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GENETIC PATTERNS IN RECOVERED SPECIMENS OF *CARETTA CARETTA* FROM THE ADRIATIC SEA

Tesi di laurea in Habitat marini: rischi e tutela

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ABSTRACT

The Adriatic Sea is considered a feeding and developmental area for Mediterranean loggerhead turtles, but this area is severely threatened by human impacts. In the Adriatic Sea loggerhead turtles are often found stranded or floating, but they are also recovered as by-catch from fishing activities. Nevertheless, information about population structuring and origin of individuals found in the Adriatic Sea are still limited. Cooperation with fishermen and a good network of voluntary collaborators are essential for understanding their distribution, ecology and for developing conservation strategies in the Adriatic Sea. In this study, a comparative analysis of biometric data and DNA sequence polymorphism of the long fragment of the mitochondrial control region was carried out on ninety-three loggerheads recovered from three feeding areas in the Adriatic Sea: North-western, North-eastern and South Adriatic. Differences in turtles body sizes (e.g. Straight Carapace Length) among the three recovery areas and relationship between SCL and the type of recovery were investigated. The origin of turtles from Mediterranean rookeries and the use of the Adriatic feeding habitats by loggerheads in different life-stages were assessed to understand the migratory pathway of the species. The analysis of biometric data revealed a significant difference in turtle sizes between the Southern and the Northern Adriatic. Moreover, size of captured turtles resulted significantly different from the size of stranded and floating individuals. Actually, neritic sub-adults and adults are more affected by incidental captures than juveniles because of their feeding behavior. The Bayesian mixed-stock analysis showed a strong genetic relationship between the Adriatic aggregates and Mediterranean rookeries, while a low proportion of individuals of Atlantic origin were detected in the Adriatic feeding grounds. The presence of migratory pathways towards the Adriatic Sea due to the surface current system was reinforced by the finding of individuals bearing haplotypes endemic to the nesting populations of Libya, Greece and Israel. A relatively high contribution from Turkey and Cyprus to the Northwest and South Adriatic populations was identified when the three sampled areas were analyzed independently. These results have to be taken in account in a conservative perspective, since coastal hazards, affecting the population of turtles feeding in the Adriatic Sea may also affect the nesting populations of the Eastern Mediterranean with a unique genetic pattern.

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1. INTRODUCTION

Caretta caretta

Taxonomy

The genus *Caretta* (order Testudines, family Cheloniidae) is now considered to be monotypic, containing the single species *Caretta caretta*, but in the past this group has been more inclusive. The name *Caretta* is a latinized version of the French word "caret" meaning turtle, tortoise, or sea turtle (Smith and Smith 1980). The species *Caretta caretta* was first described by Linnaeus in 1758 and named *Testudo caretta* (Conant et al. 2009). Over the next two centuries more than 35 names were applied to the species (Dodd 1988), but there is now agreement on *Caretta caretta* as the valid name (Conant et al. 2009). The common name of *Caretta caretta* is loggerhead, which derived for its relatively large and triangular head, supporting powerful jaws that enable the species to feed on hard-shelled prey (Valente et al. 2008).

Morphological features of Loggerheads

Shape and color of turtle carapace represent a distinctive feature between different species. About Loggerheads, carapace and plastron undergo substantial changes after hatching (Dodd 1988). Hatchling colors vary from light to dark brown to dark gray dorsally (Fig. 1A); and lack the reddish-brown coloration of adults and juveniles. Flippers are dark gray to brown above with distinct white margins. The ventral coloration of the plastron and other areas of the integument are generally yellowish to tan (Conant et al. 2009). Hatchlings have three typical dorsal keels (Fig. 1B) on the roughly heart-shaped carapace and there are two longitudinal ridges on the plastron, which disappear with age (Dodd 1988).

The carapace of adult and juvenile loggerheads (Fig. 1C-D) is reddish-brown. The dorsal and lateral head scales and the dorsal scales of the flippers are also reddish-brown, but with light to medium yellow margins. The unscaled areas of the integument (neck, shoulders, limb bases, inguinal area) are dull brown dorsally and light to medium yellow laterally and ventrally. The plastron is medium to light yellow (Conant et al. 2009), with diffuse dark margins. There are different descriptions of loggerhead coloration coming from distinct areas, however geographic color variation is difficult to assess because no quantitative or qualitative standards have been established for comparison (Kamezaki 2003).

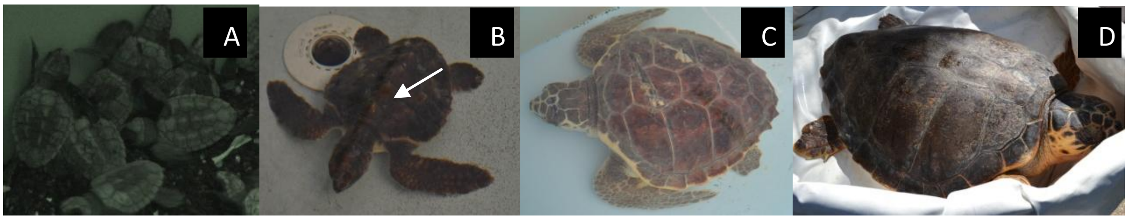


Fig. 1 Loggerhead sea turtle. A: Hatchlings; B: small juvenile with dorsal keels indicated by the arrow; C-D: juvenile and adult loggerheads, respectively.

In loggerheads growth is allometric. Both hatchlings and small juveniles have the vertebral scutes wider than long, but as the turtle grows, the length increases relatively greater than the width (Dodd 1988). There usually are 11 or 12 pairs of marginal scutes, five pairs of costals, five vertebrals, and a nuchal (precentral) scute that is in contact with the first pair of costal scutes (Fig. 2). However anomalous arrangements are occasionally observed (Kamezaki 2003). The plastron is composed of paired gular, humeral, pectoral, abdominal, femoral, and anal scutes and connected to the carapace by three pairs of poreless inframarginal scutes (Kamezaki 2003; Conant et al. 2009). Both the carapace and plastron of the loggerhead are heavily keratinized as a protective barrier against attacks and the environment (Dodd 1988).

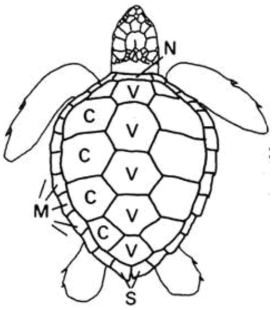


Fig. 2 External morphology of the loggerhead turtle. V: vertebral scute; C: costal scute; M: marginal scute; N: nuchal scute; S: supra-caudal scute (From Kamezaki 2003).

Since it is necessary to asses some biological parameters like age, growth rate and turtle size frequency in order to identify the demographic structure of a population, carapace length and width, turtle weight and other measures are taken using different appropriate methods and equipments. In particular, on nesting beaches adult body size is measured to be related to the reproductive output, to determine minimum size at sexual maturity and to monitor nesting female size for a particular rookery. Marine turtles are measured on foraging grounds to determine the frequency of size classes of turtles present as well as to monitor growth rates. Analyses of growth rates can indicate habitat quality and physiological status.

Dodd (1988) separated size classes of loggerhead sea turtles as follows:

Hatchling- averages 45 millimeters (mm) SCL (see Sample Collection for information related to the measures) at emergence and attains about 10 cm to the first few weeks of life. The weight is approximately 20 grams (g) soon after hatching.

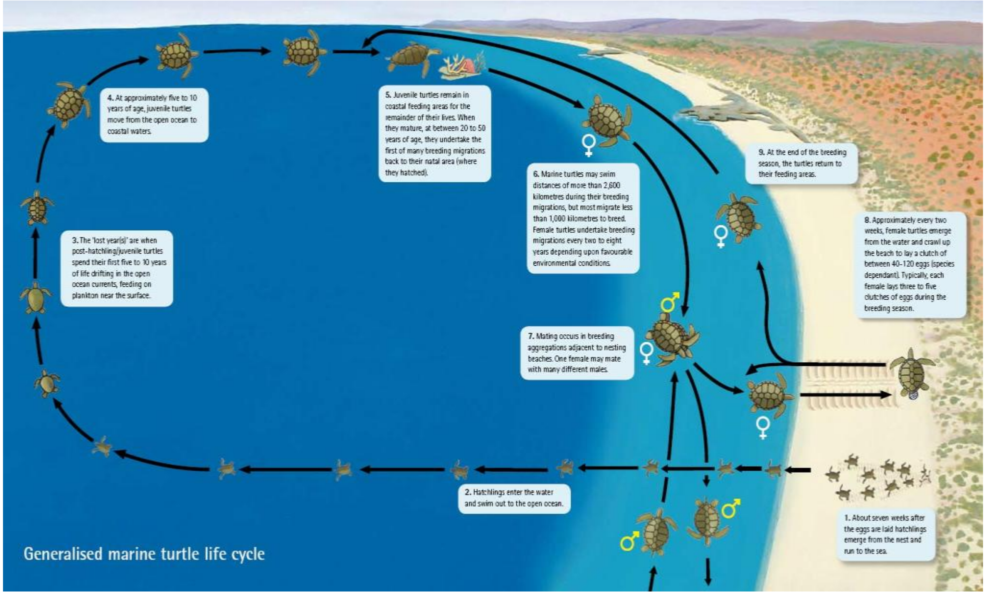
Juvenile- from 10 centimeters reaches approximately 40 cm SCL. In this stage the center of dorsal scutes is elevated forming the sharp keels.

Subadult- SCL is about 70-90 cm in females before arriving at the onset of sexual maturity, but it depends on the population.

Adult-attains reproductive maturity at >70-90 cm SCL; the size of nesting females depends on populations however, in the Atlantic Ocean adult SCL is the largest one, while it is smaller in some other areas, the smallest adult being in the Mediterranean (to about 90 cm; Margaritoulis et al. 2003). The size at sexual maturity for males is assumed to be similar to that of females (Dodd 1988).

Biology of the species

The generalized marine turtle life cycle is showed in the Figure 3.



1. About seven weeks after the eggs are laid hatchlings emerge from the nest and run to the sea.

2. Hatchlings enter the water and swim out to the open ocean.

3. The 'lost year(s)' are when post-hatchling/juvenile turtles spend their first five to 10 years of life drifting in the open ocean currents, feeding on plankton near the surface.

4. At approximately five to 10 years of age, juvenile turtles move from the open ocean to coastal waters.

5. Juvenile turtles remain in coastal feeding areas for the remainder of their lives. When they mature, at between 20 to 50 years of age, they undertake the first of many breeding migrations back to their natal area (where they hatched).

6. Marine turtles may swim distances of more than 2,600 kilometres during their breeding migrations, but most migrate less than 1,000 kilometres to breed. Female turtles undertake breeding migrations every two to eight years depending upon favourable environmental conditions.

7. Mating occurs in breeding aggregations adjacent to nesting beaches. One female may mate with many different males.

8. Approximately every two weeks, female turtles emerge from the water and crawl up the beach to lay a clutch of between 40-120 eggs (species dependant). Typically, each female lays three to five clutches of eggs during the breeding season.

9. At the end of the breeding season, the turtles return to their feeding areas.

Fig. 3 Generalized marine turtle life cycle (From http://www.sharkbay.org/Loggerheadturtlefactsheet.aspx).

Caretta caretta is a widespread sea turtle living in the temperate, subtropical and tropical waters of the Atlantic, Pacific and Indian Oceans. It is a generalist species, probably the most ecologically generalized compared to the other sea turtle species. As a matter of fact, loggerheads have the greatest geographic range in their nesting beaches, which are found in both tropical and temperate latitudes; their foraging range (Fig. 4) also extends seasonally from temperate to tropical waters (Bolten 2003a). They inhabit open oceanic waters as well as shallow near-shore waters and live in a variety of environments for a relative long time. Principal habitats are open deep waters, continental shores of warm seas or waters around islands, as Masirah in Oman and Zakinthos in Greece. Moreover, turtles are frequently associated with brackish waters of costal lagoon or river mouths. During the winter, loggerheads may remain dormant and buried in muddy bottom of sounds, bays and estuaries characterized by moderate deep waters. Apparently the limit of distribution is water of about 10°C, although they could be encountered in colder waters of high latitudes during the summer season of warm years (Márquez 1990).

Loggerheads migrate intermittently throughout their life. As hatchlings, turtles swim frenzy from their natal beaches into the open ocean, often taking refuge in circular current systems, that represent moving open-sea nursery grounds (Lohmann and Lohmann 2003).

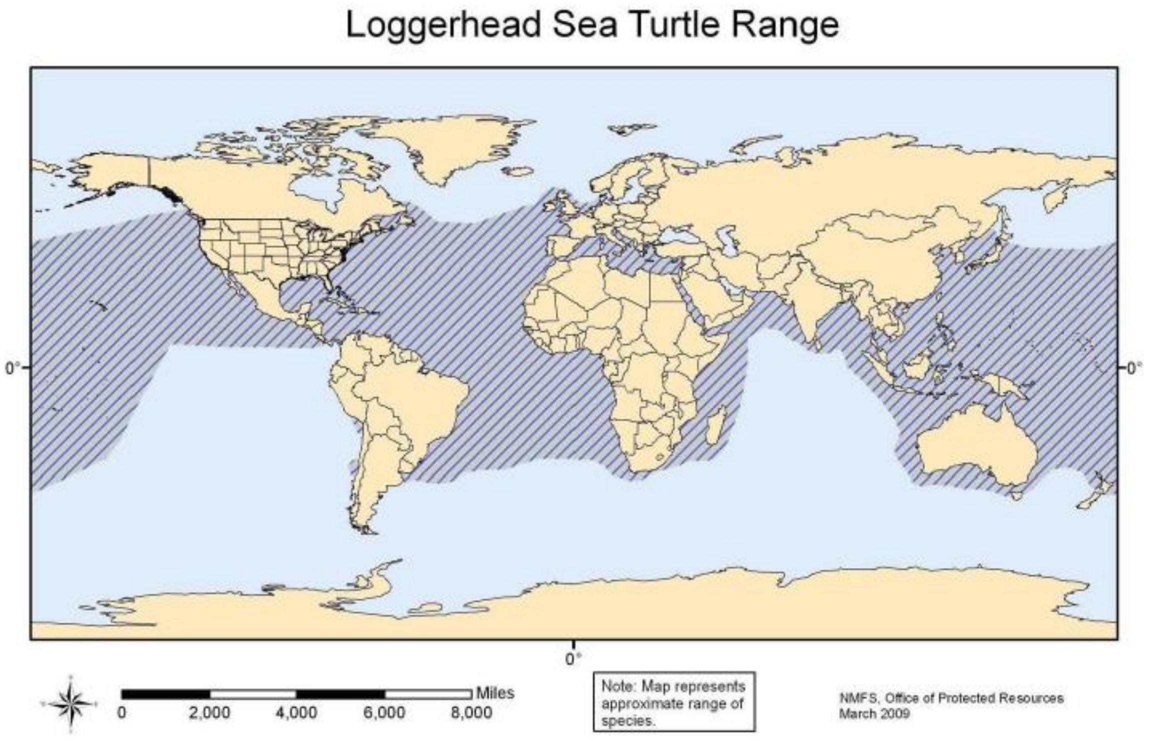


Fig. 4 *Caretta caretta* distribution maps. The grey lines represent the geographical distribution of the species (From http://www.nmfs.noaa.gov/pr/images/rangemaps).

The post-hatchling transitional stage consists in turtles that are still in the neritic zone and begin to feed before entering the open waters. After this transition, hatchlings theoretically tend to follow warm currents such as the Gulf Stream, or may enter big gyre of currents as the North Equatorial current system, consequently of their limited swimming capacity that let them often to be considered passive drifters (Casale and Mariani 2014). They possibly aggregate in sea fronts (ocean current convergences), down-welling zones and eddies, where remain feeding and growing because of the presence of floating resources (Márquez 1990). This phase is the oceanic juvenile stage in which turtles are epipelagic for 75% of the time and occasionally dive to grater depths than 5 meters (Bolten 2003b) cause of the presence of floating resources under the surface. In this life stage individuals are difficult to observe (Márquez 1990) and for this reason researchers have named "*lost years*" the first turtle life period (Casale and Mariani 2014). A previous research (Dodd 1988) suggested that the 'lost years' may cover a range of about 3/5 years but other authors (Musick et al. 1997) have extended this range to ten years or longer. Bjorndal et al. (2000) estimated that the time elapsed as juveniles in feeding and growing pelagic nurseries ranges between 6.5 and 11.5 years for loggerheads of Atlantic rookeries. However, the duration of this transitional stage before entering the neritic zones is extremely variable (Musick et al. 1997) and the cause of this variation is not really known. It may depend on different geographic regions in which this phase is spent, on current regimes and probably on the availability of food resources (Bolten 2003b). Furthermore, even the mean size of turtles leaving the pelagic zone for benthic habitats may vary among populations; in the western North Atlantic, individuals of *Caretta caretta* make this habitat shift at a smaller size (and so younger age) than do populations in the western South Pacific (Musick et al. 1997). Whilst loggerheads of North Atlantic origin leave the pelagic habitat to recruit in the neritic zone over a range of sizes from 46 to 64 cm curved carapace length (CCL) (Bjorndal et al. 2000), juveniles in the Western South Pacific recruit to coastal foraging habitats across a wide range of sizes from 66.7 to 93.9 cm (Limpus and Limpus 2003). Hence, age and size at which loggerheads make this habitat shift differs between and within populations. The reason is that individuals are characterized by different growth rates. These differences are due to several factors, for example environmental or genetic matters (Casale et al. 2009), and variations of growth rates may be relevant in determining the occurrence of the transition from a stage to another. Thermal and trophic characteristics of different oceanic areas may influence the growth of an individual or a group of individuals. Juveniles that recruit the same nursery ground have probably a similar development, using the same resources, and so a similar size at which they make this ontogenetic shift. However, also individual genetic patterns have a function in establishing the variability of turtle growth rates.

