbacks found in North-Adriatic waters could probably have originated from the western Atlantic, judging from the evaluation of the frequencies of their haplotype in the possible source rookeries.



# NATIONAL ACTION PLAN FOR THE CONSERVATION OF MARINE TURTLES ALONG THE EGYPTIAN MEDITERRANEAN COAST

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**Abdelwarith, M. S. & Jribi, I.: National Action Plan for the Conservation of Marine Turtles along the Egyptian Mediterranean Coast. Nat. Croat., Vol. 29, Suppl. 1., 37-44, 2020, Zagreb.**

Marine turtles are highly migratory reptiles that utilize both terrestrial and aquatic environments during their life. As in other regions of the Mediterranean, marine turtles in Egypt are affected by numerous human activities, such as deterioration of critical habitats and migratory routes, incidental capture, boat strikes, pollution and intentional killings. In accordance with the commitment of Egypt to the conservation of marine turtles in its national waters, underlined in national legislation and by way of ratification of related international conventions, the National Action Plan for the Conservation of Marine Turtles (NAP) was developed and adopted with the support of UNEP-MAP Regional Activity Centre for Specially Protected Areas. The participatory approach was applied in the preparation of the NAP, carried out through the concentration meetings with stakeholders. The main goal of the NAP is to achieve favorable conservation status for marine turtle species and their habitats, and enhance their protection along the Egyptian Mediterranean Coast. This goal will be implemented through specific actions and sub-actions, including legislation, management, research, capacity building, and awareness and education.

**Key words:** marine turtles, action plan, Egypt, Mediterranean Sea, critical habitats, marine conservation, policy

**Abdelwarith, M. S. & Jribi, I.: Nacionalni akcijski plan za zaštitu morskih kornjača duž egipatske sredozemne obale. Nat. Croat., Vol. 29, Suppl. 1., 37-44, 2020, Zagreb.**

Morske kornjače su vrlo migratorni gmazovi, koji tijekom života koriste i kopnena i vodena staništa. Kao i u drugim dijelovima Sredozemlja, morske kornjače u Egiptu pod utjecajem su brojnih ljudskih aktivnosti, kao što su uništavanje značajnih staništa i migratornih ruta, slučajni ulovi, sudari s plovilima, zagađenje i namjerno ubijanje. U skladu s obvezama Egipta prema zaštiti morskih kornjača u nacionalnim vodama, što je naglašeno i u nacionalnom zakonodavstvu i ratifikacijom odgovarajućih međunarodnih konvencija, razvijen je i usvojen Nacionalni akcijski plan za zaštitu morskih kornjača (NAP), uz potporu UNEP-MAP Regionalnog akcijskog centra za posebno zaštićena područja. U pripremi NAP-a primijenjen je participatorni sustav, uz sastanke sa svim stranama u postupku. Glavni cilj NAP-a je postizanje povoljnog konzervacijskog statusa morskih kornjača i njihovih staništa, te poboljšanje njihove zaštite duž egipatske obale Sredozemlja. Taj cilj bit će implementiran kroz specifične akcije i podakcije, uključujući zakonodavstvo, upravljanje, istraživanje, podizanje svijesti i edukaciju.

**Ključne riječi:** morske kornjače, akcijski plan, Egipt, Sredozemno more, značajna staništa, zaštita mora, politika

## INTRODUCTION

The Mediterranean Sea is inhabited by a rich biota the biodiversity of which is disproportionate to its dimensions. Within less than 1% of the surface area of the world's oceans, the Mediterranean hosts approximately 17,000 species representing 4–18% of the world's marine biodiversity, with 20–30% of marine species considered endemic to the basin (BIANCHI & MORRI, 2000; BOUDOURESQUE, 2004; COLL *et al.*, 2010; MANNINO *et al.*, 2017). Three species of marine turtles inhabit the Mediterranean Sea: the most common loggerhead turtle *Caretta caretta* (Linnaeus, 1758), the green turtle *Chelonia mydas* (Linnaeus, 1758), and the leatherback turtle *Dermochelys coriacea* (Vandelli, 1761). While loggerhead and green turtles reproduce in the region, the leatherback turtles regularly enter Mediterranean from the Atlantic Ocean to feed. All three species are globally listed under the IUCN Red List of Threatened Species: the green turtle as Endangered [EN], whilst loggerhead and leatherback turtles are classified as Vulnerable [VU] (IUCN, 2020).

The Mediterranean Egyptian waters host foraging grounds and migratory corridors from/to multiple nesting areas for both the loggerhead and the green turtle (LAURENT *et al.*, 1996; CLARKE *et al.*, 2000; CAMPBELL *et al.*, 2001; BRODERICK *et al.*, 2007; REES *et al.*, 2008; NADA & CASALE, 2011; NADA *et al.*, 2013; SCHOFIELD *et al.*, 2013; STOKES *et al.*, 2015; SNAPE *et al.*, 2016), while strandings and bycatch also indicate the presence of the leatherback turtle (CLARKE *et al.*, 2000; NADA & CASALE 2008; NADA *et al.*, 2013; RABIA & ATTUM 2015). In addition, loggerhead and green turtles are known to nest along the Egyptian Mediterranean coast. The highest nesting activity is documented at the beaches of North Sinai, low nesting occurs in the western coast of Alexandria, while no nesting activity has been documented in the area from Alexandria to Port Said (RABIA & ATTUM, 2015). Overall, nesting on the Egyptian Mediterranean coasts is considered to be low (CASALE *et al.*, 2018), despite the presence of several hundred kilometers of potentially suitable nesting beaches.

Incidental catch in fisheries (bycatch), coastal development and non-human predation are the main threats to sea turtles in the Mediterranean, followed by beach restructuring and erosion, exploitation (direct take of eggs and nesting females), and pollution by plastic and biomagnifying contaminants (CASALE & MARGARITOU, 2010). A high bycatch of loggerhead turtles has been observed in the easternmost part of the Levantine Basin, off the coast of Turkey, Syria, and Egypt (CASALE, 2011; CASALE *et al.*, 2012). Incidental catch, direct use of turtles for meat and blood, and coastal development have been recognized as the main threats to sea turtles in Egypt (CLARKE *et al.*, 2000; NADA & CASALE, 2008).

Due to decades of conservation efforts on nesting beaches in the form of national regulations and active protection of clutches, coupled with the cessation of exploitation, nest counts at some major nesting sites have recently indicated positive population trends for Mediterranean loggerhead and green turtles. Implementation of on-board recovery techniques for reduction of bycatch mortality have also been introduced as a conservation measure in some regions, but mitigation of incidental catch in fisheries is still in its infancy (CASALE *et al.*, 2018). The recovery of the Mediterranean subpopulation of loggerhead turtles resulted in its regional delisting from the Endangered [EN] to the Least Concern [LC] category, but such status is highly dependent on the continued implementation of conservation measures (IUCN, 2020).

Because they are highly migratory organisms that utilize different habitats throughout their life cycle, the conservation of sea turtles is dependent upon the cooperation of countries sharing their critical habitats and the inclusion of stakeholders in conservation decision-making, such as fishers, the tourism industry, conservationists, researchers and different sea-users, as well as local and national decision-makers (CAMIÑAS *et al.*, 2020). This is particularly true for the semi-enclosed Mediterranean Sea, surrounded by 21 countries (UNEP/ MAP 2012). The international commitment of Egypt to the conservation of marine turtles was underlined by the ratification of the Barcelona Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean in 1976 and its amendments in 2000, including the Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean. Egypt also adopted the updated Action Plan for the Conservation of Marine turtles in the Mediterranean (UNEP-MAP RAC/SPA, 2007). Many other regional and international conventions were ratified, such as the Convention of Biological Diversity (CBD), Convention on Migratory Species (CMS), Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and RAMSAR Convention on Wetlands. In addition, the commitment of Egypt to the conservation of marine turtles in its national waters was underlined within national legislation in the field of environmental (the Law of Environment 9/2009 and its executive regulation), biodiversity (the Law of protected areas and biodiversity 102/1983), and fisheries regulation (the Law regarding fishing, aquaculture and fish farms regulations 124/1983).

