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Mitochondrial DNA reveals regional and interregional importance of the central Mediterranean African shelf for loggerhead sea turtles (*Caretta caretta*)

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SUMMARY: The wide north African continental shelf in the central Mediterranean is known to be one of the few important areas in the basin for loggerhead turtles in the neritic stage. In order to assess the origin of these turtles, sequences of the mtDNA control region were obtained from 70 turtles caught by bottom trawlers in the area, and compared with known sequences from turtles from Mediterranean and Atlantic nesting sites. Five haplotypes were identified (Haplotype diversity = 0.262; nucleotide diversity = 5.4×10^{-3}). Specific haplotypes indicate contributions from distant rookeries such as Turkey and the Atlantic, which shows that Atlantic turtles entering the Mediterranean while in the oceanic phase use at least one Mediterranean continental shelf as a neritic foraging ground. A new haplotype and another one previously found only in foraging areas, highlight the genetic information gaps for nesting sites, which undermine powerful mixed stock analyses. Despite these limitations, the results reveal the regional importance of the study area as a neritic foraging ground for turtles that are probably from most of the Mediterranean nesting aggregates. Therefore, reducing turtle mortality resulting from the high fishing effort in the area should be regarded as key for Mediterranean turtle conservation and is also possibly important for Atlantic populations.

Keywords: *Caretta caretta*, mtDNA, Mixed Stock Analysis, conservation, Mediterranean.

RESUMEN: DNA MITOCONDRIAL REVELA LA IMPORTANCIA REGIONAL E INTERREGIONAL DE LA PLATAFORMA CONTINENTAL MEDITERRÁNEA AFRICANA PARA LA TORTUGA BOBA (*CARETTA CARETTA*). – La amplia plataforma continental africana en el Mediterráneo se reconoce como una de las pocas áreas importantes en la cuenca para la tortuga boba en su estadio nerítico. Para evaluar el origen de estas tortugas se obtuvieron secuencias de mtDNA de la región control de 70 tortugas capturadas en el área por arrastreros y se compararon con las conocidas de localidades de anidación en el Mediterráneo y el Atlántico. Se identificaron cinco haplotipos (diversidad de haplotipos = 0.262; diversidad de nucleótidos = 5.4×10^{-3}). Los haplotipos específicos indican contribución de localidades alejadas como Turquía y el Atlántico, mostrando que las tortugas atlánticas que entran en el Mediterráneo en la fase oceánica usan, al menos, una plataforma continental mediterránea como territorio de anidación y alimentación. Un nuevo haplotipo y otro más, previamente encontrado sólo en áreas de alimentación, destacan el vacío de información genética de los sitios de anidación, lo que socava el poder de los análisis de stocks mixtos. A pesar de estas limitaciones, los resultados revelan la importancia regional del área de estudio como área de alimentación para las tortugas de, probablemente, la mayor parte de los agregados de anidación mediterráneos. Por tanto, la reducción de la mortalidad de tortugas debida al alto esfuerzo de pesca en el área debería ser considerado clave para la conservación de la tortuga mediterránea y, posiblemente, importante también para la poblaciones atlánticas.

Palabras clave: *Caretta caretta*, mtDNA, análisis de stock mezclados, conservación, Mediterráneo.

INTRODUCTION

The loggerhead turtle (*Caretta caretta*) has a circumglobal distribution (Marquez, 1990) and is the most abundant of the three marine turtle species occurring in the Mediterranean Sea (Groombridge, 1990).

Loggerheads undergo two ecological stages, first an oceanic one in which they feed on pelagic preys, then a neritic one in which they feed mainly on benthic preys (Bolten, 2003). Due to the higher reproductive value of large juveniles and adults and the longer duration of the neritic stage, changes in the survival rate in this stage (due to threats or turtles being removed for conservation measures) have the greatest effect on population growth (Heppell, 1998).