Approaching the demersal areas, juveniles enter neritic habitats and recruit actively continuing to develop till maturity. In temperate areas juvenile loggerheads move from higher latitude foraging grounds in summer to lower latitudes in winter, while in tropical areas migrations are more localized (Musick et al. 1997; Bolten 2003b). Oceanic juveniles, migrated to near shore coastal grounds (neritic zone), forage and continue maturing until adulthood. In some cases neritic juvenile habitats may be geographically distinct from those of adults, but in others they may overlap or coincide (Musick et al. 1997; Bolten 2003b). Sub-adults may stay long time, up to 10 years, in foraging habitats before reaching sexual maturity. Age and size of loggerheads at maturity, as at the previous stage, differs between and within populations (Dodd 1988). This fact may depend on geographic location of rookeries or origin of individuals, but essentially on turtle growth rates that show considerable variation during their life. Overall, the SCL of nesting females ranges from approximately 70 to 109 cm (Dodd 1988). Studies made on specimens in captivity showed that ages at maturity might range from 6 to 20 years, with a carapace length (SCL) always over about 70 cm within a size range. Instead, capture-recapture data of tagged nesting females in the southeastern coasts of the United States have produced age ranges from 12 to 30 and more years through the back calculation technique (Márquez 1990).

Nesting of *Caretta caretta* usually occurs in spring and summer, with variations according to the latitude and geographical characteristics of the coast (Márquez 1990). Approaching the tropics, the nesting season becomes more extended. Loggerheads are known to nest anywhere from one to six times in a nesting season. The inter-nesting interval of loggerheads varies, but it is generally about 14 days depending on locations (Dodd 1988). Females usually deposit on each occasion from 40 to 190 eggs. A single female could lay a maximum of 560 eggs per season. In general, there is much confusion about nesting cycles in sea turtles. The major pattern of the reproductive cycle is two or three years, but some individuals of the population may shift from one cycle to another, including to a yearly cycle (Márquez 1990). Nesting is found mainly on open beaches or along narrow bays having suitable sand, and it may be in association with other species of sea turtles. They show strong fidelity to their nesting areas, which are usually the same where they were born (natal homing) (Bowen et al. 1993; Casale et al. 2007a).

Regarding feeding areas, coral reefs, rocky places, and shipwrecks are often used as foraging grounds. During the foraging stage, adult turtles are active and feed on the bottom (epibenthic/demersal) or sometimes throughout the water column (Bolten 2003b). They exhibit natal philopatry to colonial nesting beaches as well as high levels of fidelity to migratory routes towards foraging areas and wintering sites both between and within years and after successive breeding migrations. Loggerhead migrations between foraging areas and mating or nesting sites are considerable, typically hundreds or thousands of kilometers. In a study by Broderick et al. (2007), adult females tracked for two consecutive migrations after lying have shown to use highly similar routes to return to the same foraging locations. In another work made by Lazar et al. (2000), the longest migration recorded from eleven tagged loggerheads nesting in Greece and recovered in the Northern Adriatic was of about 1,200 km. It is likely that foraging habitats are the same ones where turtles recruited as juveniles (Casale et al. 2007a).

As the nesting season approaches, adults upon maturity migrate towards the nesting beaches from mating areas that may occur either relatively close compared to feeding grounds or lightly remote from nesting beaches (Musick et al. 1997).

However, the current ontogenetic model of loggerhead life history does not reflect the tendency of this species to create temporary association to specific oceanic or neritic zones according to food availability and oceanographic features in the foraging or migratory areas, independently of the stage performed. In this matter, a relaxed model has been proposed by Casale et al. (2008a) suggesting that the life history model for *Caretta caretta* would be characterized by an early short obligate epipelagic stage due to limited diving capacity, followed by the main opportunistic amphi-habitat stage.

Distribution of the nesting areas

The major nesting grounds are generally located in warm temperate and subtropical regions, with the exception of Masirah Island, Oman (Fig. 5).

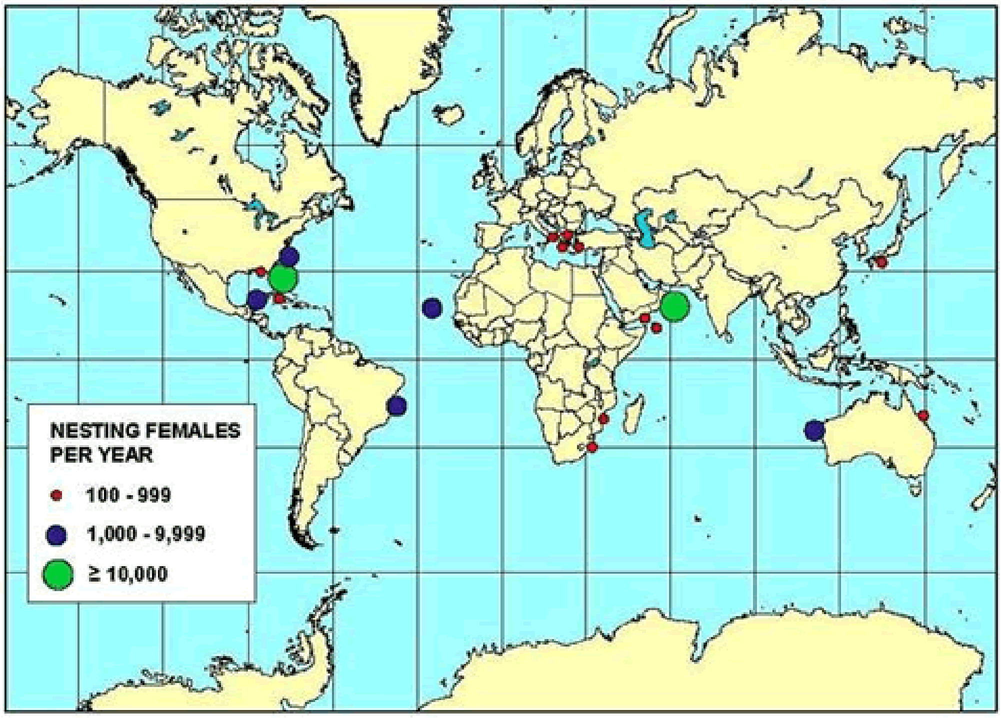


Fig. 5 Global distribution of Loggerhead Sea Turtle nesting assemblages. From NMFS and USFWS 2008.

The most recent reviews for the status of loggerhead nesting distribution show that only two nesting beaches have greater than 10,000 females nesting per year: South Florida (U.S.) and Masirah Island (Oman) (Ehrhart et al. 2003; Conant et al. 2009). In the Atlantic the majority of loggerhead nesting is at the western rims of the Ocean (Conant et al. 2009). Total estimated nesting in the U.S. Atlantic and Gulf coasts of Mexico is approximately 68,000 to 90,000 nests per year. Brazil supports an intermediately-sized loggerhead nesting assemblage, estimated by publised and unpublished accounts. There are about 4,000 nests per year contrywide. Throughout the Caribbean loggerhead nesting is sparse and rare compared with the green turtle and the hawksbill. The Cape Verde Islands support a loggerhead nesting assemblage of intermediate size. In 2000, researchers tagged over 1,000 nesting females on just 5 km of beach on Boavista Island. There may be as many as 5,000 nesting females per year in the entire archipelago, but this needs to be confirmed. The Atlantic coast of the african mainland represents a minor nesting area, with infrequent loggerhead nests and where informations are scant (Ehrhart et al. 2003).

In the Mediterranean (Fig. 6), loggerhead nesting is confined almost exclusively to the eastern portion of the basin. The main nesting assemblages occur in Cyprus, Greece, and Turkey, but small numbers of loggerhead nests have been recorded in Egypt, Israel, Italy, Lebanon, Syria, Tunisia and occasionally in Spain (Margaritoulis et al. 2003). Substantial nesting was also discovered in Libya (Laurent et al.1995) but the nesting effort there needs to be quantified (Margaritoulis 2003). Based on the recorded number of nests per year in Cyprus, Greece, Israel, Tunisia, and Turkey, loggerhead nesting in the Mediterranean ranges from about 3,375 to 7,085 nests per season (Margaritoulis et al. 2003). However, these numbers are considered minimum figures as they do not include nests outside the monitored areas or in countries where regular monitoring has not yet been initiated (e.g. Libya) (Margaritoulis 2003).

Fig. 6 Annual nesting effort per country in the Mediterranean Sea. Numbers represent average value of nests per season. Solid triangles values derived from monitoring project, while open triangles are estimates. Quadrates are exceptional nests. (From Margaritoulis et al. 2003).

Within the North Pacific, loggerhead nesting has been documented only in Japan. In the South Pacific, nesting beaches are restricted to eastern Australia and New Caledonia (Limpus and Limpus 2003).

Most trends in loggerhead nesting populations in the Indian Ocean are unknown (Conant et al. 2009).

Endangered species

Loggerheads are listed as endangered in the IUCN Red List of Threatened Species and their populations are globally decreasing in a drastic way especially cause of human impact (IUNC, 2011; Conant et al. 2009). Loggerhead turtles face threats on both nesting beaches and in the marine environment. The greatest cause of decline and the primary threat to their populations worldwide are incidental captures in fishing gear, primarily in long lines and gillnets, but also in trawls, traps, pots and dredges because of their feeding behavior and their habit of overwintering in shallow waters. Populations in Honduras, Mexico, Colombia, Israel, Turkey, Bahamas, Cuba, Greece, Japan, and Panama have been declining. This decline continues and it is attributed beyond to by-catch also to directed harvest, coastal development, increased human use of nesting beaches and pollution. Furthermore, directed harvest of loggerheads still occurs in many places as the Bahamas, Cuba, and Mexico (NMFS and FWS 2008).

Caretta caretta in the Mediterranean and Adriatic Sea

Caretta caretta represents the most abundant sea turtle species in the Mediterranean Sea, having evolved local populations (Margaritoulis et al., 2003b). It seems that the latest colonization occurred about 12,000 years ago from stocks of the western Atlantic (Bowen et al. 1993). One of the most distinctive characteristics of Mediterranean loggerheads is the significantly smaller adult size in comparison with other populations, primarily the Atlantic ones (Margaritoulis 2003). This may be an adaptation to particular conditions and it could be due to earlier sexual maturation and/or slower growth. Differences in female sizes and clutch sizes are considerable even among the Mediterranean colonies. Individual nesting females range between 60 and 99 cm CCL, while average sizes in different nesting sites range from 66.5 to 84.7 cm CCL (Margaritoulis et al. 2003). Although individuals of Atlantic populations enter the Mediterranean and share foraging habitats with local population, there is a limited gene flow between turtles of different origins (Encalada et al. 1998; Laurent et al. 1998).

Reproductive habitats concentrate in the eastern basin but turtles may disperse widely in the entire Mediterranean. The average number of documented reported in literature is over 7200/y (Casale and Margaritoulis 2010). The majority of loggerheads nesting in the Mediterranean occur between the beginning of June and the early August with sparse nest from mid-May and early September (Margaritoulis et al. 2003). This basin hosts the northernmost record for a loggerhead turtle nest worldwide.

The oceanic and neritic features of the Mediterranean make these waters very advantageous both for juvenile and adult loggerheads. The analyses of tag recoveries provide evidence of active passage of turtles in both eastern and western directions of the basin. Turtles captured in pelagic fisheries (Casale et al. 2007b), as well as recovery of tagged juveniles of *C. caretta* (Casale et al. 2007a), suggest that the Mediterranean habitats are widely exploited by pelagic juvenile loggerheads (Laurent et al. 1998). They frequently occur in the oceanic zones from the Alboran Sea to the Balearic Islands, where there are also many individuals of Atlantic origin (Carreras et al. 2006). The Strait of Sicily represents the connection between the two sides of the basin, featuring either a key route or a trophic area for loggerheads (Casale et al. 2007a). Recently, genetic markers demonstrated a demographic link between the nesting colonies in the eastern basin and the oceanic feeding juvenile aggregates of the western waters (Margaritoulis et al. 2003). The turtle distribution in the open basin is merely driven by the large current system (Bentivegna et al. 2007).

The occurrence of incidental captures in bottom trawl fisheries demonstrates that loggerheads are very abundant in the Mediterranean neritic zones as well. These feeding habitats are in the north Adriatic, off Tunisia-Libya, off Egypt and off the southeast coast of Turkey (Casale and Mariani 2014). The two major areas suggested as the main benthic habitats for adult and juvenile loggerheads are Gulf of Gabès and northern Adriatic Sea (Fig. 7). These very extensive shallow waters host important developmental grounds for juveniles in the benthic phase and a feeding area for adult females, a part of which comes from the Greek rookeries (Casale and Margaritoulis 2010; Lazar et al. 2000). Genetic tags have revealed that these areas are also frequented by individuals from other Mediterranean nesting colonies such as those of Cyprus and Turkey (Casale et al. 2008b).

In conclusion, the Mediterranean Sea hosts both oceanic and neritic habitats for loggerhead populations and for juveniles that migrate from Atlantic and return to their nesting sites to breed (Laurent et al. 1998; Carreras et al. 2006).

Fig. 7 Map of the Mediterranean region showing approximate locations of nesting areas and of known oceanic and neritic habitats. The 200 isobath delimits the continental shelf. Oblique lines indicate pelagic and demersal habitats. Triangles represent nesting areas. (From Margaritoulis et al. 2003).

Threats on Mediterranean individuals

The Mediterranean is a major destination of millions of tourists during the summer, in concurrence with the loggerhead-nesting season. As a consequence, several nesting areas are very threatened (Casale and Margaritoulis 2010). However, the major cause of turtle mortality in the Mediterranean is represented by incidental catches in fisheries. The capture of turtles in various fishing gears occurs practically in all Mediterranean countries and it seems to be also high in “less industrialized” fisheries, as set gill nets (Margaritoulis 2003). A recent review of sea turtle by-catch in the Mediterranean Sea estimated over 150,000 captures per years within the basin (over 50,000 by pelagic longlines, 40,000 by trawls, 35,000 by demersal longlines and 30,000 by set nets), and in excess by 50,000 deaths per year (Casale 2008; Casale and Margaritoulis 2010). Although these numbers seem exhaustive, quantification of turtle captures in the widely spread fisheries is very difficult to assess. Moreover, mortality following incidental capture has not been fully documented. Usually, turtles hooked in drifting long-lines are released by cutting the branch line, but the effect of hooks left on turtles are not actually known. Regarding turtles caught in trawl nets, they may suffocate and die, but if released in a comatose state they surely drawn later cause of the inability of swim in that condition (Margaritoulis 2003). Therefore, it is necessary to take into account a high degree of uncertainty of available data relative to mortality rate of loggerheads in the Mediterranean.

Other important reasons of turtle mortality are boat strikes; oil pollution and ingestion of debris (Margaritoulis 2003), but no quantitative data are available.

Incidental captures and strandings: evidence of loggerhead migration to the Adriatic Sea

Incidental captures and strandings indicate that loggerhead turtles occur in high numbers in the Adriatic primarily during the warm seasons. A ten year study along the coasts of Croatia and Slovenia assessed that adult sea turtles (CCL>70cm) were caught at high frequency in trawls during the fishing season, while smaller individuals (from 30 to 50 cm CCL) were affected in higher proportion by gill nets (Lazar et al. 2003) cause of their pelagic behavior. These data indicate that even turtles smaller than 40 cm inhabit the Adriatic waters. A study about the feeding behavior of loggerheads in the Nord Adriatic confirmed that loggerheads with CCL n-t as small as about 30 cm recruit the neritic zones of the basin (Lazar et al. 2005). Probably, these individuals have already shifted from pelagic nursery ground to benthic habitat and this fact might be ascribed to the presence of transitional habitats, favorable sea temperatures and rich benthic communities in the basin. Juveniles shift to the following ontogenetic stage in a smaller size than in most of other populations worldwide (Lazar et al. 2005). The presence of these small individuals has been widely demonstrated (Affronte and Scaravelli 2001; Vallini et al. 2011), however the occurrence of small turtles (<40 and even <20) in shallow waters is not really expected (Casale and Margaritoulis 2010).

According to a research based on a wide dataset of records in the Italian waters (Casale et al. 2010), the highest number of stranded loggerheads was recorded in the Northwestern Adriatic in spring, summer and autumn, with a density of an order of magnitude higher than in the other areas investigated (Tyrrhenian, Ionian, Sicily Channel and South Adriatic). With regard to the South Adriatic, strandings were not so numerous as in the North, and the major proportion of records concerned turtles smaller than 30 cm

CCL, and/or in the first four years of life. Conversely, the monitoring of bottom trawler activity in the Gulf of Manfredonia showed that a high density of sub-adults is caught in the neritic zone. The average size of sub-adults turtles captured in this area (mean CCL: 56.1 cm, Casale et al. 2012b) was similar to specimens caught (mean size: 55.4 cm, Casale et al. 2014) or stranded dead (51.5 cm CCL; Casale et al. 2010) in the North Adriatic basin. These results suggest that juvenile sea turtles select both the North and the South Adriatic demersal habitats with a wide size range. Data collected along the west coast of the Adriatic Sea confirmed that the main represented classes, considering CCL classes of 10 cm, are that of 40-49 and 50-59 cm CCL (Scaravelli and Affronte 2003).