The National Action Plan (NAP) for the conservation of marine turtles was prepared in order to implement national and international legislation directed at the conservation of marine turtles in Egypt, and address the continuous and increasing impacts of human activities along the Egyptian Mediterranean coast that are likely to negatively affect marine turtles inhabiting Egyptian Mediterranean waters.

## DEVELOPMENT OF THE NATIONAL ACTION PLAN

The following rationale for the development of the NAP were identified: (i) Egyptian territorial waters host habitats for marine turtles, but there is a lack of knowledge on the biology of the species in the Egyptian waters; (ii) marine turtles are under increasing pressure due to the direct and indirect effects of human activities which impact both nesting and feeding habitats in Egypt; (iii) enforcement of existing national legislation alone, without an increase in public awareness and shifts in attitudes towards the need for conservation of marine turtles, would not guarantee efficient protection since illegal takes would still exist; (iv) Egypt is bound by national and international legislation to protect marine turtles.

The National Action Plan was developed by a team of experts formed in 2017 with the support of the UNEP/MAP Regional Activity Centre for Specially Protected Areas (RAC/SPA) and in collaboration with the Egyptian Environmental Affairs Agency (EEAA). The expert team initially reviewed published information and “grey literature” (e.g. national surveys reports), and collected additional information through surveys and interviews in markets and in areas known to host important habitats for marine turtles in Egypt. The participatory, bottom-up approach was implemented in the preparation of the NAP, enabling the inclusion of different stakeholder groups. The National Action plan was finalized through concentration meetings with relevant national governmental and management institutions (the Nature Conservation Sector of

EEAA, General Authority for Fish Resources Development, managers of the relevant protected areas, environmental offices in coastal governorates), research bodies (the National Institute of Oceanography and Fisheries and the Arab Academy of Technology and Maritime Transport), civil society organizations (fishermen societies) and others.

RESULTS AND DISCUSSION

The Action Plan aims to achieve a favorable conservation status for marine turtle species and their habitats in the Mediterranean Sea, and enhance their protection in the Egyptian Mediterranean area through legislative and management actions, research, capacity building, and awareness-raising and education (Tab. 1). In order to reach these conservation goals, the following targets were set: (i) Identifying anthropogenic impacts on marine turtles and their habitats, and undertaking mitigation measures; (ii) ensuring that human activities are managed, so that human-induced mortalities are reduced; (iii) monitoring, protecting and restoring marine turtle habitats, if needed; (iv) enhancing the governance and capacity framework for marine turtle conservation, and (v) enhancing cooperation and coordination at national and regional levels. The National Action Plan was adopted at national level in consultation with

Tab. 1. Structure of the National Action Plan (NAP) for the conservation of marine turtles

Categories / Actions	Sub-actions
1. Legislative actions	
	1.1 Harmonizing national legislation with the SPA/BD Protocol, ratified conventions, obligations and provisions
	(a) Analyzis of the current relevant national legislation concerning marine turtles' conservation
	(b) Harmonization of the legislation with the National Action Plan, if needed
2.2 Management actions	
	2.1 Launching national stranding network
	(a) The preparation of a stranding programme
	(b) The establishment of a tissue bank
	(c) Periodical publication of an Egyptian Mediterranean Coast stranding report
	2.2 Implementation of nesting monitoring programme
	(a) Preparation of a national monitoring programme
	(b) Training of Egyptian personnel into on-beach monitoring techniques
	(c) Implementation of the national monitoring programme
	(d) Annual publication of the activities carried out by the programme
	2.3 Establishing a tagging programme
	(a) Launch of a national tagging programme
	(b) Organization of a training course on tagging techniques, nesting monitoring, fishing interaction assessment and strandings
	2.4 Establishment of Marine Turtle First Aid Centre
	(a) Search for an appropriate local for the center
	(b) Acquisition of materials and necessary equipment

Tab. 1. Continued

Categories / Actions	Sub-actions
	(c) Training of veterinary personnel into sea turtle medical care
	(d) Selection of personnel for training on running and managing the rehabilitation center
	2.5 Establishing specially protected areas for marine turtles
	(a) Identification of hot-spots for marine turtles' in Egyptian Mediterranean waters
	(b) Evaluation of the existing and potential threats to marine turtles in identified critical habitats
	(c) Establishing of specially protected areas, if needed
3. Research	
	3.1 Investigating turtle biology and mortality based on strandings cases including pathology, mortality, biology and genetics
	(a) Creation of a tissue bank for pathological, toxicological and genetic studies
	(b) Necropsical analysis of all possible stranded turtles for the determination of the cause of death
	(c) Promot of studies by Egyptian specialists on the pathology, mortality, biology, and genetics of marine turtles
	3.2 Studying marine turtles – fishery interactions
	(a) Conduct interview-based surveys with the local fishing community along the entire Mediterranean coastline of Egypt
	3.3 Studying nesting activity
	(a) Assignment of a national coordinator to oversee the teams, gather the data and prepare annual nesting reports
	(b) Tagging programme underway and operating
4. Capacity building	
	4.1 Institutional capacity building
	Aimed at public administration, management bodies of existing protected areas, research and teaching intitutions, and advocacy organizations
	4.2 Individual capacity building
	Aimed at law enforcers, researchers and media
5. Awareness and education	
	Aimed at decision makers, fishing communities, schools and general public

relevant stakeholders at the validation workshop held in Cairo in October 2017, for the period of five years.

Due to its geographic location, and ecological and oceanographic characteristics, Egypt is known to contain critical terrestrial and marine habitats for Mediterranean marine turtles (RABIA & ATTUM, 2015; CASALE *et al.*, 2018). However, there are significant gaps in our knowledge related to fundamental aspects of biology, such as total number of clutches laid annually, population trends, mortality and predation rates, at-sea distribution, natal origin, movements and habitat use. Collection of missing, key biological information through research and monitoring actions set by NAP (Tab. 1) is hence critical for implementation of science-based conservation actions and evaluation of their efficiency. The continuous increase in human activities along the Egyptian coast and in the sea affects nesting and feeding grounds of marine turtles. Quantitative assessment of threats, fisheries interactions in particular, is also crucial for the guiding of the national conservation management, and is also addressed by NAP actions.

Egypt is bound by both national and international legislation to protect marine turtles and to maintain populations in a ‘favorable conservation status’. However, merely enforcing and abiding by these regulations will not be sufficient to ensure adequate conservation given the level and nature of threats affecting them nationally. A particular problem is presented by illegal killings and use of marine turtle meat and blood for consumption and religious purposes (CLARKE *et al.*, 2000; NADA & CASALE, 2008). Changing attitudes towards sea turtles will therefore present a major conservation challenge, and can be achieved only by an adequate long-term public awareness-raising programme focused on specific target groups. Therefore, education and awareness actions, together with capacity building, are integral parts of the adopted NAP.

The Egyptian NAP outlines actions according to their priority and feasibility, with different activities mutually reinforcing and acting synergistically (Fig. 1). It is consistent with activities proposed by NADA & CASALE (2008) and CASALE & MARGARITOU (2010), which included reduction of sea turtle killing in Alexandria and in other areas, introduction of turtle-based ecotourism, implementation of education campaigns aimed at fishermen and stakeholders, reduction of bycatch and minimization of anthropogenic impacts on nesting beaches through legal protection, law enforcement, and long-term population monitoring.