In these two stages turtles can be affected by different threats. For instance, oceanic turtles are mainly captured by pelagic fishing gear such as the pelagic longline, while neritic turtles are caught more by gears like bottom trawls that target benthic fish.

The species displays a homing behaviour (i.e. adults return to natal sites to reproduce), which is stronger in females and results in a varying degree of reproductive isolation among nesting aggregates ("rookeries") that become genetically distinct with time (Bowen *et al*., 2005; Carreras *et al.*, 2007).

This is particularly relevant for the conservation of this species (listed as Endangered in the IUCN Red List of Threatened Species), because it represents an additional factor of vulnerability. In fact, homing determines lack of compensation among rookeries and higher risk of extinction at rookery level due to stochastic phenomena or to anthropogenic threats that affect certain rookeries more than others. Therefore, rookeries should be treated as distinct conservation Management Units (MUs), as their population growth rates depend more on natality/mortality than on immigration/emigration (Allendorf and Luikart, 2007).

Turtles from different rookeries usually share common marine areas and these areas may be affected by different threats. Therefore, for conservation purposes it is important to assess the contribution of the different rookeries to the different marine areas, and in this way understand the vulnerability of rookeries to the specific threats occurring there.

Since different rookeries are often characterized by different mtDNA haplotype frequencies, which is a consequence of female homing, Mixed Stock Analyses (MSA) can be used to estimate differential rookery contribution to a specific turtle aggregation in a marine area (e.g. Carreras *et al*., 2006).

In the Mediterranean Sea, the major loggerhead turtle rookeries are found in Greece, Turkey, Cyprus and Libya (Margaritoulis *et al*., 2003). Genetic studies have revealed the population structure in terms of MUs (Carreras *et al*., 2007), although this knowledge is still incomplete due to the limited sampling coverage and the low mtDNA haplotype differentiation among rookeries.

At sea, loggerhead turtles occur all over the basin, and have important oceanic foraging areas at least in the western Mediterranean (from the Strait of Gibraltar to the Balearic Islands) and in the area from the Sicily Strait to the south Adriatic, while the most important neritic foraging areas are found in the wide continental shelves of the eastern Mediterranean (Tunisia and Libya; north Adriatic; Egypt; southeast Turkey) (Margaritoulis *et al*., 2003).

While contributions from several Mediterranean and Atlantic rookeries were found in open sea areas in the western and central Mediterranean (Laurent *et al*., 1998; Carreras *et al*., 2006), a lower number of contributing rookeries (though the study was based on a small sample size) was detected in two eastern Mediterranean neritic areas: Egypt and Tunisia (Laurent *et al*., 1998). In particular, Laurent *et al*. (1998) suggested that distant rookeries make a low contribution to neritic areas, with no contribution of Atlantic turtles to Mediterranean neritic areas and only a Greek contribution to the Tunisian continental shelf (Laurent *et al*., 1998). However, Casale *et al*. (2007a) suggested that the distance from the oceanic foraging area is more important than the distance from the rookery to determine the possibility of a turtle frequenting a neritic area in the Mediterranean.

Since turtles from distant rookeries, such as the Atlantic and Turkish ones, were found in oceanic areas in the western and central Mediterranean (Laurent *et al*., 1998; Carreras *et al*., 2006), the present study aims to use a larger sample size to verify the rookeries that contribute to the neritic area off Tunisia, and in particular the contribution of rookeries other than Greece, not found so far (Laurent *et al*., 1998). This information is particularly necessary due to the high number of turtles captured by Tunisian and Italian trawlers fishing in this area (possibly even 10000 per year) (Casale *et al*., 2007b; Jribi *et al*., 2007).

FIG. 1. – The Mediterranean Sea. The circle shows the African continental shelf off Tunisia and Libya (study area). The 200-m isobath is shown. Vertical and horizontal lines show known oceanic and neritic foraging grounds for loggerhead turtles respectively.