Incidental catches (Casale et al. 2004; Lazar et al. 2003) occurring in bottom trawls during the winter months suggest that part of loggerheads overwinters in the Adriatic as well. There is evidence of a seasonal migration of turtles from a small area in the northernmost part of the Adriatic where temperature in winter drops even to 8°C, which is within the cold-stunning temperatures for loggerheads. Hence, the overwintering occurs south of about 45° N and in the northeast part of the North basin where temperatures are higher (between 12.5 and 13.5°C) compared to the west. It is likely that loggerheads are lethargic in such low temperature regimes and this fact would explain the statistical association between by-catch in bottom trawls and the cold period (Lazar et al. 2003).

The occurrence of loggerheads in the Adriatic Sea is related to immigrations from Mediterranean rookeries, mainly belonging to the Greek nesting population (Lazar et al. 2004; Lazar et al. 2000;). As showed in many investigations (Lazar et al. 2000; Lazar et al. 2003; Casale et al. 2004; Casale et al. 2012b; Affronte and Scaravelli 2001; Scaravelli and Affronte 2003), the Adriatic habitats are shared by the majority age classes of loggerheads and represent a main migratory corridor. Satellite telemetry has played an important role in determining migratory routes and assessing habitat utilization (Vallini et al. 2006; Zbinden et al. 2008; Casale et al. 2012a). Together with tag recoveries (Casale et al. 2007a; Lazar et al. 2000; Lazar et al. 2004), satellite tracks described the main migratory routes of *C. caretta* in the Adriatic that lead mainly along the eastern coast toward the north (Lazar et al. 2004). This pathway overlaps with the current that enters the Adriatic and surely has an influence on the direction of loggerhead migrations. However, migration against the prevailing currents cannot be excluded (Lazar et al. 2000; Vallini et al. 2006). At the light of 29 recoveries of adult females originally tagged in Greece, the eastern Adriatic Sea results the preferred migration corridor for part of this nesting population. However Zbinden et al. (2008) demonstrated that adult females nesting on Zakynthos might travel throughout the western Adriatic too. Juveniles and adults have either a small residential behavior remaining in the Adriatic throughout the year or perform seasonal migrations to other areas (Casale et al. 2012a). In other words, they are not just travelers through the basin but they may reside into it for a period of time. They do not exhibit site fidelity for specific foraging habitats, but essentially for a wider area such as the entire Adriatic Sea (Lazar et al. 2004).

Threats on Adriatic migrating specimens

The principal threat on loggerheads at sea remains fishing activities. In the eastern Adriatic, bottom trawl by-catch by Croatian fishery was conservatively estimated at a minimum of 2,500 captures per year, with a much higher by-catch rate in the northern sub-basin (Lazar and Tvrtković 1995). However, it is likely that the CPUE is higher than that originally reported by fishermen (Casale et al. 2004). The capture trend of turtles in northeastern waters has usually a peak in the cold season with a direct mortality (12.5%) higher than in the northwestern basin (9.4%; Casale et al. 2004). The cause might be associated to the longer haul duration of trawling in the Croatian waters (Lazar et al. 2003). A higher mortality percentage should be expected if delayed deaths would be included in the estimation. Another fishery with concerning impact on loggerheads in the eastern Adriatic is gill net fishery. Numbers of captures per year (658 with direct mortality of 74.4%) are low if multifunctional vessels are not considered. Otherwise, total gill net by-catch may potentially be as high as 4,038 turtles/year, suggesting a significant conservation concern for loggerheads in the northern Adriatic (Lazar 2010).

The Italian Peninsula has one of the largest fishing fleets of the Mediterranean, with several type of fishing gear used (Casale 2008). Its activity at sea represents a major threat to sea turtle loggerheads on a regional scale. The uncertainty in the total fishing effort, due to an important illegal fishing activity, make it difficult to estimate the total number of turtle captured (Casale and Margaritoulis 2010). However, the conservative estimation of turtle caught in the Northern Adriatic by Italian bottom trawls is about 4,300 per year, and 161 by mid-water trawls (Casale et al. 2004). In the south Adriatic, Gulf of Manfredonia, the catch rate per bottom trawler vessel was estimated 8.6 turtles per year (Casale et al. 2012b). Generally, mortality rate induced by pelagic long lines is unknown because of the many parameters involved and the difficulty of monitoring turtles after the captures (Casale and Margaritouli 2010). No estimates are available in the whole Adriatic about pelagic fishing activity, except in the northeastern waters (Lazar et al. 2003). Even regarding demersal longline and set net, the overall mortality induced by these fishing gears may be very high. However, trawling represents the principal fishing activity because of the shallow water features of the Adriatic at least in the northernmost part. The main cause of turtle death seems to be in general the prolonged forced apnea. Casale et al. (2014) suggested that the CCL range of turtles caught by trawlers is 23.5-85.0 cm in the North Adriatic and 21.3-92.0 cm in the South Adriatic. All age and size classes are affected by fishery-related mortality.

In the last year, the molecular analysis has supported tag return, satellite telemetry and mark-recapture techniques (Bowen et al. 2004) assaying which rookery undergo population decline due to the human impact. In fact, the genetic tag carried using a portion of the mitochondrial genome has been applied as innovative tools to assess the rookeries of origin of turtles found on feeding grounds, to establish migratory routes and to define population structure (Avise and Bowen 1994).

The importance of Mitochondrial DNA in sea turtles

Molecular techniques to define philopatry and matrilines

Genetic techniques have illuminated several aspects of marine turtle life history in order to manage and preserve marine turtle stocks. Genetic tools allow identifying discrete breeding populations on the nesting beaches and in the corresponding feeding habitats. Migrations in sea turtles may cause confusion when discriminating populations, due to the presence in the foraging aggregates of individuals from different rookeries. Then it is fundamental designate the geographic range of feeding habitats that supports a specific breeding colony and, conversely, assess proportions of different breeding populations in those areas (FitzSimmons et al. 1999).

Mitochondrial DNA (mtDNA) has proved to be a natural source of genetic markers to detect population structure in marine turtles and to infer patterns of dispersal. The highest degree of resolution for population discrimination can be obtained by examining sequence variation in the rapidly evolving portion of mtDNA such as the control region (Norman et al. 1994), a non-coding origin of replication (D-loop). D-loop polymorphisms and haplotype identification have been extensively applied in both older and more recent studies on population structure, ecology and phylogeography of *Caretta caretta* populations (Drosopoulou et al. 2012). The main purpose of genetic studies on *C. caretta* and other sea turtles is to establish independent units suitable for the conservation management of rookeries threatened and affected by demographic decline. The application of these genetic tags has substantially demonstrate the natal homing behavior of turtle females that return to nest on their natal beaches and the philopatry of juveniles as well (FitzSimmons et al. 1997; Bowen et al. 2004). In some cases these markers have been used to document long migrations in juvenile loggerheads throughout the Atlantic (Bolten et al.1998) and from Atlantic to Mediterranean Sea.

The mtDNA molecule is maternally transmitted, meaning that male offspring inherit their mother's mtDNA but do not pass it on to subsequent generations. Nonetheless, the finding of genetic differences between nesting populations in the female lineages makes it possible to determine which rookeries contribute to a particular feeding area, migratory pathway or harvest that are affected by mortality (FitzSimmons 1999). The reproducibility of mtDNA sequence data has been a boon to sea turtle genetic surveys, and registries of known haplotypes are maintained in the Archie Carr Center for Sea Turtle Research (http://accstr.ufl.edu/).

Previous genetic studies on *Caretta caretta* in the Mediterranean Sea

In the Mediterranean Sea loggerheads constitute a population genetically isolated from the Atlantic one (Carreras et al. 2007). The current genetic composition of *C. caretta* rookeries in the Mediterranean would be the result of different colonization events from the Atlantic during the Pleistocene (Clusa et al. 2013) and the Holocene (Bowen et al. 1993). During Pleistocenic glaciations local extinction and re-colonization from glacial refuge may be occurred. Although few haplotypes (i.e. CCA2 – CC-A3) are shared with the Western Atlantic nesting aggregates, the Mediterranean nesting populations are highly distinct in terms of haplotype frequencies (Carreras et al. 2011). The shared haplotypes may indicate recent contact, otherwise a Mediterranean colonization from Atlantic during the current interglacial interval (Bowen and Karl 2007).

In the Mediterranean, the distribution of mtDNA haplotypes on nesting beaches has been compared to the haplotype frequencies on the feeding grounds, and this comparison has been used to calculate the most likely contribution from each nesting beach (Bowen et al. 1993). According to several investigations (Laurent et al. 1998; Carreras et al. 2006; Maffucci et al. 2006; Carreras et al. 2011) the Mediterranean basin seems to be exploited by loggerheads from Atlantic and Mediterranean rookeries. MtDNA analysis has revealed that juveniles from Atlantic occur mainly off the North African coasts in the southwestern basin, while the western European shore seem to be inhabited by turtles from eastern Mediterranean cohorts in accordance with sea-surface current patterns. Endemic haplotypes from Atlantic rookeries (CC-A1, CC-A5, CC-A7) confirmed that individuals recruiting the southwestern Mediterranean basin have an Atlantic origin (Carreras et al. 2006). On the other hand, specimens with Mediterranean endemic haplotypes (i.e. CC-A6), detected from rookeries confined in the eastern side of the basin (Greece), are located mainly in Mediterranean foraging habitats distant from Atlantic water masses. Anyway, individuals from Atlantic that enter the Mediterranean may also share foraging habitats with local populations but nonetheless turtles of Mediterranean origin remain genetically isolated (Carreras et al. 2011).

Within the Mediterranean Sea populations are also genetically differentiated. The existence of genetic structure within the Mediterranean was previously detected with short sequences (~380bp) of the mtDNA control region (Laurent et al. 1998; Encalada et al. 1998). The first studies by Laurent et al. (1998) reported that Turkish colonies are genetically distinct from nesting areas of Cyprus, Greece and southeastern basin. This genetic tag has been also employed to identify the contribution of different nesting areas to foraging grounds shared by turtles of different origins, throughout the mixed stock analysis (MSA) technique (Carreras et al. 2006).

Further evidence of genetic diversity within the Mediterranean has been provided in a recent work conducted by Carreras et al. (2007), where haplotypes CC-A29 and CC-A32, not previously found in any nesting beach but only reported from Western Mediterranean feeding grounds, have been unveiled in Israel and Zakynthos respectively. Even concerning the haplotype CC-A31, it has been described once in a specimen sampled off the south-eastern Italian coast (Mafucci et al. 2006) and after recorded in a nesting site located among coasts of Calabria (Garofalo et al. 2009). Although exclusive haplotypes were considered rare in the Mediterranean compared to the Atlantic ocean (Encalada et al. 1998), the overall diversity in the Mediterranean may be significantly underestimated due to either the existence of unknown nesting population or the incomplete sampling of known colonies (Garofalo et al. 2009). Numerous published works (Mafucci et al. 2006; Carreras et al. 2007; Giovannotti et al. 2010; Chaieb et al. 2010) adopted the short mtDNA fragment as genetic marker for molecular differentiation between loggerhead populations (Fig. 8). However, the limited assignment power of this short sequence marker has yet precluded a fine-scale genetic assessment of Mediterranean rookeries and their contribution to foraging grounds (Clusa et al. 2014).

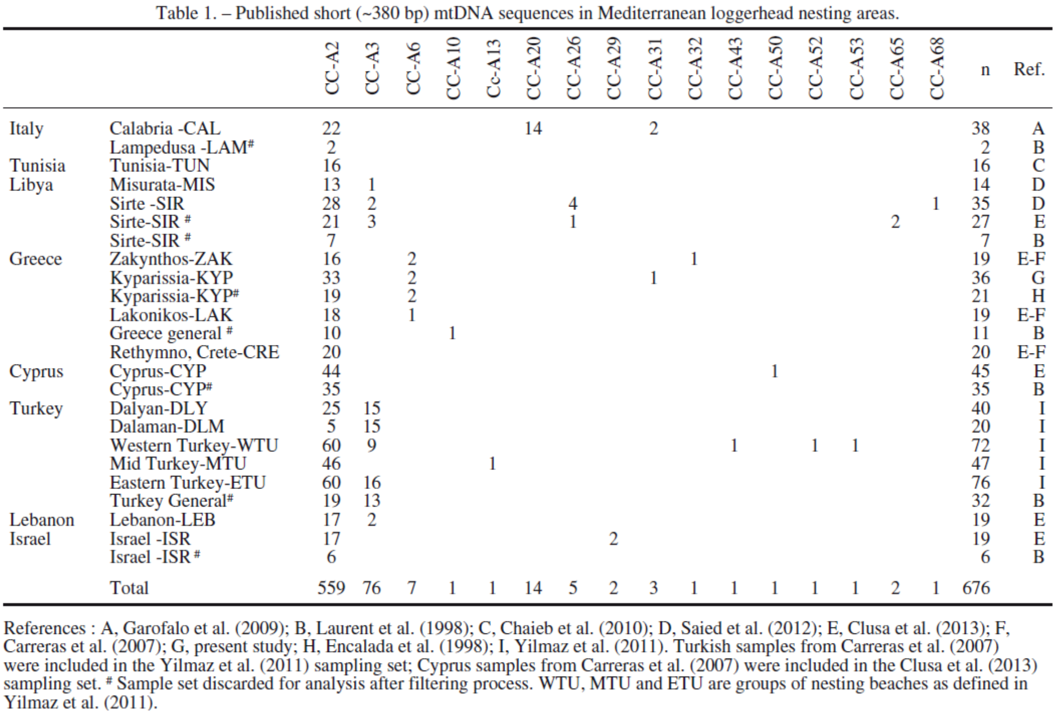


Table 1. – Published short (~380 bp) mtDNA sequences in Mediterranean loggerhead nesting areas.

		CC-A2	CC-A3	CC-A6	CC-A10	Cc-A13	CC-A20	CC-A26	CC-A29	CC-A31	CC-A32	CC-A43	CC-A50	CC-A52	CC-A53	CC-A65	CC-A68	n	Ref.
Italy	Calabria -CAL	22					14			2								38	A
	Lampedusa -LAM#	2																2	B
Tunisia	Tunisia-TUN	16																16	C
Libya	Misurata-MIS	13	1															14	D
	Sirte -SIR	28	2					4									1	35	D
	Sirte-SIR #	21	3					1								2		27	E
	Sirte-SIR #	7																7	B
Greece	Zakynthos-ZAK	16		2							1							19	E-F
	Kyparissia-KYP	33		2						1								36	G
	Kyparissia-KYP#	19		2														21	H
	Lakonikos-LAK	18		1														19	E-F
	Greece general #	10			1													11	B
	Rethymno, Crete-CRE	20																20	E-F
Cyprus	Cyprus-CYP	44											1					45	E
	Cyprus-CYP#	35																35	B
Turkey	Dalyan-DLY	25	15															40	I
	Dalaman-DLM	5	15															20	I
	Western Turkey-WTU	60	9									1		1	1			72	I
	Mid Turkey-MTU	46				1												47	I
	Eastern Turkey-ETU	60	16															76	I
	Turkey General#	19	13															32	B
Lebanon	Lebanon-LEB	17	2															19	E
Israel	Israel -ISR	17							2									19	E
	Israel -ISR #	6																6	B
	Total	559	76	7	1	1	14	5	2	3	1	1	1	1	1	2	1	676	

References : A, Garofalo et al. (2009); B, Laurent et al. (1998); C, Chaieb et al. (2010); D, Saied et al. (2012); E, Clusa et al. (2013); F, Carreras et al. (2007); G, present study; H, Encalada et al. (1998); I, Yilmaz et al. (2011). Turkish samples from Carreras et al. (2007) were included in the Yilmaz et al. (2011) sampling set; Cyprus samples from Carreras et al. (2007) were included in the Clusa et al. (2013) sampling set. # Sample set discarded for analysis after filtering process. WTU, MTU and ETU are groups of nesting beaches as defined in Yilmaz et al. (2011).

Fig. 8 Mediterranean nesting areas with mtDNA haplotype frequencies as summarized from Carreras et al. 2014.

In more recent investigations, a new designed set of primers (Abreu- Grobois et al. 2006) notably increased the length of the fragment sequenced (815 bp), allowing enhanced description of management units, inter-population connections and genetic barriers within the Mediterranean (Carreras et al. 2014). The increase in genetic resolution has improved at regional and fine-scale levels the origin assignment power of juveniles from Mediterranean foraging grounds, potentially unveiling previously unknown distribution patterns. The sequence differentiation based on the 815 bp has been defined through the sub-division of the short sequence nomenclature in long haplotype variants. In other words, the use of the longer sequence allowed the splitting for instance of the short CC-A2 common haplotype into the long haplotypes CC-A2.1, CC-A2.8, CC-A2.9 etc. which revealed further structuring within the Mediterranean (e.g Crete and Israel with CC-A2.8 and CC-A2.9 respectively, Clusa et al. 2013). The distribution of these long haplotypes in the Mediterranean nesting areas is showed in Fig. 9 provided from a recent work of Carreras et al. (2014).