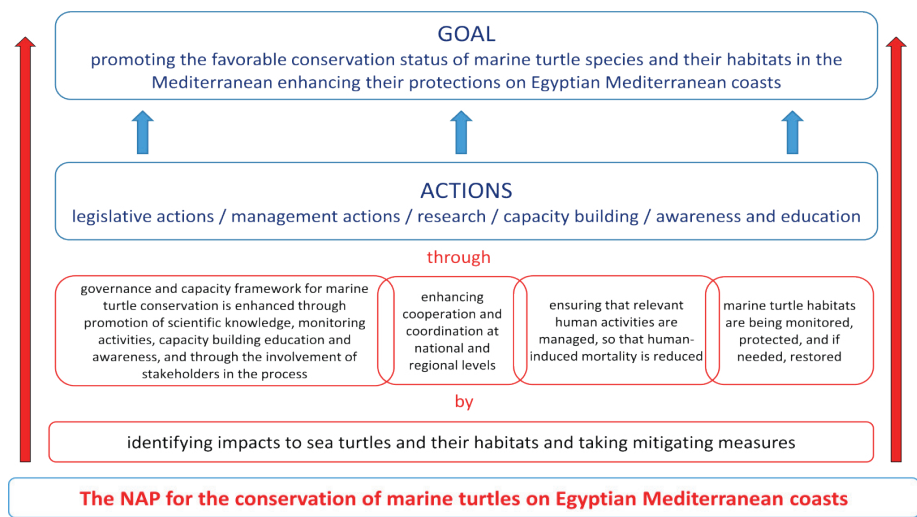


Fig. 1. The Egyptian NAP actions diagramme, according to their priority and feasibility, with different activities synergistically taking place.

As large-bodied, highly migratory, keystone species, marine turtles play an important role in shaping the biodiversity, structure and functions of marine ecosystems (ESTES *et al.*, 2016; HAMMERSCHLAG *et al.*, 2019). Protection and monitoring of nesting areas, assessment and mitigation of interactions with fisheries, and management of foraging grounds are some of the key conservation actions defined by NAP. Such a science-based strategy is crucial for achieving ‘a favorable conservation status’ for marine turtles in Egyptian Mediterranean waters, and if successful, it has the potential to enhance the biodiversity and resilience of the ecosystems marine turtles use. Estab-



lishment of a collaborative network of stakeholders, capacity building and increased public awareness will furthermore enhance national marine conservation and contribute to building a more conservation-orientated society. Moreover, the NAP will practically implement national and regional conservation commitments of Egypt, contributing to the restoration of sea turtle populations in the Mediterranean basin. In addition, it has the potential to enhance regional cooperation between UNEP MAP-RAC/SPA and EEAA aimed at conservation of marine biodiversity. At the end of 5 years, the period proposed for the implementation of NAP, a review process will critically evaluate accomplishments, problems and shortcomings in order to redirect and reformulate a new set of actions for the future.

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# PROGRESS OF WORK FOR MONITORING MARINE TURTLES ALONG THE EGYPTIAN MEDITERRANEAN COAST

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**Naguib, N.M., Abdelwarith, M.S. & Jribi, I.: Progress of work for monitoring marine turtles along the Egyptian Mediterranean Coast. Nat. Croat., Vol. 29, Suppl. 1., 45-49, 2020, Zagreb.**

Since 2016 steps towards the monitoring and conservation of marine turtles along the Egyptian Mediterranean coast were taken, to determine other possible nesting sites. A survey was implemented with the relevant stakeholders to identify possible nesting areas along the Egyptian Mediterranean coast from Port Said to El-Sallum. By the adoption of the National Action Plan for the Conservation of Marine Turtles in the Egyptian Mediterranean Coast, activities related to category (ii): management action, category (iii): research, and category (iv): capacity building were implemented. Monitoring and tagging programmes were established; protocols for sampling and genetic analyses were established, in addition to a survey of the relevant stakeholders. Training programmes were conducted at regional and national levels and then the national team was identified.

**Key words:** marine turtles, Egyptian Mediterranean coast, national action plan, loggerhead (*Caretta caretta*), green turtle (*Chelonia mydas*), conservation

**Naguib, N.M., Abdelwarith, M.S. & Jribi, I.: Napredak rada na monitoringu morskih kornjača duž egipatske obale Mediterana. Nat. Croat., Vol. 29, Suppl. 1., 45-49, 2020, Zagreb.**

Od 2016. poduzimaju se duž egipatske obale Mediterana koraci prema monitoringu i zaštiti morskih kornjača da bi se identificirala ostala moguća mjesta za gniježđenje. Poduzeto je istraživanje koje je uključivalo sve zainteresirane strane, a u svrhu identificiranja mogućih mjesta za gniježđenje duž egipatske obale Mediterana između Port Saida do El-Salluma. Usvajanjem Nacionalnog akcijskog plana za zaštitu morskih kornjača na egipatskoj obali Mediterana, određene su aktivnosti u kategoriji (ii): akcijsko upravljanje; (iii): istraživanje, i (iv): jačanje sposobnosti. Uspostavljeni su programi za monitoring i označavanje životinja; protokoli za prikupljanje i genetičke analize; te ispitivanje relevantnih zainteresiranih strana. Provedeni su treninzi na regionalnoj i nacionalnoj razini te je uspostavljen nacionalni tim sudionika.

**Ključne riječi:** morske kornjače, egipatska obala Mediterana, nacionalni akcijski plan, glavata želva (*Caretta caretta*), zelena želva (*Chelonia mydas*), zaštita

## INTRODUCTION

Egypt's Mediterranean coastline extends for 1,050 km and is considered a significant area for the nesting and foraging of the Loggerhead turtle (*Caretta caretta*) and the Green turtle (*Chelonia mydas*); there are also sporadic records for the Leatherback turtle (*Dermochelys coriacea*), referring to stranding but not to nesting activity (LAURENT *et al.*, 1996;

CLARKE *et al.*, 2000; BRODERICK *et al.*, 2007; REES *et al.*, 2008; NADA & CASALE, 2011; NADA *et al.*, 2013; SCHOFIELD *et al.*, 2013; STOKES *et al.*, 2015; SNAPE *et al.*, 2016).

Monitoring and conservation of marine turtles have always focused on Zaranik Protected area (El-Arish beach), where nests for Loggerhead and Green turtles were recorded from 1998 until 2012 (CLARKE *et al.*, 2000; CAMPBELL *et al.*, 2001, RABIA & ATTUM, 2015); KASPAREK (1993 a,b) identified nests for the loggerhead in El Salloum. Green turtles are most frequent in the region from Port Said to Rhafa, while Loggerhead turtles are typically found in the area between Alexandria and Port Said.

Through the last 10 years, there was a significant increase in tourism development activities along the Egyptian Mediterranean coast which might adversely affect both species and habitat, in addition to interaction with fisheries characterised by bycatch and other threats. In 2017, the National Action Plan for the Conservation of Marine Turtles in the Egyptian Mediterranean Coast was adopted and a regional action plan implemented. The national action plan has five broad categories: category (i): legislative actions, category (ii): management action, category (iii): research, category (iv): capacity building and category (v): awareness and education.

This paper constitutes a brief on the progress made by international and national experts in cooperation with the relevant stakeholders in the field of conservation of marine turtles along Egyptian Mediterranean coast.

## MATERIAL AND METHODS

A survey was done from Port Said (the eastern part of the Mediterranean coast in Egypt) to El-Sallum (the western part of the Mediterranean coast in Egypt), in collaboration with the relevant stakeholders (Nature Conservation Sector, National Institute for Oceanography & Fisheries – Alexandria, scouts, Faculty of Agriculture – Cairo University) to report possible locations for nesting areas from Port Said to El-Sallum through meetings and a rapid field survey. In addition, steps were taken in order to implement certain activities in line with the national action plan for marine turtle conservation on the Egyptian Mediterranean coast, in line with the following:

Capacity building at the institutional and individual level for representatives of the relevant stakeholders, NGOs, management bodies of existing MPAs, universities and research institutions in line with category (iv). Monitoring and tagging programme were established, in line with category (ii).

Meetings with stakeholders were organized to prioritize points for the research programme, such as studying interaction with fisheries, genetic studies programme, microbiological studies, diseases & medical studies and ecological studies; in line with category (iii).

## RESULTS AND DISCUSSION

By the end of the survey that was conducted in 2017 (Fig. 1) it was estimated that there had been no change in nesting activity for either loggerhead or green turtles from the situations recorded by CLARKE *et al.* (2000), NADA & CASALE (2008, 2010), NADA *et al.* (2013), and RABIA & ATTUM (2015) in the eastern part of the Egyptian Mediterranean coast and on top of that new potential nesting sites along the western part was identified and a monitoring programme planned.