MATERIALS AND METHODS

Sample collection

Skin samples were collected for genetic analyses from 70 turtles and stored in 95% ethanol. Turtles were incidentally captured in the period 2005-2006 by trawlers fishing in the north African continental shelf between Tunisia and Italy (Fig. 1) and landed at Lampedusa harbour.

Turtles ranged from 36.5 to 85 cm CCLn-t (mean: 58.8; n=70) (Fig. 2) and can be assumed to be mostly juveniles with possibly some adults, since on average Mediterranean loggerhead turtles mature at a size larger than 70 cm CCL (Margaritoulis *et al*., 2003; Casale *et al*., 2005) and in the Atlantic at an even larger size (ca. 100 cm CCL; Dodd, 1988).

Since trawl nets capture organisms close to the sea bottom, these turtles were assumed to be in the neritic stage or in transition between the oceanic and neritic stages (as defined by Bolten, 2003). Before release, turtles were measured (curved carapace length notch-to-tip; CCLn-t; Bolten, 1999) and double tagged on the front flippers with inconel tags, style 681 (National Band and Tag, Newport, KY, USA) to avoid sample duplication in the case of recapture.

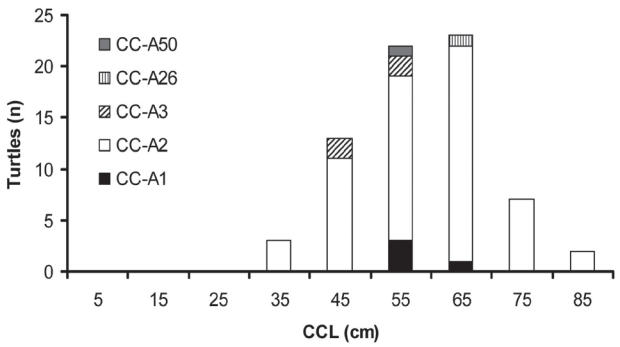

FIG. 2. – Size frequency distribution of turtles with different haplotypes (n=70). CCL: Curved Carapace Length.

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted following a standard proteinase K/SDS digestion and phenol/chloroform/ethanol extraction/precipitation protocol (Hillis *et al*., 1990; Sambrook and Russel, 2000) with a slight modification as previously described for molluscs by Oliverio and Mariottini (2001). DNA from difficult samples was extracted with the QIAGEN QiAmp Extraction Kit, according to manufacturer's instructions.

Partial sequences (ca. 520 bp) of the D-Loop of the mitochondrial control region were PCR amplified, with the primers L71+ (5'-TGCCCAACA-GAATAATATCCATAAT-3') and H599– (5'-TGCACGGCCAATCATTTTGAACGTAG-3') (Laurent *et al*., 1998). Amplification conditions after an initial 5' denaturation were as follows: 94°C for 40 seconds, 55°C for 40 seconds, 72°C for 60 seconds (35 cycles); followed by 7 min of final extension.

The PCR products were purified using the Exo-Sap enzymatic method (USB Corporation, Cleveland, OH, USA), double strand sequenced with the BigDye v 2.0 kit (Applied Biosystems, Foster City, CA, USA) using the PCR primers and sequences determined with an automatic sequencer. Sequencing was performed by Macrogen Inc. (Seoul, Korea). Chromatograms were analyzed by Staden Package (Version-1.6.0, Staden *et al*., 1998, 2005).

Data analysis

Sequences obtained were aligned using ClustalX (Thompson *et al*., 1997) with the default settings. The alignments obtained did not need manual editing. All sequences were compared with the 47 haplotypes found for the species in December 2007 (haplotype sequences and codes are available at the Archie Carr Center for Sea Turtle Research web site, University of Florida, USA; http://accstr.ufl.edu/ccmtdna.html) (Encalada *et al*., 1998; Carreras *et al*., 2007). Haplotype diversity (Hd) and nucleotide diversity (π) were calculated using the program DNAsp (Rozas *et al.,* 2003).