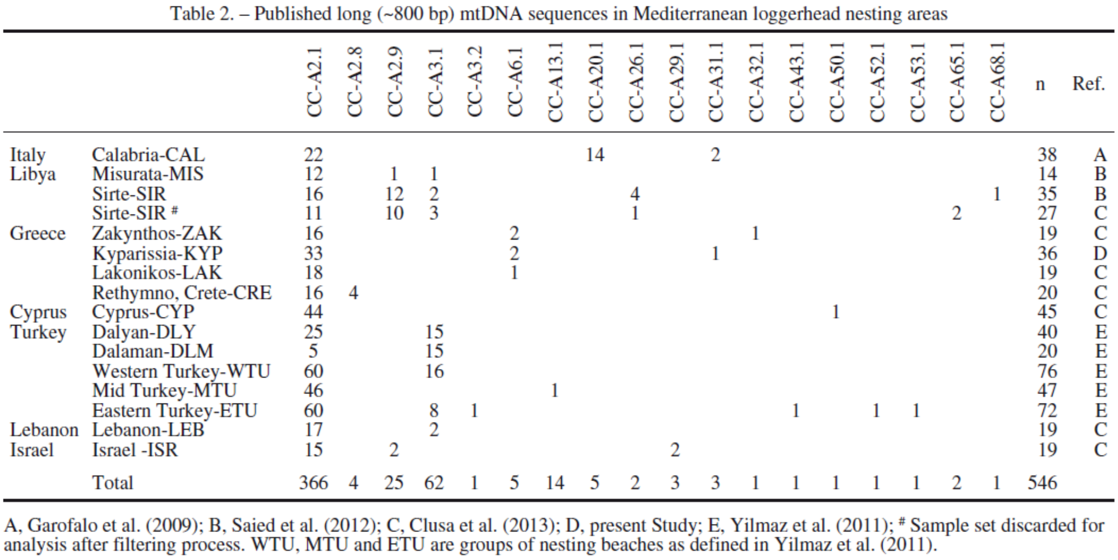


Table 2. – Published long (~800 bp) mtDNA sequences in Mediterranean loggerhead nesting areas

		CC-A2.1	CC-A2.8	CC-A2.9	CC-A3.1	CC-A3.2	CC-A6.1	CC-A13.1	CC-A20.1	CC-A26.1	CC-A29.1	CC-A31.1	CC-A32.1	CC-A43.1	CC-A50.1	CC-A52.1	CC-A53.1	CC-A65.1	CC-A68.1	n	Ref.
Italy	Calabria-CAL	22							14			2								38	A
Libya	Misurata-MIS	12		1	1															14	B
	Sirte-SIR	16		12	2					4									1	35	B
	Sirte-SIR #	11		10	3					1								2		27	C
Greece	Zakynthos-ZAK	16					2						1							19	C
	Kyparissia-KYP	33					2					1								36	D
	Lakonikos-LAK	18					1													19	C
	Rethymno, Crete-CRE	16	4																	20	C
Cyprus	Cyprus-CYP	44													1					45	C
Turkey	Dalyan-DLY	25			15															40	E
	Dalaman-DLM	5			15															20	E
	Western Turkey-WTU	60			16															76	E
	Mid Turkey-MTU	46						1												47	E
	Eastern Turkey-ETU	60			8	1								1		1	1			72	E
Lebanon	Lebanon-LEB	17			2															19	C
Israel	Israel -ISR	15		2							2									19	C
	Total	366	4	25	62	1	5	14	5	2	3	3	1	1	1	1	1	2	1	546	

A, Garofalo et al. (2009); B, Saied et al. (2012); C, Clusa et al. (2013); D, present Study; E, Yilmaz et al. (2011); # Sample set discarded for analysis after filtering process. WTU, MTU and ETU are groups of nesting beaches as defined in Yilmaz et al. (2011).

Fig. 9 Published long (~800 bp) mtDNA sequences in Mediterranean loggerhead nesting areas from Carreras et al. (2014).

AIMS

The Adriatic Sea, due to its great availability of food and shallow waters, represents an ideal foraging and over-wintering environment for the loggerheads. In the last years human-related impact on loggerheads in the Adriatic Sea increased, as observed by the number of incidental catches and strandings along all the coasts. These impacts derive mainly from trawl fishery operations, and affects loggerheads that migrate in the Adriatic. Moreover, these impacts also affect the Mediterranean nesting populations with a decline of rookeries, that represent distinct genetic units and that need to be conserved.

The aim of the study is to unveil the biometric and genetic characterization of loggerhead specimens recovered in three different foraging grounds along the Italian and Croatian coasts of the Adriatic Sea.

Differences in turtle sizes among the three foraging grounds, and the use of the Adriatic Sea by loggerheads at different life stages have been analysed using biometric data, essentially the SCL. Moreover, the description of the finding data could be helpful to understand the proportion of loggerheads affected by the different threats, and the relationship between turtle size classes and threats.

The long sequence mtDNA analysis was implemented for the first time on loggerheads recovered from the Adriatic Sea. Previously, only short haplotypes were detected from individuals found along the Italian and Croatian coasts of the North Adriatic Sea. The long fragments of the mitochondrial control region have been used to:

1) describe the mtDNA haplotype distribution within the Adriatic Sea and to infer the migratory pathway;

2) quantify the contribution of Mediterranean and Atlantic nesting groups to the Adriatic populations, using Bayesan mixed-stock analysis (MSA).

2. MATERIALS AND METHODS

Study Area

Adriatic Sea: ecological role for loggerhead sea turtles

The Adriatic Sea is the northernmost basin of the Mediterranean Sea, lying between the Italian and Balkan Peninsulas. It extents northwest from 40° to 45° 45’ N, connecting to the Ionian Sea to the south. It is about 800 km long, with an average width of 160 km and an area of 130.000 km². Three regions could be identified: the northernmost region extending to the latitude of Ancona, Italy, that is shallow with depths of no more than 100 meters. The second region departs from the south of Ancona where the topography drops quickly to more than 200 m in the Jabuka Pit (also called the Pomo Depression). Finally, the third and deepest part of the Adriatic reaches 1.324 m of depth and rises up again to 780 m depth in the Strait of Otranto, where meets the rest of Mediterranean Sea (Venegas li 2009).

The Northern and Central Adriatic constitutes a wide continental shelf. In the west and east side, shelves are long (shore parallel) and narrow. The substrate is characterized by muddy to sandy bottoms. The western coast is lower and generally sandier than the eastern one that is rugged with islands and coves (Kourafalou 1999). Owing to river runoff and oceanographic conditions, the region exhibits a decreasing trend of nutrient concentration and production from north to south and from west to east. Circulation is mainly controlled by wind stress, river discharge, topography and interaction with the deep basin flow. The mean surface circulation consists of a basin-wide cyclonic gyre. Water enters on the east from the Ionian Sea and floes northward along the Balkan coast. Along the Italian side, water flows south, re-entering the Ionian Sea on the west part of the Otranto strait (Venegas li 2009). The western Adriatic current, flowing southward, is intense because of the rivers emissions that fuel and sustain the cyclonic circulation. On the other side, the eastern Adriatic current, less influenced by river discharges, is weak, warm, salty and more oligotrophic with relevant remineralization processes. The water column stratification in the Northern Adriatic, caused by freshwater buoyancy and heating of the sea surface, occurs from spring to mid autumn, whereas in winter cooling and cold north-easterly wind cause intense mixing and the formation of dense waters (Giani et al. 2012). However oceanographic conditions are subject to great seasonal and inter-annual variations (Venegas li 2009). The geographical location of the Adriatic Sea and the geomorphology of the basin dictate intensive variability in the wind field (Kourafalou 1999), while natural and anthropogenic pressures are responsible for modifying environmental conditions. These oceanographic and trophic features and the current system make the Northern-central Adriatic an important feeding area for juveniles of *C. caretta.* Furthermore, its shallow waters host an extensive neritic usable habitat even for sub-adult and adult turtles. The temperature conditions of the eastern side in the cold season allow sea turtles to overwinter in this basin as well.

The Southern Adriatic is considered a more oceanic developmental habitat (Casale et al. 2012a), nevertheless it has been demonstrated (Casale et al. 2012b) that its shallow bottoms, as the Gulf of Manfredonia, may represent a neritic feeding area too.

As a consequence, turtles with different body size that may be in both neritic and oceanic life stage use the entire Adriatic, depending on habitats they are recruiting within the basin.

Sample collection

Ninety-three loggerhead turtles were recovered along the Adriatic coast of Italy and Croatia, from 2005 to 2014 (Table 1) thanks to the help of three organizations. These three associations (Fondazione Cetacea, Rescue Center of Manfredonia and Pola Acquarium) are actively involved in rescue and conservation of marine vertebrates.

Fondazione Cetacea is a no profit organization that attends to the protection of marine ecosystems and the assistance of large marine animals, but especially it is involved in the hospitalization of marine turtles. The Foundation is located in Riccione and the mainly field of its activity is the North Central Adriatic Sea. It is also occupied in data collection of specimens found stranded or caught as by-catch and at sea.

The CRTM of Manfredonia is situated in the Gargano National Park and act to rescue and monitor marine turtles affected with fishery and other human threats. Its work is based on the rehabilitation of injured animals and on the awareness and scientific divulgation about this endangered species.

Table 1 Sampling data on loggerhead turtles recovered in the Adriatic Sea.

Sample Name	Rescue Association	Recovery Location	Date of Recovery
Agata	FondazioneCetacea	Cesenatico(FC)	21/02/2014
Alice	FondazioneCetacea	PortoCorsini(RA)	22/01/2013
Amleto	FondazioneCetacea	Chioggia(VE)	22/07/2014
Andreina	FondazioneCetacea	Ravenna(RA)	27/03/2013
Angelo	FondazioneCetacea	FalconaraMarittima(AN)	14/04/2013
Ani	FondazioneCetacea	PortoGaribaldi(FE)	03/12/2013
Benedetta	FondazioneCetacea	Bellaria(RN)	05/06/2013
Biondina	FondazioneCetacea	Casalborsetti(RA)	31/08/2014
Catarina	FondazioneCetacea	Cesenatico(FC)	21/02/2014
Cdr	FondazioneCetacea	Riccione(RN)	07/04/2013
Chicca	FondazioneCetacea	FalconaraMarittima(AN)	27/06/2013
Christian	FondazioneCetacea	Ravenna(RA)	04/02/2014
Cleo	FondazioneCetacea	Bellaria(RN)	05/12/2013
Cristina	FondazioneCetacea	BaiaVallugola(PU)	17/08/2014
Daniel	FondazioneCetacea	Ravenna(RA)	04/02/2014
Davide	FondazioneCetacea	PortoCorsini/Savio(RA)	04/12/2012
Dente	FondazioneCetacea	MarinaRomea(RA)	12/06/2014
Diamantina	FondazioneCetacea	Cesenatico(FC)	21/02/2014
Dory	FondazioneCetacea	PortoSanGiorgio(FM)	15/03/2014
Edo	FondazioneCetacea	Portonovo(AN)	11/03/2014
EmanuelaII	FondazioneCetacea	Cesenatico(FC)	09/06/2014
Fegghy	FondazioneCetacea	SanBenedettodelTronto(AP)	21/11/2005
Fortunato	FondazioneCetacea	Ravenna(RA)	23/01/2013
FrancescaMaria	FondazioneCetacea	Grado(GO)	15/09/2013
Francesco	FondazioneCetacea	PortodiAncona(AN)	08/09/2014
Giada	FondazioneCetacea	LidoAdriano(RA)/Cesenatico(FC)	18/07/2013
Gigi	FondazioneCetacea	Ravenna(RA)	23/07/2014
Gioele	FondazioneCetacea	Bellaria(RN)	24/06/2013
Giulia	FondazioneCetacea	Cesenatico(FC)	13/02/2014
Graziano	FondazioneCetacea	Bellaria(RN)	10/12/2013
Iside	FondazioneCetacea	Bellaria(RN)	15/01/2013
Jane	FondazioneCetacea	Cesenatico(FC)	13/02/2014
Kiss	FondazioneCetacea	Ravenna(RA)	09/12/2013
Lisa	FondazioneCetacea	Cesenatico(FC)/MilanoMarittima(RA)	02/01/2014
Lucia	FondazioneCetacea	PortoCorsini(RA)	05/12/2012
Martina	FondazioneCetacea	Cesenatico(FC)	07/01/2014
Matteito	FondazioneCetacea	n/a	29/09/2013
Melissa	FondazioneCetacea	Cesenatico(FC)	04/07/2014
Michelino	FondazioneCetacea	Senigallia(AN)	10/12/2013
Milagro	FondazioneCetacea	Ancona(AN)	21/08/2013
Montone	FondazioneCetacea	Ravenna(RA)	13/11/2014
Natale	FondazioneCetacea	Ravenna(RA)	20/12/2013
Paola	FondazioneCetacea	Riccione(RN)	27/11/2013
Patroclo	FondazioneCetacea	Bellaria(RN)	30/01/2013
Piccolina	FondazioneCetacea	Cesenatico(FC)	17/09/2014
Pilone	FondazioneCetacea	Ravenna(RA)	13/12/2012
Pimpa	FondazioneCetacea	LidodelleNazioni(FE)	27/04/2014
Pipino	FondazioneCetacea	Ravenna(RA)	13/12/2012
Polifemo	FondazioneCetacea	Ravenna(RA)	04/01/2013
Quasimodo	FondazioneCetacea	Numana(AN)	13/06/2009
Radia	FondazioneCetacea	Ravenna(RA)	23/12/2013
Richi	FondazioneCetacea	SanBenedettodelTronto(AP)	28/08/2014

Salina	FondazioneCetacea	LidodegliScacchi(FE)	17/08/2013
Senigallia	FondazioneCetacea	Senigallia(AN)	17/08/2013
Serebirra	FondazioneCetacea	Cesenatico(FC)	05/12/2013
Serena	FondazioneCetacea	MarinadiMontemarciano(AN)	07/08/2014
Shakira	FondazioneCetacea	Ancona(AN)	14/03/2014
SperdutaSperanza	FondazioneCetacea	PortoD'Ascoli(AP)	26/08/2014
Steam	FondazioneCetacea	Cesenatico(FC)	06/12/2013
Stephen	FondazioneCetacea	LidodelleNazioni(FE)	27/11/2014
Steve	FondazioneCetacea	LidoAdriano(RA)	10/07/2013
Susy	FondazioneCetacea	Ancona(AN)	07/09/2014
Theta	FondazioneCetacea	Ravenna(RA)	20/09/2011
Tommaso	FondazioneCetacea	MiramarediRimini(RN)	07/08/2014
Valeria	FondazioneCetacea	Cesenatico(FC)	13/12/2013
Vanity	FondazioneCetacea	Bellaria(RN)	30/12/2013
Venerdì	FondazioneCetacea	Ravenna(RA)	25/11/2014
864	CRTM Manfredonia	6MNVignanotica-Vieste(FG)	30/09/2014
865	CRTM Manfredonia	4MNVignanotica-Vieste(FG)	30/09/2014
866	CRTM Manfredonia	4MNMergoli-Mattinata(FG)	30/09/2014
867	CRTM Manfredonia	4MNMattinatella-Mattinata(FG)	02/10/2014
868	CRTM Manfredonia	1MNZapponeta(FG)	07/10/2014
869	CRTM Manfredonia	6MNMattinata(FG)	07/10/2014
870	CRTM Manfredonia	3MNManfredonia(FG)	08/10/2014
871	CRTM Manfredonia	4MNZapponeta(FG)	08/10/2014
873	CRTM Manfredonia	4MNMattinata(FG)	09/10/2014
874	CRTM Manfredonia	4MNMattinata(FG)	09/10/2014
875	CRTM Manfredonia	4MNMattinata(FG)	09/10/2014
876	CRTM Manfredonia	4MNMattinata(FG)	09/10/2014
877	CRTM Manfredonia	4MNMattinata(FG)	09/10/2014
878	CRTM Manfredonia	4MNMattinata(FG)	09/10/2014
879	CRTM Manfredonia	6MNManfredonia(FG)	13/10/2014
880	CRTM Manfredonia	4MNZapponeta(FG)	14/10/2014
Croazia1	Pula Aquarium	Spalato	05/06/2014
Croazia2	Pula Aquarium	Savudrija	18/09/2014
Croazia3	Pula Aquarium	Umag	20/07/2014
Croazia4	Pula Aquarium	Savudrija	11/08/2014
Croazia5	Pula Aquarium	Savudrija	22/08/2014
Croazia6	Pula Aquarium	SudDalmatia	17/08/2014
Croazia7	Pula Aquarium	MaliLošinj	18/06/2014
Croazia8	Pula Aquarium	Savudrija	18/09/2014
Croazia9	Pula Aquarium	BaiadiKoria,Zaklopatica,IsoladiLastovo	17/09/2014
Croazia10	Pula Aquarium	Savudrija	19/10/2014

The Pola Acquarium cooperates with the Brijuni national park for the recovery of marine turtles and participate, as the Fondazione Cetacea, in the NETCET project that have the aim to realize a strengthened network of organizations involved in help and treatment of injured and sick sea turtles and other protected species.