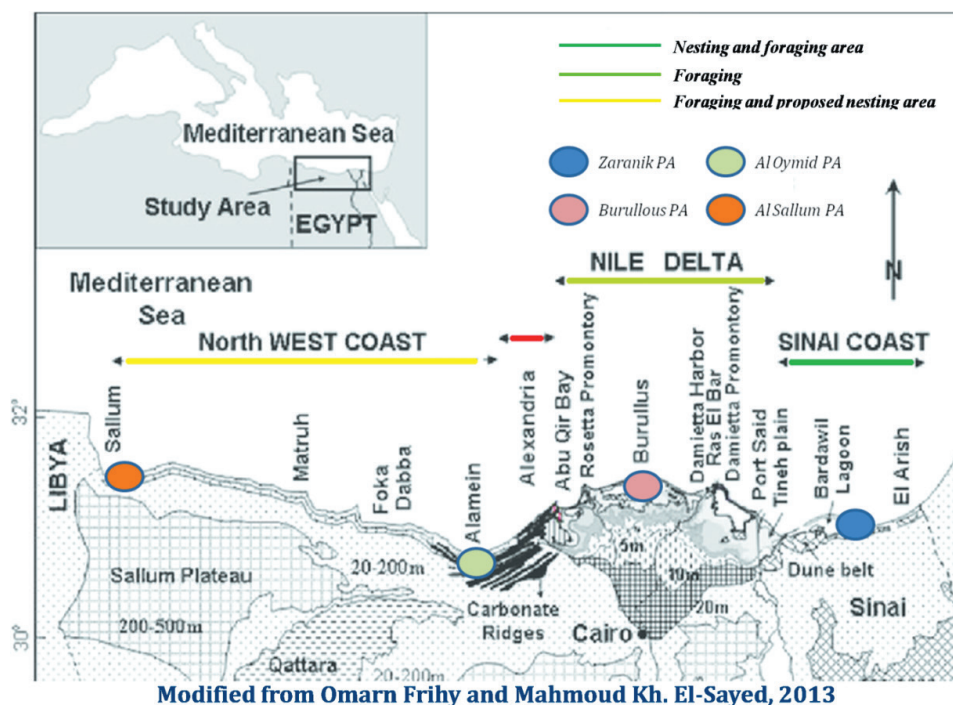


Fig. 1. Location of nesting and foraging areas

During this survey, sequential meetings with the relevant stakeholders were organized to prioritize points for research programmes, such as interaction with fisheries, a genetic studies programme, microbiological studies, diseases & medical studies and ecological studies.

The National Team (21 participants representing different stakeholders governmental, research, universities, NGOs, fishermen, and Scouts) was determined and trained at national and at international level on the rescue & rehabilitation of marine turtles, monitoring, necropsies, sample collection and data analysis supported by SPA-RAC (Special Protected Areas—Regional Activity Center). Network among stakeholders and implementing bodies was established.

Monitoring and tagging programmes were established, in concert with the implementation of a limited monitoring survey. Loggerhead and green turtle individuals were recorded from Marsa Matrouh and El-Sallum. In addition stranded turtles along the Egyptian Mediterranean coast were reported by fishermen especially in Damietta and Burullus (Fig. 1). More than 10 turtles were released by scouts, NGOs and EEAA in the last 2 years in cooperation with the National Institute of Oceanography and Fisheries with two newly tagged turtles. Fishermen observed loggerhead and green turtles in Marsa Matroh and El-Salum, which indicates that these areas could well be considered as nesting sites. Strandings were reported in Port Said, Alexandria and El-Salum.

Sample collection started in 2017 for both the Loggerhead turtle (*Caretta caretta*) and the Green turtle (*Chelonia mydas*) for the purpose of establishing a tissue bank for marine turtles in Egypt, and protocol was prepared for genetic analyses.

## CONCLUSION

Along the Egyptian Mediterranean coast, the monitoring and conservation of marine turtles has always focused on the Zaranik Protected Area, neglecting the other possible nesting sites. Over the last 10 years, there was significant increase in water pollution along the Egyptian Mediterranean coastline that might affect both species and habitat. A survey was established in 2017 to report possible locations for nesting areas from Port Said to El-Salum, and by the end of this survey the National Action Plan for the Conservation of Marine Turtles in the Egyptian Mediterranean Coast had been adopted.

The survey emphasized that despite the presence of several hundred kilometers of seemingly suitable nesting beaches nesting activity is considered low compared to other Mediterranean sites. Interviews with fishermen and local communities tended to support the hypothesis that the region from north Sinai to Alexandria (Fig.1) offers foraging habitats for green turtles and loggerheads. Some observations by fishermen and local communities suggest a possible site for loggerheads in El-Salloum.

Furthermore, identification of the national team representing different stakeholders governmental, research, universities, NGOs, fishermen, and Scouts. Sample collection started in last 2 years for both the Loggerhead turtle (*Caretta caretta*) and the Green turtle (*Chelonia mydas*) for the purpose of establishing a tissue bank for marine turtles in Egypt, a system was established for tagging and a protocol was prepared for genetic analysis. Two training programmes were conducted supported by SPA-RAC and funded by the MAVA foundation: a regional training in Turkey and a national one in Egypt. More than 10 turtles were released by scouts, NGOs and EEAA in the last 2 years in cooperation with the National Institute of Oceanography and Fisheries with 2 tagged individuals. Fishermen observed loggerhead and green turtles in Marsa Matroh and El-Salum. Stranded turtles were reported in Port Said, Alexandria and El-Salum. The National Programme is managing the monitoring marine turtles in the rest of Egyptian Mediterranean coast, with supporting programmes for necropsies and sample analyses (medical, microbiological and genetic analyses).

The monitoring programme will continue for 3 years to be able to assess the newly proposed nesting sites in parallel with raising public awareness.

## Challenges during the implementation of the national action plan:

- Difficulties in obtaining the right samples at the right time
- Costs
- Overlap of responsibilities among stakeholders
- Overlapping between researchers and those working in field and/or NGOs.

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# MARINE TURTLE NESTING SURVEY AND STRANDING ASSESSMENT FROM TARTUS TO SYRIA'S BORDER WITH LEBANON

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Saad, A., Soulaiman, A. & Alkusairy, H.: Marine turtle nesting survey and stranding assessment from Tartus to Syria's border with Lebanon. Nat. Croat., Vol. 29, Suppl. 1., 235-242, 2020, Zagreb.

The nesting of sea turtles (*Caretta caretta* and *Chelonia mydas*) was studied along the beach south of Tartus (i.e. *the Beach of Dreams "Al Ahlam"*) as far as the border of Syria and Lebanon to the south, a distance of about 40 km. It includes a length of about 10 km suitable for sea turtle nesting. The beach was divided into 7 subsections, each of them between 0.3 and 3 km long. The nesting success was 25% for the loggerhead turtle and 40% for the green turtle. A total of 106 dead marine turtles were observed, including 95 (92%) loggerhead turtles and 11 (8%) green turtles.

**Keywords:** Sea turtle nesting, stranding, threats, Tartus beach, Syria

Saad, A., Soulaiman, A. & Alkusairy, H.: Istraživanje gniježđenja morskih kornjača i procjena nasukavanja od Tartusa do granice Sirije i Libanona. Nat. Croat., Vol. 29, Suppl. 1., 235-242, 2020, Zagreb.

Istraživano je gniježđenje morskih kornjača (*Caretta caretta* i *Chelonia mydas*) od plaže južno od Tartusa (tj. *Plaža snova "Al Ahlam"*) sve do granice Sirije i Libanona na jugu, duljine od oko 40 km. To područje uključuje oko 10 km prikladnih za gniježđenje morskih kornjača. Plaža je podijeljena u 7 dijelova, svaki dugačak od 0,3 do 3 km. Uspjeh gniježđenja bio je 25% za glavatu želvu i 40% za zelenu želvu. Zabilježeno je ukupno 106 mrtvih morskih kornjača, uključujući 95 (92%) glavatih želvi i 11 (8%) zelenih želvi.