The contribution of different nesting sites (rookeries) to the turtle sample was estimated through a Mixed Stock Analysis (MSA) based on haplotype frequencies of this sample and those available for rookeries in the Atlantic and Mediterranean (Table 1).

TABLE 1. – Haplotype frequency and average number of nests laid annually in Atlantic and Mediterranean rookeries. P: Peloponnesus; Z: Zakynthos. *Mediterranean rookeries included in the MSA (see text). Sources: a, Ehrhart *et al*., 2003; b, Margaritoulis *et al*., 2003; c, Cross *et al*., 2006; d, Mingozzi *et al*., 2007; e, Laurent *et al*., 1999; f, Bowen *et al*., 2004; g, Encalada *et al*., 1998; h, Carreras *et al*, 2007; i, Laurent *et al*., 1998; j, Kaska, 2000.

Rookery	Nest/yr	Source	N	CC-A1	CC-A2	CC-A3	CC-A4	CC-A5	CC-A6	CC-A7	CC-A8	CC-A9	CC-A10	CC-A11	CC-A14	CC-A20	CC-A29	CC-A32	Source
Atlantic																			
NWFL	600	a	49	38	7	2				2									f
SFL	67100	a	109	52	45	4		1		3				1	2	1			f
NEFL-NC	6200	a	105	104	1														f
Dry Tortugas	217	a	58	4	50							2	2						f
Mexico	1800	a	20		11	2					1	1	5						f
Brasil	2400	a	11				11												f
Mediterranean																			
Greece - Kyparissia Bay (P)	581	b	21		19				2										g
Greece - Lakonikos Bay (P)	192	b	19		18				1										h
Greece - Zakynthos (Z)	1301	b	20		17				2									1	h
Greece - total P-Z*	2074		60		54				5									1	
Greece - Crete - Rethymno	502	b	19		19														h
Turkey*	1366	b	32		19	13													i
Cyprus*	572	b	35		35														i
Greece - Zakynthos			10		9								1						
Turkey - Fethiye	124	b	16		15	1													h
Cyprus			10		10														h
Cyprus			10		10														j
Lebanon	60	c	9		9														h
Israel*	33	b	20		17												3		h
Israel	33	b	6		6														i
Italy - Lampedusa	2	d	2		2														i
Libya	>1000	e	7		7														i

Since the traditional Maximum Likelihood approach can be biased by rare haplotypes, which often happens in turtle studies, we preferred to use a Bayesian approach (implemented by the program BAYES) as it is less affected by such factors (Pella and Masuda, 2001). Moreover, this approach allows population size to be considered, which minimizes potential biases due to haplotype frequencies from small populations.

For the Atlantic, rookeries and relative genetic data were obtained from Bowen *et al*. (2004), while population size data (number of nests) for each rookery were obtained from Ehrhart *et al*. (2003). For the Mediterranean, rookeries and respective genetic data were obtained from Encalada *et al*. (1998), Laurent *et al*. (1998) and Carreras *et al*. (2007), while population size data (number of nests) for each rookery were obtained from Margaritoulis *et al*. (2003). Only the four independent units identified by Carreras *et al.* (2007) were considered in the MSA: (i) Peloponnesus and Zakynthos island, Greece (P-Z); (ii) Turkey; (iii) Israel; (iv) Cyprus.Only the largest dataset available for each rookery was considered to avoid possible duplication due to different sampling campaigns at Mediterranean nesting sites. Since genetic data from Turkey collected by Laurent *et al*. (1998), were from several nesting sites along the coast (mapped in Schroth *et al.,* 1996) (L. Laurent, pers. comm.), they were considered as representative of Turkey as a whole. Genetic data from Cyprus were also considered as representative of Cyprus as a whole.

In BAYES, prior parameters were set as different contributions from rookeries in relation to population size (average nests per year). One chain per baseline stock (n=10) was run (50000 iterations), setting most contributions by that stock.