The specimens were divided based on their foraging ground of provenience: North Central Adriatic east (NCAeast) recovered from Grado (45°40'41"88 N; 13°23'44"88 E) in the North to Porto D’Ascoli (42°55′00″N 13°53′00″E); North Central Adriatic west

(NCAwest) from Savudrija, in the Northern Croatia, to Dalmacija, a region in the South of Croatia; South Adriatic (SA) recovered in the Gulf of Manfredonia.

The specimens were caught alive and nearly all turtles were released at sea after the hospitalization, even though few individuals died during treatments.

Blood samples were collected and stored in Queen’s lysis buffer (Seutin et al. 1990) with contribution of the organizations that recovered turtles and then conserved at 4°C for the molecular analysis.

Biometric data

Body size of all captured individuals was measured following Bolten (1999). Over-the-curve measurements are taken with a non-stretching tape measure. Two Curved Carapace Length (CCL) are generally used: minimum and notch to tip. Minimum CCL is measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supra-caudals (Fig. 10a). CCL n-t (notch to tip) is measured from the anterior point at midline to the posterior tip of the supra-caudals (Fig.10b). An alternative measure is straight carapace length (SCL) that is taken with caliper. It is measured in one of three ways: minimum SCL, SCL notch to tip or maximum SCL taken from the anterior edge of the carapace to the posterior tip of the supra-caudals (Fig.10c). Anterior and posterior locations must be on the same side of the carapace (Bolten 1999).

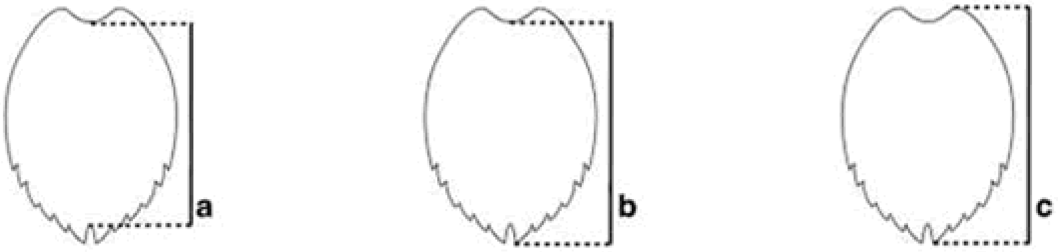


Fig. 10 Anatomical points for straight (SCL) and curved (CCL) carapace length measurements. (a) minimum SCL and minimum CCL; (b) SCL notch to tip and CCL notch to tip; (c) maximum SCL. From Bolten (1999).

In the present work the measure techniques used to collect sample sizes were the minimum CCL and the Curved Carapace Length notch to tip (CCL n-t).

To figure out differences in biometric parameters depending on the foraging ground of provenience, measures were standardized. Although variations exist in CCL measurements, we assumed discrepancies to be minimal. Minimum CCL measures taken from CRTM of Manfredonia and from the Pola Acquarium and CCL notch to tip measures taken from Fondazione Cetacea on recovered individuals were converted in SCL measures. Data conversion to SCL parameter was accomplished using an empirical equation (SCL = 0.948 * CCL – 1.442) based on linear regression of paired CCL and SCL data for *Caretta caretta* (Teas 1993).

A size frequency distribution was carried out distributing individuals into 10 cm size classes following Piovano et al. (2011). The size class frequency distribution was analyzed in relation to the different recovery modalities and to the three foraging areas.

A One–way ANOVA (R command "aov," package "stats," R Core Team 2014) was performed to test the effect of the Factor "Site" with three levels (three feeding areas) on SCL. When a difference was detected, a post hoc test was performed (R command "Tukey HSD," package "stats," R Core Team 2014). Before carrying out these analyses, normality and homogeneity of variances were tested with Bartlett's test.

Significant differences in the mean SCL of samples depending on the type of recovery were examined as well. In this case, the Factor "Recovery" with four levels (by-catch, floating, stranded and not classified recovery) was tested on SCL of turtle individuals.

DNA extraction and amplification

Genomic DNA from blood samples was extracted with the NucleoSpin® kit (MACHEREY-NAGEL), following the manufacturer's instructions. 815 base pair (bp) fragment of the mtDNA control region was amplified by the polymerase chain reaction (PCR) using the primers LCM15382 (5'-GCTTAACCCTAAAGCATTGG-3') and H950 (5'-TCTCGGATTTAGGGGTTTG-3') designed by Abreu-Grobois et al. (2006). The resulting fragment contains the 380 bp fragment traditionally used for population studies on this species (Carreras et al. 2006; Giovanotti et al. 2010).

PCR reactions were performed in 20 µL volumes under the following conditions: an initial denaturation step at 95 °C for 3 min; followed by 35 cycles of 95 °C for 30 seconds, 56 °C for 1 min, 72 °C for 30 s; with a final extension at 72 °C for 10 min.

All amplifications were sent to Macrogen Europe for sequencing reaction using both forward and reverse primers to confirm the nucleotide polymorphisms.

Electropherograms were checked using Bioedit Sequence Alignment Editor v.7.0.9 and sequences were aligned using the ClustalX program (Thompson et al. 1997).

Data analysis

The data sets were divided in short and long data set based on the short (~380 bp) and long (~815 bp) fragments of the mitochondrial control region. The haplotypes were compared to previously identified haplotypes from nesting and foraging locations in the Eastern Atlantic and Mediterranean (Carreras et al. 2006; Revelles et al. 2007; Clusa et al. 2013; Clusa et al. 2014; Garofalo et al. 2013), according to the standardized nomenclature of the Archie Carr Center for Sea Turtle Research (ACCSTR; http://accstr.ufl.edu/ccmtdna.html).

Long Haplotype diversity (*h;* Nei 1987) and nucleotide diversity (π; Nei 1987) were estimated using ARLEQUIN version 3.5 (Excoffier and Lischer 2010) to analyze the genetic diversity of each foraging ground (NCA west, NCA east and SA).

Relationships between the haplotypes were visualized in unrooted haplotype networks, calculated by Median Joining with the software Network v. 4.6.1.1 (Bandelt et al. 1999).

The statistical significance of haplotype frequency differentiation among the three feeding grounds was tested on long sequences with ARLEQUIN software package. Pairwise genetic distances (F_{ST}) were calculated with 1,000 permutations setting P value on the 0.01 significance level. Significant differences in haplotype frequencies were also tested between the three feeding grounds and published Mediterranean foraging grounds (see Table S1 in Supplementary materials for references). When the same geographical area was analyzed in different published studies considered for the comparison, data of absolute haplotype frequencies were combined (i.e. Tyrrhenian Sea). The feeding aggregates within the Mediterranean Sea included in the analysis are showed in Table S1.

Significance levels were corrected using a false discovery rate (FDR) methodology (Benjamini and Hochberg 1995).

To assess the proportion of individuals in the Adriatic feeding ground (all the three sampling site altogether) coming from different rookeries, the mixed sock analysis (MSA) was applied as implemented in the program ‘Bayes’ (Pella and Masuda 2001). The analysis was performed on the basis of haplotype frequency differences between baseline (nesting populations) and our Adriatic sample. This baseline (Table S2) was obtained from published data from 13 Mediterranean nesting areas (Garofalo et al. 2009; Saied et al. 2012; Clusa et al. 2013; Carreras et al. 2014; Yilmaz et al. 2011) and 10 Atlantic rookeries (Monzón-Argüello et al. 2010; Shamblin et al. 2012) reporting the absolute haplotype frequencies analyzed using the same primer pair (LCM 15382; H950).

The test was performed in two ways: first combining all the Atlantic and all the Mediterranean nesting beaches (regional level) and then considering each nesting site as a separate potential contributor (fine scale level) (Giovannotti et al. 2010; Clusa et al. 2014).

An additional Bayesian analysis was performed to unveil differences in the proportion of individuals in each foraging stock originating from different Mediterranean rookeries. The test was applied to the three Adriatic recovery areas independently and only the Mediterranean nesting sites were included in the analysis cause of the low proportion of Atlantic rookery contribution. The Gelman-Rubin criterion was used to test for convergence in the algorithm. Values above 1.2 indicate lack of convergence and the corresponding estimates are considered unreliable (Gelman et al. 1996).

3. RESULTS

3.1 Analysis of biometric and finding data

Biometric measures (minimum CCL and CCL n-t) and date, place and recovery modality (Table 2) were recorded in 93 specimens.

The CCL of the recovered individual ranged between 11 and 87.5 cm.

Table 2 Data set of recovered turtles. Data not recorded are indicated as n/a (not available).

Name	**Finding modality**	**CCL (cm)**
Chicca	stranded	11
Paola	stranded	16
Edo	stranded	20
Susy	floating	20
Cristina	floating	21
Angelo	floating	23.5
Matteito	n/a	24
Salina	floating	24
Pipino	trawling	24.5
Dente	floating	27
Sperduta speranza	stranded	27
Biondina	floating	28
Cdr	stranded	28
Michelino	mussel line	28
Piccolina	trawling	28
Senigallia	floating	28
Serena	stranded	28
Steve	trawling	28
Croazia10	trawling	28.7
Davide	trawling	29
Croazia1	n/a	31.7
Croazia8	trawling	31.8
Giada	trawling	33
Kiss	trawling	35
Venerdì	trawling	35
Gioele	trawling	36
Croazia2	trawling	36.2
Tommaso	floating	36.5
865	trawling	37.5
Croazia5	trawling	39.2
Dory	stranded	40

Richi	floating	40
880	trawling	40.8
Stephen	stranded	41
Gigi	trawling	41.5
Milagro	floating	42
Serebirra	trawling	43
Diamantina	trawling	48
Croazia4	trawling	48
Croazia3	trawling	49.1
879	trawling	49.8
Steam	trawling	50
Amleto	trawling	51.5
Ani	floating	52
Francesco	floating	52
Quasimodo	floating	52
Theta	stranded	52
Pilone	trawling	53
Patroclo	trawling	54
876	trawling	55
Croazia6	floating	55.7
Benedetta	trawling	57.5
Melissa	trawling	58
Montone	trawling	58
Valeria	trawling	58
Lisa	trawling	59
Martina	trawling	59
Shakira	floating	59
Croazia9	n/a	59.9
Catarina	trawling	60
869	trawling	60
867	trawling	60.2
Alice	trawling	62
Fortunato	trawling	62
Giulia	trawling	62
Iside	trawling	62
Pimpa	stranded	62
Agata	trawling	63
Cleo	trawling	63
875	trawling	63.2
Andreina	trawling	64
Christian	trawling	65
Fegghy	fishing net	65
866	trawling	65
Graziano	trawling	66

Vanity	trawling	66
868	floating	66
877	trawling	67
FrancescaMaria	floating	68
870	trawling	68.5
Jane	trawling	69
874	trawling	69
Daniel	trawling	70
871	trawling	70
Croazia7	n.d	70
878	trawling	70.5
Natale	trawling	71
EmanuelaII	trawling	73
Radia	trawling	73
864	trawling	74.2
Lucia	trawling	76
Polifemo	trawling	81
873	trawling	87.5

The size of the turtles after conversion of CCL to SCL ranged from 9.0 to 81.5 cm and the mean SCL of all the individuals was 45.6 cm (SD=16.6).

The SCL measures meet the normality and homogeneity of variances (Bartlett’s K-squared = 3.2487, df = 2, *p* = 0.197). The one–way analyses of variance showed that the SCL measures of the individuals differ significantly based on their feeding area (Table 3). The post hoc test revealed that significant differences in the SCL occurred between the NCA ($SCL_{NCAwest} = 43.3 \pm 16.9$ cm; $SCL_{NCAest} = 41.2 \pm 13.1$ cm) and the South Adriatic ($SCL_{SA} = 58.1 \pm 11.8$cm) with Tukey’s post hoc tests < 0.05 for both the comparisons (Fig. 11a).

Table 3 Results from one-way ANOVA on the straight carapace length measures between the three recovery areas

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	2	3028	1514.00	6.0456	0.003439
Residuals	90	22539	250.43		

The dominant size class was the size class 50-59 cm (23.7%), followed by the 60-69 and the 20-29 size classes (20.4% and 19.3% respectively) (Fig. 11b). The size frequency distribution in the single recovery areas, as shown in the Fig. 12, shows that size classes vary among the three feeding aggregates.

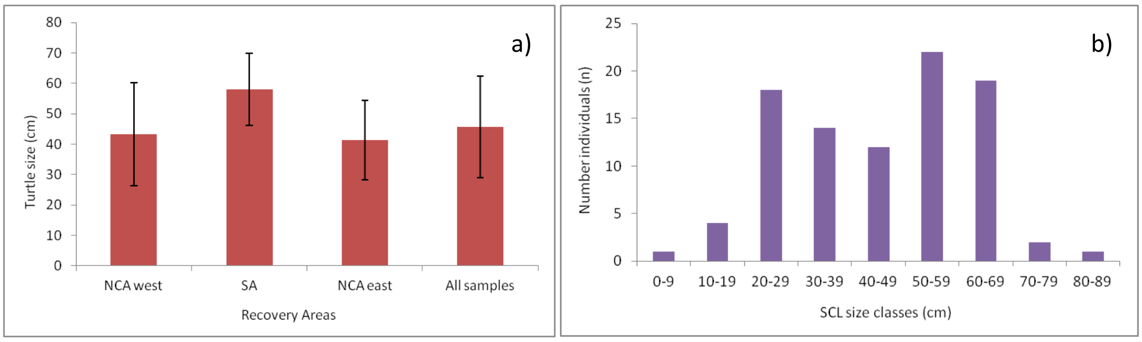


Fig. 11 a) Turtle mean SCL for each Adriatic sub-area and total sample mean size. Standard deviation (SD) is included in the bar graph and values are expressed in the text. b) Frequency distribution of measured SCL referred to the total number of collected individuals.

The frequency distribution of size classes from the NCA west sample (n=67) showed the same trend of Figure 11b, although here the 20-29 size class was more abundant than the 60-69 size class. In the NCA east the distinct size classes seemed quite homogeneously distributed, with a predominance of individuals within the 20-29 size class (Fig. 12). Regarding the South Adriatic, 8 of the 16 rescued turtles fall into the size class 60-69 cm (50%). However, the restricted sample size of recovered turtles in the Croatian waters (n=10) and in the South Adriatic (n=16) in contrast with the NCA west sample should be taken into account when analyzing the entire data set.

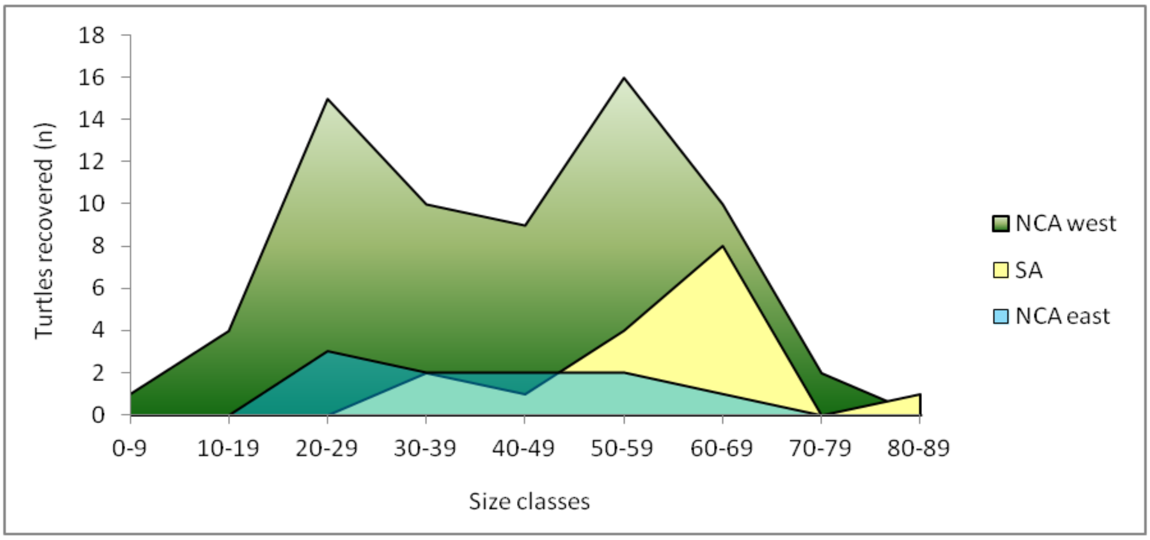


Fig. 12 Frequency distribution of measured SCL class loggerheads recovered in the three recovery areas of the Adriatic Sea.

The finding modality of the individuals was classified into stranded, floating and incidental captured turtles. The highest proportion (67.4%) of individuals was found as by-catch in trawl nets (Fig. 13a), while turtles found floating at sea and stranded were the 18.5% and 10.9% respectively. Even considering the three recovery locations independently, incidental capture was the most recurrent type of recovery (Fig. 13b).