**Ključne riječi:** gniježđenje morskih kornjača, nasukavanje, ugroze, plaža Tartus, Syria

## INTRODUCTION

Three sea turtle species occur regularly in the Mediterranean; loggerhead turtle (*Caretta caretta*), green turtle (*Chelonia mydas*) and leatherback turtle (*Dermochelys coriacea*) (CASALE *et al.*, 2018; REES *et al.*, 2004; TÜRKOZAN & KASKA, 2010). Loggerhead and green turtles both breed in the Mediterranean, while leatherback turtles from the Atlantic use the region only for foraging (KASPAREK *et al.*, 2001; MARGARITOU LIS *et al.*, 2003). The main loggerhead nesting areas are in Greece, Turkey, Cyprus, Libya and Syria (KASPAREK *et al.*, 2001; MARGARITOU LIS *et al.*, 2003; 2004; CASALE & MARGARITOU LIS, 2010; REES *et al.*, 2008) and the main foraging areas are along the North African, Adriatic and north-eastern Ionian continental shelves (CASALE & MARGARITOU LIS, 2010; STOKES *et al.*, 2015). Green turtle habitats are restricted, with a more easterly distribution. The main green turtle nesting areas are in Turkey, Cyprus and Syria (KASPAREK *et al.*, 2001; CANBO LAT, 2004; REES *et al.*, 2008; CASALE *et al.*, 2018; SAAD, 2012). The Mediterranean subpo-

pulation of loggerhead turtles was downgraded in 2015 to Least Concern (LC) from Endangered (EN) according to the IUCN red list criteria. It is assumed that the current status of the Mediterranean subpopulation represents the success of past conservation efforts at nest sites and the Mediterranean subpopulation is subsequently assessed as conservation dependent (CASALE & TUCKER, 2017). In addition, Mediterranean green turtles are ranked as part of the global classification as Endangered (SEMINOFF, 2004) and the leatherback turtle as globally Vulnerable (WALLACE *et al.*, 2004). However, the main conservation efforts were carried out on the nesting beaches of Mediterranean and the main threats in marine habitats still remain.

The presence of loggerhead (*Caretta caretta*) and green sea turtles (*Chelonia mydas*) off the coast of Syria, was first reported by GRUVEL (1931) but the nesting beaches were not indicated. The next turtle information to come out of Syria resulted from a rapid assessment survey in 1991 that identified low-level nesting concentrated on a beach south of Lattakia City (KASPAREK, 1995). Local researchers noted incidental turtle captures in beach seines, and also observed turtles stranded along the coast (SAAD *et al.*, 2003). Since 2004 a more extensive coastal survey has been undertaken, primarily to better identify Syria's actual and potential nesting populations (SAAD & REES, 2004; REES *et al.*, 2005; SAAD *et al.*, 2006; REES *et al.*, 2008). A combination of nocturnal surveys during the nesting season and co-operative efforts with fishermen afforded the first opportunities to observe turtles in the wild to obtain basic biometric data and tag the turtles before they returned to the sea after nesting or were released after being caught in fishing nets. In contrast to Lattakia Beach where nesting has been studied for 10 years (2004-2010) (SAAD *et al.*, 2010), this is the first time that nesting sites have been studied thoroughly and comprehensively on the coast stretching from the city of Tartus to the Lebanese-Syrian border.

## MATERIAL AND METHODS

The nesting and stranding of sea turtles were studied in the beach south of Tartus city (35°35'07 E; 34°51'43 N) as far as the Lebanese border in the south (35°58'24.16 E; 34°37'58.48 N), a total distance of about 40 km (Fig. 1). Assessment of marine turtle strandings was carried out by direct examination of carcasses and a questionnaire survey of fishermen to confirm their observations about the mortality of sea turtles: The study was undertaken weekly between January 2016 and December 2017. On encountering the dead turtle, the following data were recorded: Area name - date - species identification - straight carapace length (SCL) - note accompanying injury, which depends on the availability of a healthy and soft shield. Estimate the distance between dead turtles during the trip when close to each other. Turtle carapaces were painted to avoid repeat counts (GARDNER & NICHOLS, 2001). Interviews were also conducted with fishermen on the beach about the dangers facing turtles. With respect to nesting, the area was surveyed daily between 20 June to 29 August 2016. Ground patrols were carried out throughout every kilometer of the sandy coast to survey for turtles, turtle tracks and turtle nests. Nesting success and nest numbers were calculated in two ways: 1 - from initial track assessment that did not include clutch identification (Figs. 1, 2). 2 - By summing the number of nests proven by observation of eggs (Fig. 4). The stage of the mature individual and the stage of the young or non-adult individual were determined based on the body length of the dead turtle, both the loggerhead turtle and the green turtle reach sexual maturity in the Mediterranean when body length averages between 60-65 cm (<https://seaworld.org>, 2020 ; MILLER, 1997).

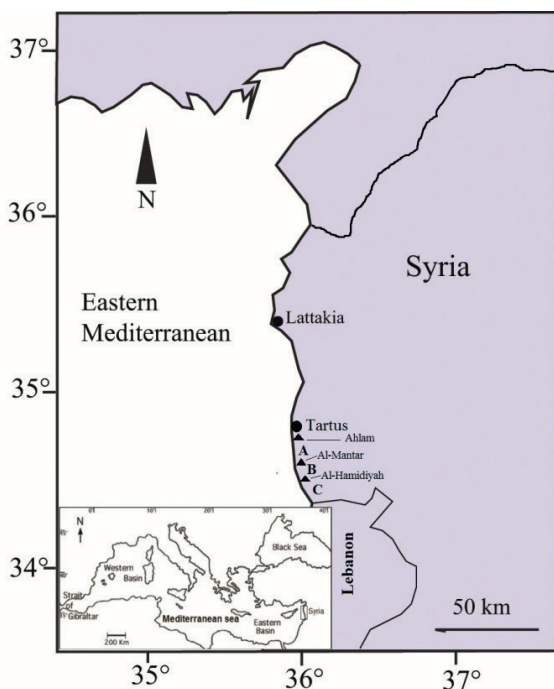


Fig. 1. Study area Between Tratus and the Lebanese border, a total distance of about 40 km



Fig. 2. Green turtle *Chelonia mydas* hatching track



Fig. 3. Track of loggerhead turtle *Caretta caretta*.

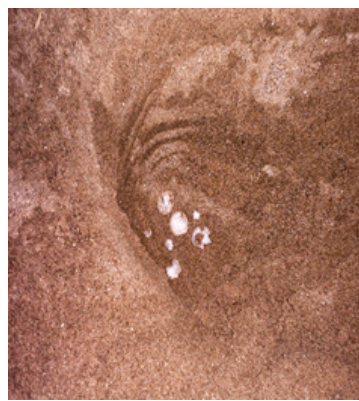


Fig. 4. Egg observation in the nest.

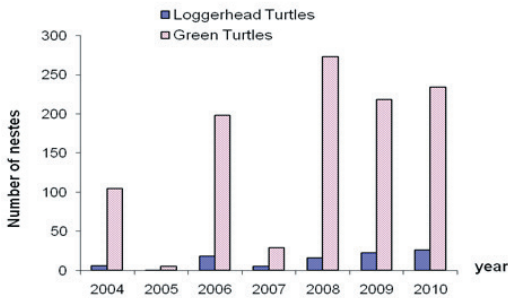
## RESULTS AND DISCUSSION

The present study provides the first sea turtle nesting and stranding data from the south of Tartus beach in Syria, and shows that a total of 36 turtle tracks including 13 nests were recorded. The majority of the nests, 11 (84%), were from the loggerhead turtle, *Caretta caretta*, and 2 (16%) were from the green turtle, *Chelonia mydas*. The nesting success rate was 25% for the loggerhead turtle and 40% for the green turtle. The nesting density at the site of the study was 1.3 nest/km, and the overall nesting success rate was 26.5%. The highest number of nests was recorded in Area A (Al Ahlam

Beach and Amrit (35°54"06 E; 34°50"08 N) (Tab. 1). The results showed that the peak of nesting occurred during the second week of July (7 nests). The results of this study reveal a reversal of species prevalence from the northern part of the country, as a similar study previously undertaken on the beach of Shkaifate, south of Lattakia (REES *et al.*, 2010; SAAD *et al.*, 2010) revealed green turtle nesting was dominant (Fig. 5).