In addition to the present study sample, MSA was also performed for two other samples reported by Laurent et al. (1998) from the same area (Tunisia) and another one (Egypt), with the new baseline (rookery) data and Bayesian approach.

Statistical comparisons of haplotype frequencies of different datasets were performed without grouping haplotypes with low frequencies, with a Chi-square test using the results from Monte-Carlo resampling, as implemented by the programme CHIRXC (Zaykin and Pudovkin, 1993).

RESULTS

The 380 bp alignment of our sequences with all known haplotypes, revealed five haplotypes (Ta-

TABLE 2. – Number of turtles with different haplotypes observed in this study and in another study (Laurent *et al*., 1998) that sampled the same (Tunisia) and another neritic area (Egypt).

Area	CC-A1	CC-A2	CC-A3	CC-A26	CC-A48	CC-A50	N
Study area	4	60	4	1		1	70
Tunisia		33		1			34
Egypt		18	2	2	1		23

TABLE 3. – Bayesian MSA results, with Mean, Standard Deviation (SD), Percentiles and Median of the estimated contributions by Atlantic and Mediterranean rookeries to the study area. Greece P-Z: Peloponnesus-Zakynthos.

Rookery	Mean	SD	2.5%	median	97.5%
NWFL	0.00	0.01	-	-	0.00
SFL	0.13	0.08	0.01	0.13	0.32
NEFL-NC	0.01	0.02	-	-	0.06
Dry Tortugas	0.00	0.08	-	-	0.00
Mexico	0.00	0.01	-	-	0.02
Brasil	0.00	0.00	-	-	0.00
Greece P-Z	0.01	0.03	0.00	0.00	0.05
Israel	0.00	0.00	0.00	0.00	0.00
Turkey	0.07	0.10	0.00	0.01	0.31
Cyprus	0.78	0.11	0.53	0.80	0.95

ble 2). Haplotype diversity (Hd) was 0.262 (SD = 0.068) and nucleotide diversity (π) was 5.4×10^{-3} (SD = 2.2×10^{-3}). Size classes of turtles with these five haplotypes are shown in Figure 2. CC-A1 turtles ranged from 50.3 to 69 cm (mean 58.85), CC-A2 turtles ranged from 36.5 to 85 cm (mean 59.4), CC-A3 turtles ranged from 41.1 to 58.5 cm (mean 50.15), while CC-A26 and CC-A50 were 60.6 and 58.9 cm respectively. No significant size differences among haplotypes were detected (Kruskall-Wallis test; p=0.18; n=70) although the sample size for each haplotype was probably too small to allow a robust test of differences. Haplotype CC-A1 has only been reported from Atlantic rookeries, while CC-A2 and CC-A3 are shared between Atlantic and Mediterranean rookeries, although CC-A2 is found in higher proportions in the Mediterranean, and CC-A3 is found in a higher proportion in Turkey (Table 1). CC-A26 has been previously reported from other Mediterranean foraging grounds (Laurent *et al*., 1998; Carreras *et al*., 2006), but not yet from a nesting site. CC-A50 was previously unknown (Genbank accession number EU352258).

MSA (Table 3) estimated relatively important mean contributions from three rookeries: Cyprus (Mediterranean) (78%), South Florida (Atlantic) (13%), and Turkey (Mediterranean) (7%), and small (1%) contributions from Greece (Mediterranean) and north-east Florida/South Carolina (Atlantic).

TABLE 4. – Bayesian MSA results, with Mean, Standard Deviation (SD), Percentiles and Median of the estimated contributions by Atlantic and Mediterranean rookeries to the sample from Tunisia reported by Laurent *et al.* (1998). Greece P-Z: Peloponnesus-Zakynthos.