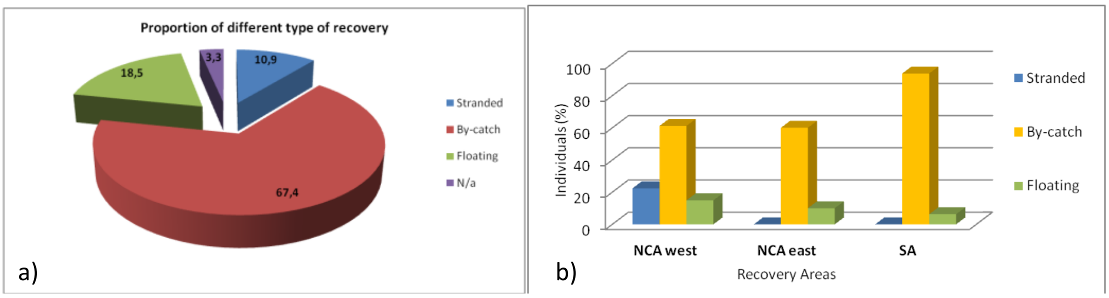


Fig. 13 Proportion of individuals associated to the three different types of recovery in the Adriatic (a) and in each study area (b).

The mean SCL of stranded specimens was 29.4 cm (SD ± 15.3), while the mean sizes of captured and floating turtles were 50.7 cm (SD ± 14.5) and 37.3 (SD ± 15.5) respectively (Fig. 14). This finding was comparable with the distribution of finding modality in each SCL classes (Fig. 15).

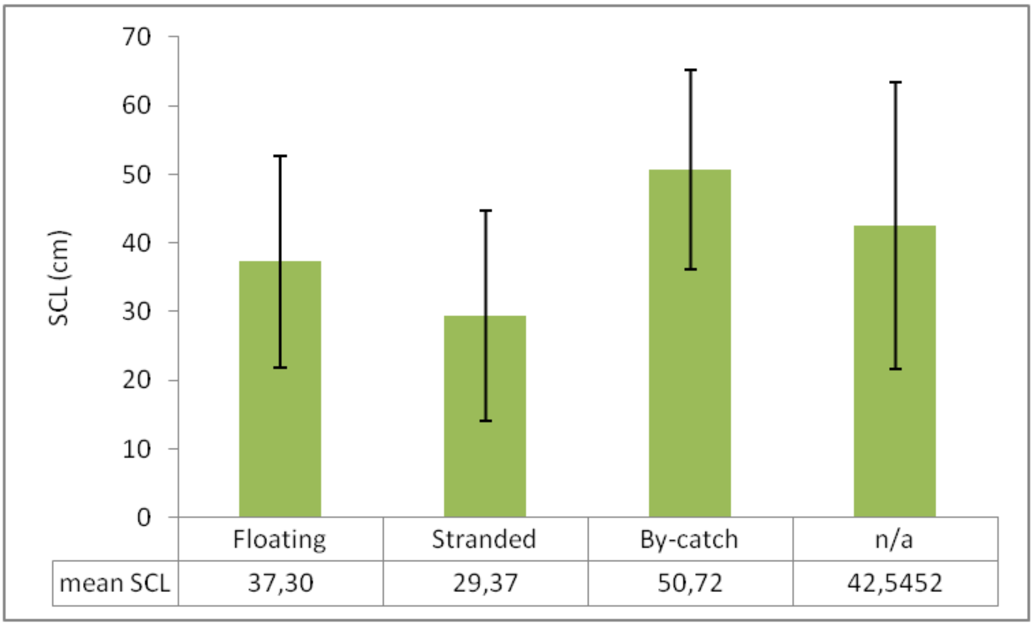


Fig. 14 The figure shows the relationship between turtle mean size expressed as SCL and the three recovery modalities. Mean SCL of not available data are also showed.

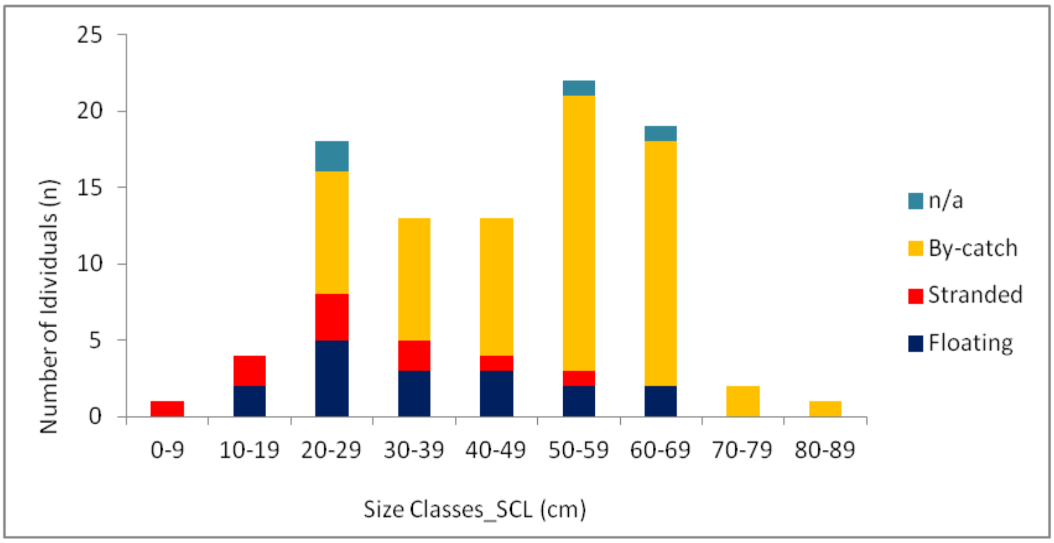


Fig. 15 Distribution of the different finding modalities in each SCL class for all sampled turtles.

After Bartlett test confirming the normality and homogeneity of variance (K-squared = 0.9367, df = 3, *p* = 0.8166), the one-way ANOVA revealed statistically significant differences in the mean SCL of samples depending on the type of recovery (Table 4). The Tukey analysis outcome suggested that this statistical differences concerned stranded and floating turtles versus individuals captured as by-catch with a probability of 0.0004 and 0.008398 respectively.

Table 4 One – way ANOVA results testing the statistical relationship between type of recovery and the mean SCL of turtles.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Recovery	3	5467	1822.4	8.069	8.17e-05 ***
Residuals	89	20099	225.8		

3.2 Mitochondrial DNA haplotype analysis

Data on short and long haplotypes assigned to the loggerhead turtles captured in the Adriatic study areas are displayed in Table 5.

Table 5 Long and short haplotypes assigned to each turtle specimen.

Sample Name	Recovery location	Short Haplotype	Long Haplotype
Agata	NCA west	CC-A2	CC-A2.1
Alice	NCA west	CC-A2	CC-A2.1
Amleto	NCA west	CC-A2	CC-A2.1
Andreina	NCA west	CC-A3	CC-A3.1
Angelo	NCA west	CC-A2	CC-A2.1
Ani	NCA west	CC-A2	CC-A2.1
Benedetta	NCA west	CC-A2	CC-A2.1
Biondina	NCA west	CC-A2	CC-A2.1

Catarina	NCA west	CC-A2	CC-A2.1
Cdr	NCA west	CC-A2	CC-A2.1
Chicca	NCA west	CC-A2	CC-A2.1
Christian	NCA west	CC-A2	CC-A2.1
Cleo	NCA west	CC-A2	CC-A2.1
Cristina	NCA west	CC-A2	CC-A2.1
Daniel	NCA west	CC-A2	CC-A2.1
Davide	NCA west	CC-A2	CC-A2.1
Dente	NCA west	CC-A2	CC-A2.1
Diamantina	NCA west	CC-A2	CC-A2.1
Dory	NCA west	CC-A2	CC-A2.1
Edo	NCA west	CC-A2	CC-A2.1
EmanuelaII	NCA west	CC-A2	CC-A2.1
Fegghy	NCA west	CC-A2	CC-A2.1
Fortunato	NCA west	CC-A2	CC-A2.1
FrancescaMaria	NCA west	CC-A2	CC-A2.1
Francesco	NCA west	CC-A2	CC-A2.1
Giada	NCA west	CC-A2	CC-A2.1
Gigi	NCA west	CC-A2	CC-A2.1
Gioele	NCA west	CC-A2	CC-A2.1
Giulia	NCA west	CC-A2	CC-A2.1
Graziano	NCA west	CC-A2	CC-A2.1
Iside	NCA west	CC-A2	CC-A2.1
Jane	NCA west	CC-A2	CC-A2.1
Kiss	NCA west	CC-A2	CC-A2.1
Lisa	NCA west	CC-A2	CC-A2.1
Lucia	NCA west	CC-A2	CC-A2.1
Martina	NCA west	CC-A2	CC-A2.1
Matteito	NCA west	CC-A2	CC-A2.1
Melissa	NCA west	CC-A2	CC-A2.1
Michelino	NCA west	CC-A2	CC-A2.1
Milagro	NCA west	CC-A2	CC-A2.1
Montone	NCA west	CC-A2	CC-A2.1
Natale	NCA west	CC-A2	CC-A2.1
Paola	NCA west	CC-A2	CC-A2.1
Patroclo	NCA west	CC-A2	CC-A2.1
Piccolina	NCA west	CC-A2	CC-A2.1
Pilone	NCA west	CC-A2	CC-A2.1
Pimpa	NCA west	CC-A2	CC-A2.1
Pipino	NCA west	CC-A2	CC-A2.1
Polifemo	NCA west	CC-A2	CC-A2.1
Quasimodo	NCA west	CC-A2	CC-A2.1
Radia	NCA west	CC-A31	CC-A31.1
Richi	NCA west	CC-A2	CC-A2.1
Salina	NCA west	CC-A2	CC-A2.1
Senigallia	NCA west	CC-A2	CC-A2.1
Serebirra	NCA west	CC-A2	CC-A2.1
Serena	NCA west	CC-A2	CC-A2.1

Shakira	NCA west	CC-A2	CC-A2.1
Sperdutasperanza	NCA west	CC-A2	CC-A2.9
Steam	NCA west	CC-A2	CC-A2.1
Stephen	NCA west	CC-A2	CC-A2.9
Steve	NCA west	CC-A10	CC-A10.4
Susy	NCA west	CC-A2	CC-A2.1
Theta	NCA west	CC-A2	CC-A2.1
Tommaso	NCA west	CC-A2	CC-A2.1
Valeria	NCA west	CC-A2	CC-A2.1
Vanity	NCA west	CC-A2	CC-A2.1
Venerdì	NCA west	CC-A2	CC-A2.1
864	SA	CC-A2	CC-A2.1
865	SA	CC-A2	CC-A2.1
866	SA	CC-A2	CC-A2.1
867	SA	CC-A2	CC-A2.1
868	SA	CC-A2	CC-A2.1
869	SA	CC-A2	CC-A2.1
870	SA	CC-A2	CC-A2.1
871	SA	CC-A2	CC-A2.1
873	SA	CC-A2	CC-A2.1
874	SA	CC-A2	CC-A2.1
875	SA	CC-A2	CC-A2.1
876	SA	CC-A2	CC-A2.1
877	SA	CC-A2	CC-A2.1
878	SA	CC-A2	CC-A2.1
879	SA	CC-A2	CC-A2.1
880	SA	CC-A3	CC-A3.1
Croazia1	NCA east	CC-A2	CC-A2.9
Croazia2	NCA east	CC-A2	CC-A2.9
Croazia3	NCA east	CC-A2	CC-A2.1
Croazia4	NCA east	CC-A2	CC-A2.1
Croazia5	NCA east	CC-A2	CC-A2.9
Croazia6	NCA east	CC-A2	CC-A2.1
Croazia7	NCA east	CC-A2	CC-A2.1
Croazia8	NCA east	CC-A2	CC-A2.1
Croazia9	NCA east	CC-A2	CC-A2.1
Croazia10	NCA east	CC-A2	CC-A2.1

The sequence analysis of the ninety-three loggerhead individuals examined revealed four short mtDNA haplotypes: CC-A2 (95.7%), CC-A3 (2.2%), CC-A10 (1.1%) and CC-A31 (1.1%) according to Bowen *et al*. (2004) (Fig.16).

The long sequence analysis split the short CC-A2 haplotype into CC-A2.1 and CC-A2.9 haplotypes, due to further polymorphism of the longer mtDNA fragment. Moreover, the CC-A3, CC-A10 and CC-A31 short haplotypes were resulted equal to the haplotypes CC-A3.1, CC-A10.4 and CC-A31.1 respectively based on the long sequence.

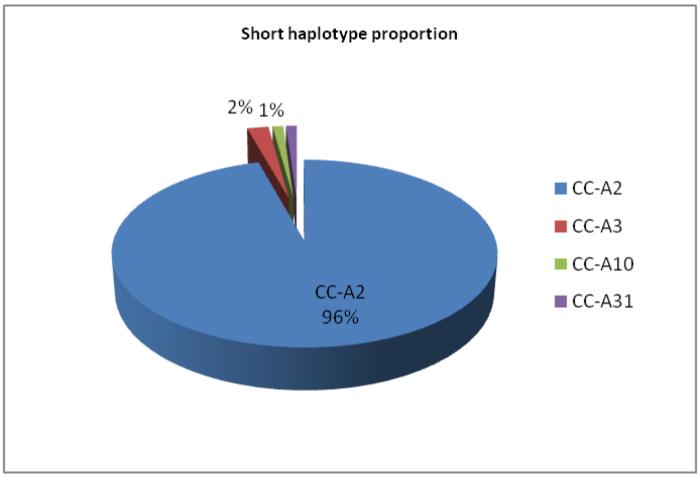


Fig. 16 Short haplotype proportion in the sampled turtles.

Table 6 Distribution of the short haplotypes for the examined loggerheads in the three sampling regions.

SHORT HAPLOTYPES	NCA west	NCA east	SA	Total
CC-A2	64	10	15	89
CC-A3	1	-	1	2
CC-A10	1	-	-	1
CC-A31	1	-	-	1

CC-A2 haplotype was the most represented in all the three foraging areas (Table 6). Regarding the long variants, the CC-A2.1 haplotype was the most abundant among the sample data set (Fig. 17), while CC-A2.9 was found only in five individuals (5.4%) collected in the North Central Adriatic. The short CC-A3 was identified in the 2.2% of examined individuals, that correspond all to the long CC-A-3.1 haplotype. CC-A10/CC-A10.4 and CC-A31/CC-A31.1 were each recorded in one specimen of the NCA west (Table 7).

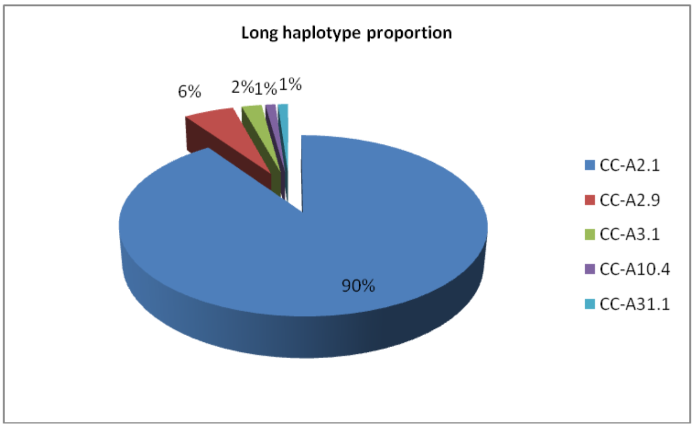


Fig. 17 Long haplotype proportion in the sampled turtles.

Table 7 Distribution of the long haplotypes for the examined loggerheads in the three sampling regions.

LONG HAPLOTYPES	NCA west	NCA east	SA	Total
CC-A2.1	62	7	15	84
CC-A2.9	2	3	-	5
CC-A3.1	1	-	1	2
CC-A10.4	1	-	-	1
CC-A31.1	1	-	-	1
n	67	10	16	93
h	0.1443 ± 0.0581	0.4667 ± 0.1318	0.1250 ± 0.1064	
π	0.037087 ± 0.059156	0.116667 ± 0.125221	0.031250 ± 0.056569	

The haplotype network (Fig. 18) showed the relationship between haplotypes. All of them differed from the most abundant CC-A2.1 by a single mutational change with a probability higher than 95%. Pie graphs, each representing one haplotype and its frequency in each foraging ground, indicated that the three feeding areas shared the principal haplotype CC-A2.1. All five haplotypes detected in the data set were present in the NCA west sample. Although the low number of turtles collected in the SA and in the NCA east, the CC-A3.1 haplotype was also found in the South Adriatic, while the other most common haplotype CC-A2.9 was found in the NCA east sample as well as in the NCA west.

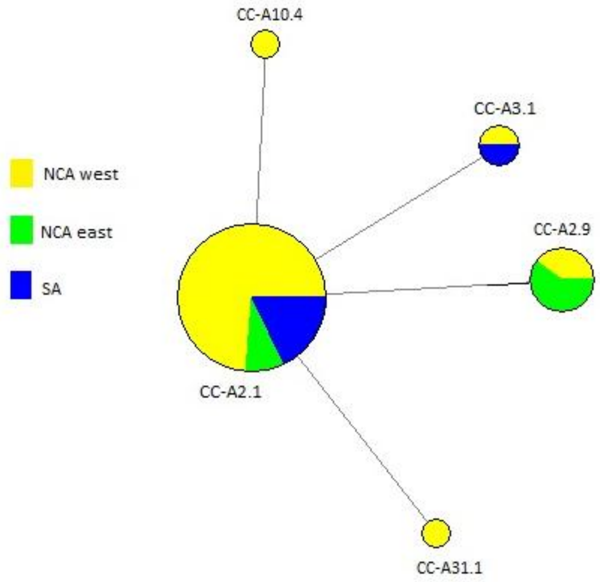


Fig. 18 Unrooted haplotype network of mtDNA long fragment for *Caretta caretta* in the Adriatic feeding grounds. Each pie graph represents one haplotype and the different colours represent it frequency in each feeding grounds. The size of each pie graph depends on its absolute frequency. Solid lines connect haplotypes by single mutational changes with a probability higher than 95%.