**Tab. 1.** Monthly variation of stranding turtles number on the three areas (A,B,C)

Month	Area			Number of stranding turtles
	A	B	C	
1	1	0	0	1
2	4	0	0	4
3	1	3	2	6
4	14	9	9	32
5	9	2	0	11
6	4	6	0	10
7	0	6	4	10
8	1	1	11	13
9	1	6	2	9
10	1	2	2	5
11	0	0	0	0
12	1	1	3	5
Total	37	36	33	106



**Fig. 5.** Variation in the number of turtle nests on Lattakia beach during seven years of survey.

The strandings indicate that the loggerhead is present year-round in this area. A total of 106 dead marine turtles were observed, including 95 (92%) loggerhead turtles and 11 (8%) green turtles. The results showed that higher numbers of dead green turtles in April ( $n = 32$ ) with a mortality rate of 0.25 carcasses/km/month (Tab. 1). About 86% of all turtles found were juveniles or sub-adults. The percentage of turtles over 60 cm in length came to 56% (it is assumed that they reach sexual maturity with a length of 60 cm); individuals over 65 cm in length accounted for 28%. BASKAL *et al.* (2018) in the Fethiye-Göcek (Turkey) reported that the majority of stranded logger heads were considered to be adults (77.45%), while the majority of stranded green turtles were immature. Timing of marine turtle mortalities and visible injuries suggest that gill net and dynamite fishing were responsible for most of the deaths.

Strandings of Loggerhead turtle were more common during spring and summer. This probably coincided with the increase in fishing activities. Our data agree partially with the observations of JRIBI *et al.* (2007) in the Gulf of Gabes (Tunisia), TOMÁS *et al.* (2008) in the Valencian Community (Spain) and CARACAPPA *et al.* (2018) on the Sicilian coast. On the other hand CORSINI *et al.* (2013) reported that a higher incidence of loggerhead turtle strandings in Rhodes Island was observed in summer, while there were more green turtle strandings in winter.

Surveys indicate that there is interaction of turtles with fisheries as it is speculated that drift net fishing offshore could be the cause of the numbers of dead stranded turtles found; also, the coast is greatly affected by the presence of sea-borne plastic and other waste. The presence of large quantities of litter was noted during this survey. This waste accumulates in some areas in such quantities as to cause hindrance to turtles wishing to come ashore to nest and subsequently for the emerged hatchlings struggling to reach the sea.



Comparing the results of the current study with the results of the numerous studies carried out in other countries bordering the eastern and southern shores of the Mediterranean Sea, it becomes clear to us that the main threats to sea turtle populations are coastal development (ILGAZ *et al.*, 2007), natural predation (ERK'AKAN, 1993; BRODERICK & GODLEY, 1996; REES *et al.*, 2008; SAAD *et al.*, 2010), incidental catch and intentional killings by fishermen (CASALE, 2011), as well as collision with marine craft (CASALE & MARGARITOU, 2010). Entanglement in plastic marine debris is also likely to be a major source of mortality (DUNCAN *et al.*, 2017). Incidental catch is considered to be the main threat in the Eastern Mediterranean (TÜRKOZAN *et al.*, 2013); the results of LEVY *et al.* (2015) also show that gillnets and trawlers are the main threats to sea turtles in the Levantine basin. In addition SONMEZ (2018) reported that the Fishing activities and marine pollution is the main cause of strandings on Samandağ Beach (eastern Mediterranean coast of Turkey). Oceanic and sub-adult stage individuals were stranded in especially high numbers due to plastic Materials (SONMEZ, 2015). CASALE (2011) estimated that over 132,000 sea turtles were captured, of which 44,000 die annually in the Mediterranean Sea.

Incidental catch is considered to be the main threat in the Mediterranean and CASALE (2011) estimated that over 132,000 sea turtles were captured, of which 44,000 die annually in the region

This study confirms that south of Tartus is likely to be an important feeding and nursery ground, with the loggerhead turtle being the most common species.

## CONCLUSION

Conservation efforts on the nesting beaches should therefore not be the only criteria for assessing a population. In this case, the population status of sea turtles in marine habitats and mortality rates, according to stranding data and observations in fisheries, are needed for a comprehensive assessment, especially in Syria. The lack of data on the number of strandings and sea turtle deaths prevents us from making a proper assessment, but the available data shows that we lost at least the same number of sea turtles in the marine habitats that we protected on the nesting beaches of the Tartus beach. Thus, conservation measures should be extended to marine habitats while conservation studies on the nesting beaches must be continued.

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# DETERMINATION OF THE FACTORS THAT MIGHT HAVE INFLUENCED THE REHABILITATION OF *CARETTA CARETTA* IN THE LAMPEDUSA SEA TURTLE RESCUE CENTER BETWEEN 2001-2016

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**Roldi, C. & Freggi, D.: Determination of the factors that might have influenced the rehabilitation of *Caretta caretta* in the Lampedusa Sea Turtle Rescue Center between 2001-2016. Nat. Croat., Vol. 29, Suppl. 1., 59-66, 2020, Zagreb.**

The Lampedusa Sea Turtle Rescue Center started its activity in 1990, and in this paper we analyze data from 2812 loggerhead turtles rescued by 2016. Here we analyse which factors might have affected the rehabilitation success of the injured sea turtles hosted in our Center. We underline the following factors: type of clinical case, animal's health condition and the presence of a qualified surgeon.

For the first factor we estimated the percentage of survival animals with the various clinical cases (n=928) and for the second factor we estimated the outcome of therapies (recovery achieved/death) and health condition (good health condition/depressed/comatose) using the Fisher exact test which confirmed how health condition can significantly affect sea turtle rehabilitation success. For the last factor, we divided the study period into five subperiods (2001-2003, 2004-2006, 2007-2009, 2010-2012, 2013-2016) based on the evolution of surgical techniques and the presence of an expert surgeon with direct experience of sea turtle surgery; with post hoc test of univariate ANOVA investigation, we confirm significantly the value of the experience of professionals involved. Bycatch and health condition appear to significantly influence rehab success and the presence of a competent surgeon is radically responsible for an increase in the survival of loggerhead turtles, as expected.

**Key words:** marine turtles, rehabilitation success, clinical cases, survival, Lampedusa

**Roldi, C. & Freggi, D.: Određivanje čimbenika koji su mogli utjecati na rehabilitaciju glavatih želvi *Caretta caretta* u Centru za spašavanje morskih kornjača Lampedusa između 2001-2016. Nat. Croat., Vol. 29, Suppl. 1., 59-66, 2020, Zagreb.**

Centar za spašavanje morskih kornjača Lampedusa počeo je s aktivnostima 1990., a u ovom radu analiziramo podatke 2812 glavatih želvi spašenih do 2016. godine. Analiziramo čimbenike koji su mogli imati utjecaja na uspjeh oporavka ozlijeđenih morskih kornjača smještenih u našem Centru. Naglašavamo sljedeće čimbenike: tip kliničkog slučaja, zdravstveno stanje životinje i prisutnost kvalificiranog kirurga.

Za prvi čimbenik procijenili smo postotak preživljavanja životinja u odnosu na razne kliničke slučajeve (n=928), a za drugi čimbenik procijenili smo ishode liječenja (postignuti oporavak/smrt) i zdravstveno stanje (dobro zdravstveno stanje/depresivna/komatozna) koristeći Fisherov egzaktni test koji je potvrdio da zdravstveno stanje može značajno utjecati na oporavak morske kornjače. Za posljednji čimbenik podijelili smo vrijeme istraživanja u 5 potperioda (2001-2003, 2004-2006, 2007-2009, 2010-2012, 2013-2016), na temelju razvoja kirurških tehnika i prisutnosti kirurga s direktnim iskustvom na operiranju morskih kornjača; koristeći post hoc test univarijatne ANOVA analize, potvrdili smo značajnu vrijednost iskustva prisutnih stručnjaka. Slučajni ulov i zdravstveno stanje značajno utječu na uspjeh oporavka, a kao što je i očekivano, prisutnost kompetentnog kirurga radikalno povećava preživljavanje glavatih želvi.

**Ključne riječi:** morske kornjače, uspjeh oporavka, klinički slučajevi, preživljavanje, Lampedusa

## INTRODUCTION

Over recent decades, the status of sea turtles and the need for their protection have increasingly become topics of public interest (CASALE *et al.*, 2008; CASALE, 2011), accompanied by a global rise in the number of sea turtle rescue centers. This is particularly true in Italy, where more than 25 of these rehabilitation centers operate. Rehabilitation based on sound conservation and biological principles (TRIBE & BROWN, 2000; FERRARO & PATTANAYAK, 2006) ensures that available resources can be allocated to the most effective conservation measures (TRIBE & BROWN 2000, FERRARO & PATTANAYAK, 2006; WIMBERGER *et al.*, 2010; FECK & HAMANN, 2013). In some cases, however, the high costs of rehabilitation or low success rates may prohibit widespread implementation (BAKER *et al.*, 2010). Thus, understanding the success rates of rehabilitation and how they vary with species biology (e.g. body size, sex) will enhance our ability to gauge how these activities can contribute to conservation (LAZAR *et al.*, 2004; BEKER *et al.*, 2015; CASALE *et al.*, 2016).

Sea turtle rehabilitation is usually achieved through medical management of sick or injured animals by veterinary surgeons in wildlife hospitals (CASAL & ORÓS, 2009; FECK & HAMANN, 2013). The majority of animals in rehabilitation are taken there because of some previous negative interaction with humans, including entanglement in fishing gear (BENTIVEGNA, 1993; ALLEN, 2000; RAC/SPA, 2004; BAGARINAO, 2011), being hit by a boat or propeller and a wide range of other causes (BAGARINO, 2011). Wide spread rehabilitation of sick or injured turtles currently supplements other conservation efforts (CASAL & ORÓS, 2009; MESTRE *et al.*, 2014), but we still have a poor understanding of the conservation potential of rehabilitation in terms of numbers of healthy animals released (even without any knowledge of their longer-term individual survival (MESTRE *et al.*, 2014). To

fill this knowledge gap, we analysed data on injured sea turtles admitted into rehabilitation facilities from 2001 to 2016 in Lampedusa Sea Turtle Rescue Center (Italy) (Fig. 1) for the rehabilitation success, using different factors like type of clinical case, health condition of the animal, and the presence of an experienced surgeon.

## MATERIALS AND METHODS

The overall rehabilitation success for all cases presented at the rescue centre was assessed quantitatively by the following formula:

$$\text{Overall rehabilitation success (\%)} = \left( \frac{\text{NrecoveredYr}}{\text{NtreatedYr}} \right) \times 100$$

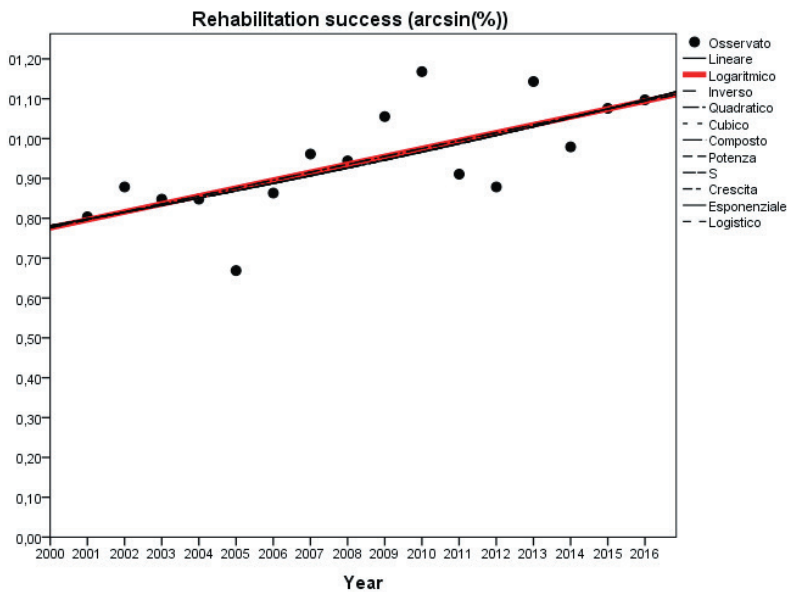
Where NrecoveredYr = number of recovered turtles after medical



Fig. 1. Study area.



treatment per year;  $N_{treatedYr}$  = number of turtles with medical treatment per year. The percentage of rehabilitation has been turned into arcsine (%). Arcsine transformation of data is appropriate for the data on proportions, i.e., data obtained from a count and the data expressed as decimal fractions and percentages; Fig. 2 shows the arcsine percentage of rehabilitation success during the years and the red line represents the best fit obtained with the interpolation line of the curve. The red line was made with a regression line.

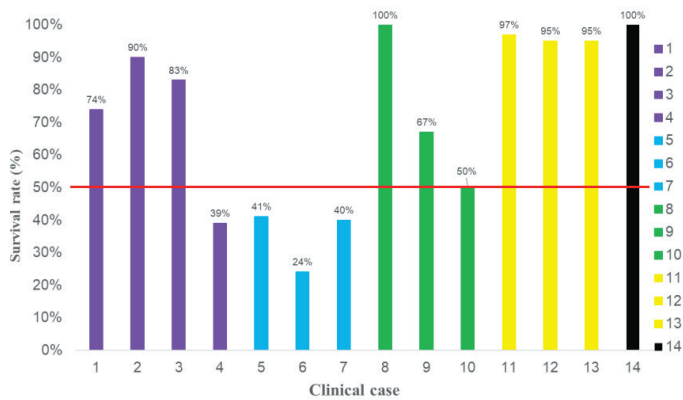


**Fig. 2.** Arcsine (%) of the rehabilitation success during the years between 2001 and 2016 ( $n=795$ ). The red line represents the regression line made by interpolation of data.

Each clinical case was first assigned a category, based on its primary manifestation, the criteria for which are represented in Tab. 1. We assigned different colors to the categories to make Fig. 3 more understandable and each clinical case was then identified with a number based on the position of the category in the body. The rehabilitation success for each of these was then calculated to determine which injuries are the most dangerous for sea turtles, using the following formula:

**Tab. 1.** Assessment criteria for categorizing each clinical case. Colours indicate the category (purple: hook, blue: line, green: fracture, yellow: lesion, black: infection) and each clinical case was then identified with a number based on the position of the category in the body (1: hook in mouth, 2: hook in oesophagus, and so on).

Hook	Line	Fracture	Lesion	14. Infection
1. mouth	5. from mouth	8. flipper	11. flipper	
2. oesophagus	6. from cloaca	9. carapace	12. carapace	
3. stomach	7. from mouth to cloaca	10. head	13. head	
4. intestine				



**Fig. 3.** Percentage of loggerhead turtles treated with recovery achieved according to the type of clinical case (%). The red line marks 50% (n=928).

**Case-specific rehabilitation success (%) = Number of turtles that survived a particular case / Total number of turtles presenting that case**

Statistical analysis was performed to compare the result of the treatment (recovery or death), against the health condition of each animal (good, depressed or comatose) by means of Fisher’s exact test. The study period was also divided into five subperiods (2001-2003, 2004-2006, 2007-2009, 2010-2012 and 2013-2016) to account for the evolution of surgical techniques and the presence of an expert surgeon with specific experience in sea turtle surgery. A post hoc test of univariate ANOVA was then used to identify the periods with a significant increase in rehabilitation success.

RESULTS AND DISCUSSION

We divided the primary factors that can influence rehabilitation in three areas: clinical case, health condition and presence of an expert surgeon.



**Clinical case:** Through analysis of the rehabilitation success for each case, it is evident that the presence of a fishing line (in blue) from the mouth (5), from the cloaca (6, Fig. 4), and from the mouth to cloaca (7) were the most dangerous injuries, with a lower than 50% survival rate. Hook in intestine (4) shows a survival rate of only 39%. This is shown above in Fig.3.