The highest haplotype and nucleotide diversities were found in the NCA east (h = 0.4667 ± 0.1318; π = 0.11667 ± 0.125221). Conversely, values of haplotype and nucleotide diversities in the NCA west and in the SA were comparable (h = 0.1443 ± 0.0581 and 0.1250 ± 0.1064; π = 0.037087 ± 0.059156 and 0.031250 ± 0.056569, respectively; Table 7).

Pairwise F_{ST} between the three foraging ground ranged from 0 to 0.25. No significant genetic structuring was found between the SA and the NCA west and east sample ($p\ F_{ST\ \mathrm{NCAest\ vs\ SA}}$ = 0.06153; $p\ F_{ST\ \mathrm{NCAwest\ vs\ SA}}$ = 0.7334). A weak statistical difference was found between the NCA west and NCA east sample ($p\ F_{ST\ \mathrm{NCAwest\ vs\ NCAeast}}$ = 0.04686) after correction with FDR (Table 8).

Table 8 Pairwise comparison (F_{ST}) of haplotype composition differences between the studied foraging areas.

	SA	NCA west	NCA east
SA	-		
NCA west	-0.01391	-	
NCA east	0.22465	0.25225*	-

Significant values were corrected with FDR methodology. ∗∗∗P <0.001; ∗∗P <0.01; ∗P <0.05

The Pairwise F_{ST} estimates between Mediterranean foraging aggregates (see Table S1 for compared feeding grounds) revealed the most significant genetic heterogeneity between Lampedusa (LAM) and all the three Adriatic feeding grounds (Table 9) with the major genetic differentiation observed between LAM and NCAwest (F_{ST} = 0.528). Even the pairwise comparison between Algerian basin (ALG) and the three Adriatic samples resulted statistically different from zero. However, no significant difference of the genetic structure was found between the Algerian basin and the NCAeast. This latter feeding ground showed a low statistical difference in haplotype frequency with Driny Bay and the sample NCAwest. A great statistically significant difference occurred between the NCAwest and the Ionian Sea (P < 0.001), while a weak heterogeneity was computed between the NCAwest and the Catalano-Balearic Sea (CAB) after correction with a false discovery rate (P<0.05)

Table 9 Genetic structure of Mediterranean feeding populations based on pairwise genetic distances (F_{ST} values).

	SA	NCAwest	NCAeast	LAM	MAL	CAB	ALG	TYR	ION	SLE	NADR	SADR	DrinyBay
SA	-												

NCAwest	-0.0261	-											
NCAeast	0.1774	0.2203*	-										
LAM	0.3767***	0.5283***	0.2257***	-									
MAL	0.0134	0.0836	0.1023	0.2736***	-								
CAB	0.0169	0.0518*	0.0188	0.2584***	0.0022	-							
ALG	0.1983***	0.2717***	0.1005	0.0738*	0.1468**	0.099***	-						
TYR	0.0001	0.0267	0.0784	0.3457***	0.0112	-0.0085	0.144***	-					
ION	0.0813	0.1438***	0.0125	0.1716***	0.0142	0.0179	0.090***	0.0568**	-				
SLE	-0.0149	0.0235	0.0905	0.3239***	-0.0183	-0.0071	0.159***	-0.0061	0.0396	-			
NADR	-0.0047	0.0367	0.0833	0.3382***	-0.0214	-0.0027	0.167***	0.0029	0.0351	-0.0160	-		
SADR	-0.0560	-0.0199	0.2288	0.4229***	0.0474	0.0375	0.222***	0.0157	0.1072*	0.0057	0.0158	-	
DrinyBay	-0.0231	-0.0108	0.2206*	0.4733***	0.0813	0.0455	0.241***	0.0228	0.128**	0.0190	0.0321	-0.0176	-

References and acronyms as in Table 2. P values were corrected with the FDR methodology and thresholds of significance are indicated as ∗∗∗P <0.001; ∗∗P <0.01; ∗P <0.05

The mixed stock analysis results showed that all the Mediterranean source populations potentially contributed to the Adriatic stock (99.5%) and that only a small proportion of specimens appeared to belong to the Atlantic stock (0.5 ± 1%) (Table 10). The weak contribution to the Adriatic mixed stock from Atlantic populations was confirmed when Atlantic rookeries were analyzed independently as single potential source. The contribution from Atlantic populations ranged from 0.05% (± 0.2%) to 1.2% (± 6%).

Within the Mediterranean rookeries the Adriatic stock is made by a contribution of 54.3% (± 36.7%) from the nesting sites of Misurata in Lybia, 10.8% (± 24.8%) from the Israel nesting population and 10.8% (± 17.9%) from the nesting site of the Western Greece. Contributions of the other Mediterranean nesting beaches were low and ranged from 0.2% (DLM) to 6.7% (LEB).

When the three Adriatic foraging grounds were considered separately, the contribution of each Mediterranean nesting population was differently distributed (Table 11). In the NCAwest, Misurata remained the major contributor (27.3 ± 34.1%), followed by Cyprus (19 ± 29.4%) and Western Greece (16.7 ± 24.9%). A low relative contribution (11 ± 22.7%) was also detected from Middle Turkey. On the other hand, most individuals of the NCAeast came from Lybia (35.7 ± 31.1 % Sirte; 12.5 ± 25.5% Misurata), while 20.3% of turtles (± 31.8%) were ascribed to the rookeries of Israel. Finally, the SA feeding ground showed a different composition, hosting a high proportion of individuals from Turkey (10.7 ± 23% ETU; 11.6 ± 23.2% MTU; 11.6 ± 23.4% WTU). Even nesting sites of Lebanon resulted important contributors (16.6 ± 28.8%) to the South Adriatic, together with rookeries of Cyprus and Misurata, which contributed with a proportion of 13.6% (± 25.3%) and 11.4% (± 24%), respectively. The main directions followed by individuals found in the Adriatic areas were reconstructed in Figure 19 according to the results of the Mixed Stock Analysis.

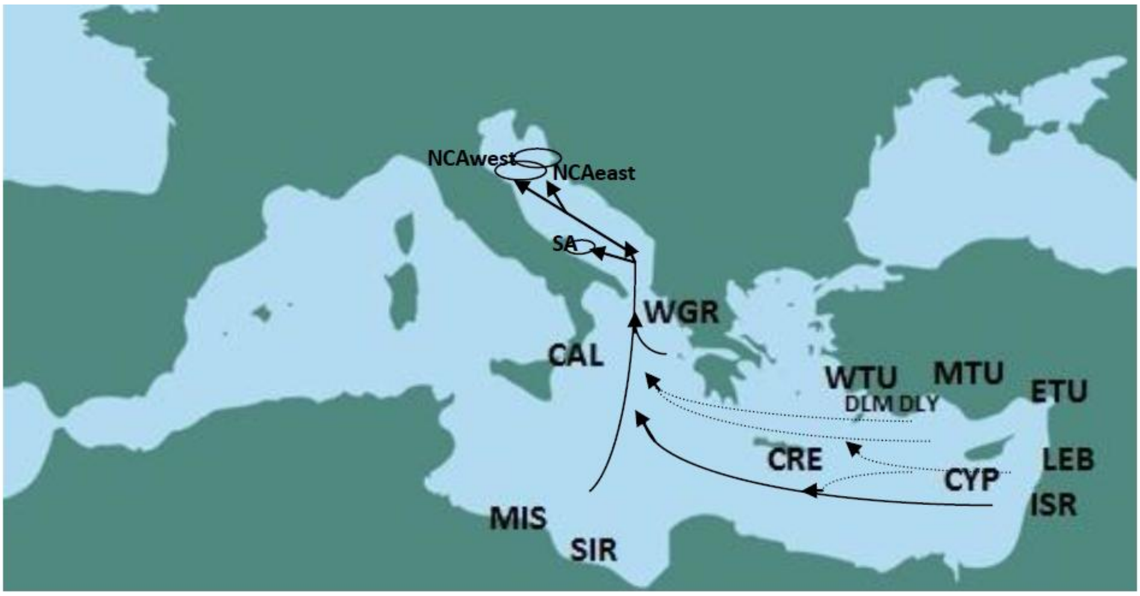


Fig. 19 Reconstruction of the main directions followed by individuals found in the Adriatic areas analyzed (circled), as emerged from the Mixed Stock Analysis. Contributions above 10% from rookeries to the Adriatic areas are shown with solid arrows. Dashed lines indicate further flows identified for each Adriatic sample. Each line is drawn to connect the origin and sampling places and does not necessarily describes the specific route followed by turtles. Rookeries: SIR (Sirte), MIS (Misurata), CAL (Calabria), WGR (Western Greece), WTU (western Turkey), MTU (Middle Turkey), ETU (Eastern Turkey), DLM (Dalaman), DLY (Dalyan), LEB (Lebanon), ISR (Israel), CYP (Cyprus), CRE (Crete).

Table 10 Results of the mixed stock analysis: estimates of contributions by the Atlantic and Mediterranean rookeries to the Adriatic feeding ground. The analysis was performed combining all the Atlantic and all the Mediterranean nesting beaches and considering each nesting site as a separate potential contributor.

STOCK	MEAN	SD	2.5%	MEDIAN	97.5%
Mediterranean rookeries[1]	**0.9953**	0.0105	0.9605	1.000	1.000
MIS	**0.5433**	0.3677	0.0000	0.6620	0.9832
SIR	0.0132	0.0373	0.0000	0.0000	0.1395
ISR	**0.1085**	0.2484	0.0000	0.0000	0.8944
LEB	0.0675	0.1847	0.0000	0.0000	0.7584
CYP	0.0568	0.1605	0.0000	0.0000	0.6528
ETU	0.0102	0.0461	0.0000	0.0000	0.1176
MTU	0.0291	0.1010	0.0000	0.0000	0.3869
WTU	0.0114	0.0447	0.0000	0.0000	0.1349
DLM	0.0022	0.0086	0.0000	0.0000	0.0266
DLY	0.0042	0.0161	0.0000	0.0000	0.0510
CRE	0.0037	0.0202	0.0000	0.0000	0.0390

WGR	**0.1082**	0.1787	0.0000	0.0133	0.6760
CAL	0.0092	0.0211	0.0000	0.0000	0.0718
Atlantic rookeries[2]	0.0047	0.0105	0.0000	0.0000	0.0395
NOR	0.0005	0.0022	0.0000	0.0000	0.0051
CEF	0.0098	0.0142	0.0000	0.0037	0.0492
SEF	0.0013	0.0062	0.0000	0.0000	0.0152
SAL	0.0117	0.0591	0.0000	0.0000	0.1213
DRT	0.0057	0.0266	0.0000	0.0000	0.0670
QMX	0.0008	0.0036	0.0000	0.0000	0.0087
SWF	0.0009	0.0043	0.0000	0.0000	0.0104
CWF	0.0008	0.0036	0.0000	0.0000	0.0087
NWF	0.0005	0.0025	0.0000	0.0000	0.0057
CPV	0.0005	0.0023	0.0000	0.0000	0.0054

[1]Sample obtained by combining all Mediterranean nesting areas; [2]sample obtained by combining all the Atlantic nesting areas; Mediterranean and Atlantic codes as in Table S2.

Table 11 Relative contribution of each Mediterranean rookery to the three Adriatic foraging grounds. In bold values above 0.100. Rookery acronyms as in Table S2.

NCA west	Rookeries	MEAN	SD	2.5% percentile	MEDIAN	97.5% percentile
	MIS	**0.2732**	0.3412	0.0000	0.0671	0.9683
	SIR	0.0167	0.0340	0.0000	0.0001	0.1191
	ISR	0.0758	0.1791	0.0000	0.0003	0.7466
	LEB	0.0974	0.2221	0.0000	0.0002	0.8517
	CYP	**0.1900**	0.2944	0.0000	0.0033	0.8920
	ETU	0.0176	0.0575	0.0000	0.0000	0.1845
	MTU	**0.1103**	0.2269	0.0000	0.0003	0.8165
	WTU	0.0152	0.0488	0.0000	0.0000	0.1527
	DLM	0.0035	0.0111	0.0000	0.0000	0.0364
	DLY	0.0073	0.0231	0.0000	0.0000	0.0737
	CRE	0.0125	0.0535	0.0000	0.0000	0.1211
	WGR	**0.1666**	0.2491	0.0000	0.0282	0.8586
	CAL	0.0139	0.0313	0.0000	0.0001	0.1082
NCA east						

	MIS	**0.1253**	0.2552	0.0000	0.0004	0.9169
	SIR	**0.3567**	0.3115	0.0000	0.3387	0.9520
	ISR	**0.2031**	0.3182	0.0000	0.0031	0.9655
	LEB	0.0415	0.1129	0.0000	0.0001	0.4379
	CYP	0.0471	0.1206	0.0000	0.0001	0.4740
	ETU	0.0335	0.0976	0.0000	0.0000	0.3706
	MTU	0.0401	0.1092	0.0000	0.0001	0.4209
	WTU	0.0323	0.0960	0.0000	0.0000	0.3531
	DLM	0.0104	0.0377	0.0000	0.0000	0.1130
	DLY	0.0215	0.0738	0.0000	0.0000	0.2365
	CRE	0.0341	0.0977	0.0000	0.0000	0.3575
	WGR	0.0366	0.1044	0.0000	0.0000	0.3964
	CAL	0.0178	0.0564	0.0000	0.0000	0.1967
SA						
	MIS	**0.1145**	0.2396	0.0000	0.0004	0.8864
	SIR	0.0110	0.0395	0.0000	0.0000	0.1184
	ISR	0.0402	0.1240	0.0000	0.0000	0.4662
	LEB	**0.1659**	0.2882	0.0000	0.0015	0.9444
	CYP	**0.1361**	0.2530	0.0000	0.0008	0.8752
	ETU	**0.1070**	0.2305	0.0000	0.0004	0.8797
	MTU	**0.1159**	0.2325	0.0000	0.0004	0.8375
	WTU	**0.1164**	0.2341	0.0000	0.0006	0.8772
	DLM	0.0179	0.0500	0.0000	0.0000	0.1721
	DLY	0.0474	0.1233	0.0000	0.0001	0.4507
	CRE	0.0415	0.1262	0.0000	0.0000	0.4729
	WGR	0.0723	0.1785	0.0000	0.0001	0.7125
	CAL	0.0138	0.0491	0.0000	0.0000	0.1442

4. DISCUSSION

Distribution of loggerheads in the Mediterranean Sea has been widely investigated. Nesting sites and pelagic and demersal feeding habitats have been described (Margaritoulis et al. 2003; Casale and Margaritoulis 2010). Few studies have only recently investigated the genetic structure based on the analysis of long fragments within the Adriatic basin. In the present work, the long haplotype frequency was analyzed from a wide sample of turtles that inhabit these waters. Then, the contribution of previously undetected nesting colonies to the Adriatic aggregates was identified. Moreover, long sequences mtDNA have been analyzed for the first time in individuals sampled along the Croatian coast.

The occurrence of both neritic sub-adults and adults (range: 52-70 cm SCL according to Giovannotti et al. 2010) in the samples analyzed supports previous observations on the role of the Adriatic Sea as foraging area for adult and sub-adult loggerheads (Margaritoulis et al. 2003). Moreover, juveniles (13-32 cm) and pelagic sub-adults (33-51cm; following the classification of Giovannotti et al. 2010) were the 28% and the 25.8%, of the total number of the individuals, respectively. These findings suggest that the Adriatic could provide suitable environments for different turtle’s life stages. In particular, these results provide new evidences that juveniles undertake developmental migrations towards this basin. The presence of different life stages in the Adriatic Sea is likely due to the occurrence of closely connected coastal and open sea ecosystems, and these features may allow the trophic shift from pelagic-omnivorous to benthic-carnivorous stages (Lazar et al. 2000; Giovannotti et al. 2010).

The recovery of juveniles and sub-adults in the North Central Adriatic is consistent with turtle size range observed previously in other investigations in the same area (Scaravelli and Affronte 2003; Lazar et al. 2003; 2005; Casale et al. 2010; 2014). However, the mean size of turtle sampled on the Eastern and Western coast of the Adriatic Sea, was lower than the mean size previously registered, suggesting that the presence of smaller individuals migrating into the North Adriatic is likely increasing. On the other hand, the significant differences in the mean turtle body size between North and South Adriatic is due to the recovery of a greater proportion of turtles ranging from 60 to 69 cm in the Gulf of Manfredonia. The South Adriatic and Ionian Sea are considered developmental areas for juveniles in the first years of life (Casale et al. 2010). However, in the South Adriatic the Gulf of Manfredonia may represent a neritic foraging ground for loggerhead sea turtles in a wide range of sizes. The results support previous evidences of the use of this area as an inter-breeding foraging ground from adult loggerheads (Casale et al. 2012).