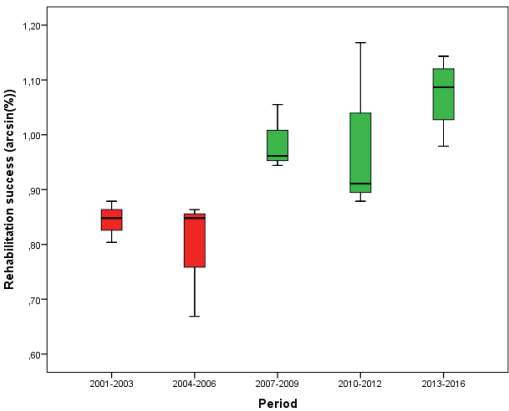
**Health condition:** Health condition was also shown to strongly influence the success of treatments (Tab. 2). The Fisher exact test proves that loggerhead turtles in bad health conditions have a lower probability of survival ( $P<0,001$ ;  $n=967$ ), as expected.

**Fig. 4.** Fishing line hanging from the turtle’s cloaca.

**Tab. 2.** Contingency table: Results of treatment and health condition. Fisher’s exact test between the outcome of the treatment and the health conditions upon arrival excluding the “unsolved cases” and the loggerhead turtles with unknown health conditions ( $P<0,001$ ;  $n=967$ ).

Contingency table: Results of treatment * health condition					
		Health condition			Total
		good	depressed	comatose	
Results of treatment	recovery	776	44	2	822
	death	36	53	56	145
Total		812	97	58	967

**Presence of an expert surgeon:** The success of rehabilitation (based on the arcsine%) between the 5 periods identified was evaluated, and revealed a significant increase in success after 2006. We investigate these factors with the post hoc test of univariate ANOVA ( $P = 0,016$ ;  $n = 1011$ ) (Fig.5; Tab. 3). The success rate continued to rise over the last 3 periods, when there was the presence of an experienced surgeon, thus suggesting that this factor is of great importance to rescued sea turtle survival.



**Fig. 5.** Arcsine (%) of therehabilitation success during 5 subperiods ( $n=1011$ ). The division into periods is given by the evolution of surgical techniques.

Post hoc Test of univariate ANOVA						
Dependent variable: Rehabilitation success (Arcsin(%))						
LSD						
(i) Period	(j) Period	difference between means (I-J)	Standard error	Sig.	Confidence range 95%	
					Lower limit	Higher limit
2001-2003	2004-2006	,05020	,07709	,528	-,1195	,2199
	2007-2009	-,14335	,07709	,090	-,3130	,0263
	2010-2012	-,14234	,07709	,092	-,3120	,0273
	2013-2016	-,23033 <sup>*</sup>	,07211	<b>,009</b>	-,3890	-,0716
2004-2006	2001-2003	-,05020	,07709	,528	-,2199	,1195
	2007-2009	-,19355 <sup>*</sup>	,07709	,029	-,3632	-,0239
	2010-2012	-,19254 <sup>*</sup>	,07709	,030	-,3622	-,0229
	2013-2016	-,28053 <sup>*</sup>	,07211	<b>,003</b>	-,4392	-,1218
2007-2009	2001-2003	,14335	,07709	,090	-,0263	,3130
	2004-2006	,19355 <sup>*</sup>	,07709	<b>,029</b>	,0239	,3632
	2010-2012	,00101	,07709	,990	-,1687	,1707
	2013-2016	-,08698	,07211	,253	-,2457	,0717
2010-2012	2001-2003	,14234	,07709	,092	-,0273	,3120
	2004-2006	,19254 <sup>*</sup>	,07709	<b>,030</b>	,0229	,3622
	2007-2009	-,00101	,07709	,990	-,1707	,1687
	2013-2016	-,08799	,07211	,248	-,2467	,0707
2013-2016	2001-2003	,23033 <sup>*</sup>	,07211	<b>,009</b>	,0716	,3890
	2004-2006	,28053 <sup>*</sup>	,07211	<b>,003</b>	,1218	,4392
	2007-2009	,08698	,07211	,253	-,0717	,2457
	2010-2012	,08799	,07211	,248	-,0707	,2467

\*. The average difference is significant at the 0.05 level

**Tab. 3.** Univariate ANOVA post hoc test on the success of rehabilitation in the five periods. Significant outcome are in bold.

## CONCLUSION

This study confirm that lines inside a loggerhead turtle's body result in high mortality, higher than the presence of hooks and this is evident from Fig. 3. The only exception is of hook in intestine, because the presence of the hook in this position must involve the presence of the line in all cases. In fact Professor Di Bello A. has shown in his studies how the presence of a line determines a higher mortality than the presence of only a hook. In fact, surgery of the lower digestive tract is certainly more challenging and further complicated by the serious injuries that hooks and lines often cause in the stomach and intestine, while the surgical approach for the extraction of hook from the oesophagus is relatively easy (DI BELLO *et al.*, 2010). In several cases single or multiple intestinal lacerations were detected, caused by the pull of the lines, in other cases the surgeon performed enterectomy of a long section of intestine because of lines injuries (DI BELLO *et al.*, 2010). Moreover health conditions can have a great influence on rehabilitation success, as we expected. We have seen that loggerhead turtles that show a higher mortality are those that present bad health conditions on arrival (depressed and comatose). Finally we noticed that, especially, the increase in the knowledge of surgical techniques over the years and therefore also the support of an expert surgeon have certainly determined an increasing success in turtle rehabilitation, for turtles have a complex anatomy and all surgical operations require a lot of experience on the part of the operator.

**TAKE-HOME MESSAGE:** The conservation status of sea turtles has been steadily improving during the last ten years; as a reason for this, we have witnessed an increase in activities aimed at the safeguarding and protection of these species. The role of rescue centers for sea turtles should be to raise public awareness, and therefore of actors in the tourism industry, of the issues concerning the possible extinction of sea turtles.

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## SUMMARY

### **Determination of the factors that might have influenced the rehabilitation of *Caretta caretta* in the Lampedusa Sea Turtle Rescue Center between 2001-2016**

C. Roldi & D. Freggi

Over the last few decades, the status of sea turtles and the need for their protection have increasingly captured the interest of citizens, and the number of sea turtle rescue centers is increasing worldwide, but especially in Italy, where we count more than 25 first aid stations.

The Lampedusa Sea Turtle Rescue Center started its activity in 1990, and in this paper we analyze data from 2812 loggerhead turtles rescued by 2016. Here we analyse which factors might have affected the rehabilitation success of the injured sea turtles hosted in our Center. We underline the following factors: type of clinical case, animal's health condition and the presence of a qualified surgeon.

For the first factor we estimated the percentage of survival animals with the following clinical cases (n=928): infections, fin/carapace/head fractures, fin/carapace/head wounds, hook in oesophagus/intestine/stomach/mouth, fishing line from mouth/cloaca, fishing line from mouth and cloaca; five of the analysed clinical cases show rehab success below 50% (head fracture, fishing line from cloaca/mouth/mouth and cloaca, hook in intestine).

For the second factor we estimated the outcome of therapies (recovery achieved/death) and health condition (good health condition/depressed/comatose) using the Fisher exact test. The Fisher test confirmed how health condition can significantly affect sea turtle rehabilitation success (Fisher test=369,894; d.f.=2;  $P < 0,001$ ;  $n=967$ ).

For the last factor, we divided the study period into five subperiods (2001-2003, 2004-2006, 2007-2009, 2010-2012, 2013-2016) based on the evolution of surgical techniques and the presence of an expert surgeon with direct experience of sea turtle surgery; with post hoc test of univariate ANOVA investigation, we confirm significantly the value of the experience of professionals involved ( $P = 0,016$ ;  $n = 1011$ ). Bycatch and health condition appear to significantly influence rehab success and the presence of a competent surgeon is radically responsible for an increase in the survival of loggerhead turtles, as expected.



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In the title of our journal the initial letter **N** in the word natura is unfinished. Symbolically that means that natural processes as well as the research of them are "in flux". All is in flux – Πάντα ῥεῖ.