The analysis of the relationship between the mean SCL and recovery modality showed that juveniles are more affected by strandings. Instead, turtles found floating were in a wide size range (17 - 63 cm SCL) suggesting that all size classes might be gathered at sea with buoyancy and diving problems. Finally, by-catch occurs mainly in larger individuals, especially the 50-59 and 60-69 cm size classes. The significant differences between turtle body size and the type of recovery is consistent with turtle behavior, as neritic sub-adult individuals feed on the sea bottom and hence they are more affected by trawling fishery operations. Differently, juveniles have a more pelagic behavior so they may undergo other threats, probably deriving from human activities. It is difficult to assess the causes of strandings or floating turtle findings unless there are evidences of the impact of fishing gears or other anthropogenic factors (boat strikes, pollution, marine debris) or natural factors (infections). However, there are estimates that anthropogenic impact is one the primary cause of floating and stranded turtle recovery (Casale et al. 2010). The mean size of captured turtles as by-catch in the Adriatic confirms previous estimates on turtle size incidental captured in the Adriatic Sea (Casale et al. 2004; 2014). The high fishing effort in the North Adriatic might explain the relevant numbers of floating, stranded or captured turtles of different sizes found in this part of the basin. Only a small portion of these turtles is recovered and rescued.

The long sequence analysis of the mitochondrial control region allowed the splitting of the short haplotypes (e.g. CC-A2) into long haplotypes (e.g. CC-A2.1, CC-A2.9) which revealed further structuring within the Adriatic loggerhead populations. The CC-A2 haplotype and its long variant CC-A2.1 are the most represented in the Adriatic feeding grounds and they are also the most common in the Mediterranean feeding aggregates (Carreras et al. 2006; Mafucci et al. 2006; Garofalo et al. 2013; Clusa et al. 2014). Despite these haplotypes are widely distributed among the Mediterranean nesting populations (Carreras et al. 2007; Garofalo et al. 2009; Yilmaz et al. 2011; Clusa et al. 2013; Carreras et al. 2014), they are common also in the Atlantic rookeries, especially in nesting beaches of Florida. However, the haplotype CC-A1 and its long variants are the most frequent haplotypes in the Atlantic nesting populations. The long haplotype CC-

A2.9, found in five individuals collected in the North Central Adriatic, was recently identified in the nesting sites of Lybia and Israele (Saied et al. 2012; Clusa et al. 2013) and represents an exclusive genotype of Mediterranean rookeries (Clusa et al. 2014). Individuals bearing this haplotype were recorded at low frequency in the west central Mediterranean feeding aggregates (Clusa et al. 2014) and only one time in the Adriatic Sea (Garofalo et al. 2013).

The long haplotyp CC-A3.1 (deriving from the short CC-A3) was recently detected at low frequency in nesting beaches of Lybia and Lebanon (Saied et al. 2012; Clusa et al. 2013) and widely among Turkish nesting populations (Yilmaz et al. 2011). CC-A3/CC-A3.1 was also found in various feeding and developmental loggerhead aggregates of the Eastern and Western Mediterranean and off the Italian coasts including the Adriatic basin (Carreras et al. 2006; Mafucci et al. 2006; Revellas et al. 2007; Giovannotti et al. 2010; Garofalo et al. 2013; Clusa et al. 2014). In this study, the long haplotype CC-A3.1 was found in two individuals rescued in the North Western Adriatic and in the Southern Adriatic. This is unexpected, since this mtDNA genotype seems more common than the haplotype CCA-2.9 in the Adriatic populations (Garofalo et al. 2013; Clusa et al. 2014). However, the lack of resolution of the short mtDNA marker used in previous studies (Mafucci et al. 2006; Giovanotti et al. 2010) might have hidden the abundance of the haplotype CC-A2.9, because of the inability of splitting the common haplotype CC-A2 into subtypes.

CC-A10/CC-A10.4 and CC-A31/CC-A31.1 are rare haplotypes and in fact they were recorded only in two individuals. These haplotypes were found at very low frequency in the Mediterranean foraging grounds. CC-A10 was identified once in the Alboran Sea (Revellas et al. 2007) and Northeastern Spain (Carreras et al. 2006). While in the Atlantic feeding grounds, it was recently detected in the Canary Islands (Monzón-Argüello et al. 2009) and previously in Madeira Island (Bolten et al. 1998). The long form CC-A10.4 was reported for the first time in the southern Adriatic basin (Driny bay; Yilmaz et al. 2012), and then in the southern Mediterranean feeding grounds (Southern Levantin Sea; Clusa et al. 2014). This haplotype seem to be an exclusive haplotype of Atlantic rookeries (Clusa et al. 2014) since the short variant was previously described in Mexico (Encalada et al. 1998; Bowen et al 2005) and the long CC-A10.4 was found recently in a nesting population of Florida (Shamblin et al. 2012). The presence of this haplotype in the Mediterranean basin is still unclear, but records of the short form in the Western Mediterranean might be explained by migration of juveniles from Atlantic to the

Mediterranean pelagic habitats. Since the long haplotype CC-A10.4 was detected from one individuals collected in the Northwestern Adriatic, we could hypothesize that Atlantic juveniles can arrive by chance in the Eastern Mediterranean coastal waters, including the Adriatic Sea, probably following favorable surface currents, as suggested by Mafucci et al. (2006). The haplotype CC-A31, bore by a single individual from the North Western Adriatic, was found previously among the feeding aggregates of the southeastern Italian coast (Carreras et al 2006; Mafucci et al. 2006). Recently, its correspondent long haplotype was described in nesting sites of Calabria and Kyparissia Bay, in western Greece (Garofalo et al. 2009; Carreras et al. 2014). It is absent in the Atlantic, and likely it represents an endemic haplotype of the Mediterranean colonies (Clusa et al. 2014). The individual recorded with this haplotype in our sample was an adult female and hence, it is reasonable to suppose that it came from mentioned nesting colonies, because of the habit of adult loggerheads to migrate, feed, and overwinter in the neighboring neritic habitats.

Pairwise comparison of haplotype composition of the three Adriatic samples revealed only a weak heterogeneity between the North Western and the North Eastern Adriatic and no statistical differences between the North and the South Adriatic. It is likely that the Adriatic basin represents a wide unique feeding habitat for loggerheads.

The significant difference in haplotype frequency observed in the individuals recorded in the North Western Adriatic compare to other Mediterranean feeding aggregates (e.g. Ionian Sea and Catalano-Balearic Sea) could be ascribed to the rare haplotypes CC-A10.4. The South Adriatic resulted significantly different only from the heterogeneous aggregates of Algerian Sea and Lampedusa, and this is likely in accordance with the abundance in the South Adriatic of the common haplotype CC-A2.1. On the other hand, the homogeneity detected between the North Eastern Adriatic and the Algerian Sea could be due to the low number of sampled turtles in the North Eastern Adriatic and to the presence of a relevant number of individuals with the haplotypes CC-A2.9 in both basins.

The analysis carried out using the long sequence of the control region allowed inferring about migration routes and the origin of the recovered turtles in the Adriatic throughout the Bayesan approach of the Mixed Stock Analysis (MSA). Results of the MSA let hypothesize short and long migratory pathways towards the Adriatic mostly from the Mediterranean nesting populations. In fact, the contribution of Atlantic rookeries is minimal and lower compare to Clusa et al. (2014). This might be explained by the fact that Clusa et al. (2014) sampled smaller turtles in several Mediterranean feeding areas hence, more Atlantic individuals may have been included. Atlantic loggerheads are supposed to migrate back to the Ocean at an average length of 58.8 cm CCL (Revellas et al. 2007) and to follow Atlantic water masses after entering the Mediterranean Sea. In addition, the lack of population size as a weighting factor for the MSA in our study could have implied an underestimation of the Atlantic rookery contribution.

The contributions from specific rookeries to the Adriatic foraging ground described here are important not only for a better understanding of the biology of this species, but also for its conservation. Fisheries bycatch stands as one of the major anthropogenic factors threatening sea turtle populations around the Mediterranean Sea (Casale 2008) and in particular in the Adriatic Sea (see results). The knowledge of which rookeries major contributes to the Adriatic feeding habitats is of great concern to design specific conservation measures for these populations in their foraging grounds.

The MSA results ascribed the highest contribution to the Adriatic feeding areas by the nesting site of Misurata in Libya. The high frequency of CC-A2.1 haplotype and the presence of CC-A2.9 and CC-A3.1 in the nesting colonies of Misurata probably emphasized the contribution of this rookery in comparison with other potential sources (i.e. Sirte). Since the mixed stock analysis is sensitive to the occurrence of rare haplotypes in rookeries, it may yield biased results when population sizes are not included. However, the haplotype CC-A2.9 is present only in rookeries of Libya and Israele (Saied et al. 2012; Clusa et al. 2013) and this could explain the prevalence of the Libyan nesting population as source of individuals for the Adriatic and also the contribution of the nesting colonies of Israele. Consistently, long-range migrations were previously reported for a low proportion of juvenile loggerheads from Libya which were supposed to drift passively through the local eddies in the eastern Mediterranean basin (Saied et al. 2012). In fact, the sizes of turtles bearing the Libyan haplotype in our sample were in the range of pelagic juveniles. The presence of this haplotype in the Adriatic supports the wide dispersal of the Mediterranean loggerhead juveniles predicted by Baran and Kasparek (1989).

Greek nesting site resulted another possible source of individuals for the Adriatic, demonstrating the occurrence of short-range migrations as well. Circulation patterns of marine water masses might influence this migratory pathway from Greece as previously observed (Lazar et al. 2004; Zbinden et al. 2008). The presence in the Adriatic of the CC-A31.1 haplotype, which was recently recorded in nesting colonies of Kyparissia Bay (Carreras et al 2014), probably highlighted the contribution from Western Greece.

When the contribution from Mediterranean rookeries to each Adriatic feeding area was computed, the contribution of Lybia and Israele was confirmed in the North East Adriatic area, since here individuals bore exclusively the haplotype CC-A2.1 and CC-A2.9. Conversely, the nesting populations of Turkey seem to be important sources of individuals for the North western and the South Adriatic and they probably migrate towards the Adriatic following the principal marine surface currents. An additional contribution from Cyprus was detected both in the North Western and in the Southern Adriatic. Moreover, Lebanese rookeries seem to contribute to the South Adriatic aggregates with a relative high proportion. The rookery of Cyprus is characterized by the prevalence of the haplotype CC-A2.1 in a restricted sample of individuals and this might have overestimated its contribution. Instead, in Lebanon the presence of the haplotype CC-A3.1 supports our finding about the contribution of these nesting colonies to the South Adriatic. The geographic location of both these rookeries is in accordance with the MSA results, since the surface circulation patterns may drive the distribution of loggerheads in the Adriatic feeding areas.

The genetic relationships between the Libya, Turkey and Greece rookeries and the Adriatic Sea suggest that conservation actions should be addressed to both nesting areas and related feeding/developmental areas. In particular, because of the unique genetic structure of rookeries in Libya, Turkey or Greece, their status is of great concern and should be kept under special protection by both national and international legislations. In this context, the use of MSA was a useful tool to understand the main migratory routes of Adriatic recovered specimens. However, this technique should be implemented integrating rookery sizes as weighting factor and other methods should be associated to improve the knowledge of genetic and demographic structure of loggerheads in their foraging habitats. Here, the importance of the use of long mtDNA fragments was highlighted to increase the genetic resolution. Future management plans should include updated genetic assessments of loggerhead populations to unveil marine regions with peculiar genetic structure and to establish independent management units.

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Supplementary materials

Table S1 Absolute long (~815 bp) mtDNA haplotype frequencies found in the Mediterranean foraging areas for loggerheads. Sample size (n) and total number of individuals bearing the different haplotypes are also shown. Data of haplotype frequencies from different published papers were combined when the same geographical area was analyzed.

	LAM[a]	MAL[a]	CAB[b]	ALG[b]	TYR[ab]	ION[ab]	SLE[b]	NADR[ab]	SADR[b]	Drini Bay[c] (SADR)	NCA west[d]	NCA east[d]	SADR[d]	Total
CC-A1.1	6		2	21	5									34
CC-A1.3	2		1	2	1		1							7
CC-A1.4	1													1
CC-A1.6					1									1
CC-A2.1	4	17	30	31	59	33	28	43	20	37	62	7	15	386
CC-A2.8						2								2
CC-A2.9			2	4	1	5		1			2	3		18
CC-A3.1	6	4	2	4	3	9	3	6	1		1		1	40
CC-A5.1			1											1
CC-A6.1								2		1				3
CC-A10.3						1								1
CC-A10.4							1			1	1			3
CC-A14.1			1	3										4
CC-A20.1					2									2
CC-A26.1	2													2
CC-A28.1						2				1				3
CC-A29.1							1							1
CC-A31.1					1						1			2
CC-A32.1			1					1						2
CC-A50.1	1													1
CC-A55.1						2								2
n	22	21	40	65	73	54	34	53	21	40	67	10	16	516

[a] Data from Garofalo et al. (2013); [b] Data from Clusa et al. (2014); [c]Data from Yilmaz et al. (2012) ; [d] present study. Feeding ground codes: LAM (Lampedusa); MAL (Malta); CAB (Catalano-Balearic Sea); ALG (Algerian basin); TYR (Tyrrhenian Sea); ION (Ionian Sea); SLE (Southern Levantine Sea); NADR (Northern Adriatic Sea); NCA (North Central Adriatic east and west); SADR (Southern Adriatic Sea).

Table S2 Published long (~815 bp) sequence haplotype frequencies in the Atlantic and Mediterranean rookeries that represent possible source populations for loggerheads foraging in the Adriatic Sea. Total number of haplotypes per rookery (n) and rookery sizes (mean nests per year) are included. References for each Atlantic and Mediterranean rookery considered for the present MSA are introduced below in the table.

	MIS	SIR	ISR	LEB	CYP	ETU	MTU	WTU	DLM	DLY	CRE	WGR	CAL	NOR	CEF	SEF	SAL	DRT	QMX	SWF	CWF	NWF	CPV
CC-A1.1														141	668	27		1		16	248	94	
CC-A1.2															15	1					9		
CC-A1.3															3	16		1	1		2		79
CC-A1.4															20	7	1		13		7	2	6
CC-A1.5																							3
CC-A2.1	12	27	15	17	44	60	46	60	5	25	16	67	22		275	123	18	28	64	80	108	12	2
CC-A2.2																1							
CC-A2.3															1	3			6		2		
CC-A2.4															1	8					3		
CC-A2.5															1	1			10		3		
CC-A2.8											4												
CC-A2.9	1	22	2																				
CC-A2.11																					1		
CC-A3.1	1	5		2		8		16	15	15					37	14	1		3	13	46	1	
CC-A3.2						1																	
CC-A5.1															2				2				
CC-A6.1												5											
CC-A7.1															7	2					6		
CC-A7.2															2								
CC-A8.1																			7		1		
CC-A9.1															2	2		2	8	1	1	1	
CC-A1.1															9	7			41		7		
CC-A1.4															1								
CC-A11.2																							1
CC-A11.3																	1		7		1		
CC-A11.5																			1				
CC-A12.1																			2				
CC-A13.1							1								1	1			2		2		
CC-A14.1															15	1			7	1	5		
CC-A17.1																							30
CC-A17.2																							6
CC-A2.1													14		2	2				5	1		
CC-A21.1																					2		
CC-A26.1		5																				1	
CC-A27.1																							

CC-A29.1			2																				
CC-A31.1												1	2										
CC-A32.1												1											
CC-A36.1															1								
CC-A36.2																			1		1		
CC-A41.1															1								
CC-A42.1																			1				
CC-A43.1						1										1							
CC-A47.1																							1
CC-A5.1					1																		
CC-A51.1																				2			
CC-A52.1						1																	
CC-A53.1						1	1																
CC-A59.1																						1	
CC-A6.1																			1				
CC-A65.1		2																					
CC-A68.1		1																					
n	14	62	19	19	45	72	48	76	20	40	20	74	38	141	1064	217	21	32	177	208	456	112	128
REF.	a	a/b	b	b	b	c	c	c	c	c	b	b/d	e	f	f	f	f	f	f	f	f	f	g

References: a) Saied et al. 2012 b) Clusa et al. 2013; c) Yilmaz et al. 2011; d) Carreras et al. 2014; e) Garofalo et al. 2009; f) Shamblin et al. 2012; g) Monzón-Argüello et al. 2010. Mediterranean and Atlantic nesting areas and acronyms: Misurata (MIS), Sirte (SIR), Israel (ISR), Lebanon (LEB), Cyprus (CYP), eastern Turkey (ETU), middle Turkey (MTU), western Turkey (WTU), Dalaman (DLM), Dalyan (DLY), Crete (CRE), WGR (western Greece, consisting in Zakynthos, Lakonikos and Kyparissia bay), Calabria (CAL), South Carolina and Georgia (NOR), central eastern Florida (CEF), south eastern Florida (SEF), Cay Sal Bank- Bahamas (SAL), Dry Tortugas-Florida (DRT), Isla Cozumel and mainland Quintana Roo-Mexico (QMX), south western Florida (SWF), central western Florida (CWF), north western Florida (NWF) and Cape Verde (CPV).