Rookery	Mean	SD	2.5%	median	97.5%
NWFL	0.00	0.00	0.00	0.00	0.00
SFL	0.04	0.05	0.00	0.03	0.17
NEFL-NC	0.00	0.01	0.00	0.00	0.02
Dry Tortugas	0.00	0.00	0.00	0.00	0.00
Mexico	0.00	0.01	0.00	0.00	0.02
Brasil	0.00	0.01	0.00	0.00	0.01
Greece	0.16	0.35	0.00	0.00	0.99
Israel	0.00	0.00	0.00	0.00	0.00
Turkey	0.00	0.01	0.00	0.00	0.01
Cyprus	0.79	0.35	0.00	0.95	1.00

The haplotypes reported by Laurent *et al*. (1998) from Tunisia and Egypt are shown in Table 2. Pairwise comparison of haplotype frequencies shows no significant differences between these two groups and the haplotypes in the present study (Chi-square test; Tunisia: p=0.289; Egypt: p=0.155). Haplotypes CC-A26 and CC-A48 are still unknown from nesting sites and so were not considered by BAYES.

Contributions to the Tunisia foraging area were estimated to come mostly from Cyprus and to a lesser extent from Greece (Table 4). The main contributions to the Egyptian foraging area were estimated to come from Turkey and Cyprus, and to a lesser extent from Greece (Table 5). MSA also estimated some contribution from Atlantic nesting sites for both areas, although this was not based on a haplotype that is exclusive to the Atlantic.

TABLE 5. – Bayesian MSA results, with Mean, Standard Deviation (SD), Percentiles and Median of the estimated contributions by Atlantic and Mediterranean rookeries to the sample from Egypt reported by Laurent *et al.* (1998). Greece P-Z: Peloponnesus-Zakynthos.

Rookery	Mean	SD	2.5%	median	97.5%
NWFL	0.00	0.01	0.00	0.00	0.00
SFL	0.11	0.12	0.00	0.07	0.43
NEFL-NC	0.00	0.01	0.00	0.00	0.04
Dry Tortugas	0.02	0.11	0.00	0.00	0.38
Mexico	0.07	0.23	0.00	0.00	0.95
Brasil	0.00	0.01	0.00	0.00	0.02
Greece	0.15	0.28	0.00	0.00	0.89
Israel	0.00	0.00	0.00	0.00	0.00
Turkey	0.42	0.39	0.00	0.30	0.99
Cyprus	0.22	0.35	0.00	0.00	0.96

DISCUSSION

Haplotype frequencies are not yet available for some of the known major nesting sites in the Mediterranean (Margaritoulis *et al*., 2003), while the sam-

ple sizes for other sites is still small (Table 1). For instance, Libya is suspected to host a very important nesting area, which is possibly comparable to those located in Greece and Turkey (Laurent *et al*., 1999), and its coastline (about 1000 km of sandy beaches) might well host different major nesting sites, which may have specific haplotype frequencies. Unfortunately, genetic data from Libyan nesting sites are not yet available (except for a very small sample; Table 1). As evidence of these gaps, haplotypes CC-A26 and CC-A50 have not yet been reported from any nesting sites; the former was found in oceanic-stage turtles in the western and central Mediterranean (Laurent *et al*., 1998; Carreras *et al*., 2006), while the latter has never been reported. These haplotypes might well be from nesting sites that have not been sampled for genetic assessment, such as Libya, or they may occur at low frequencies in sampled nesting sites. Moreover, the nesting sites that have already been sampled show a low variation of haplotype frequencies (Table 1), probably due to relatively recent colonization (less than 12000 years; Bowen *et al*., 1993), possibly by a low number of colonizers, and a low mutation rate. Indeed, pairwise comparison of haplotype frequencies (the most common haplotype, CC-A2, with the others) shows no significant differences among three of the four Mediterranean rookeries considered (Greece P-Z, Cyprus, Israel) (Fisher Exact Test), while Turkey has a higher differentiation due only to haplotype CC-A3. Under such circumstances, the capacity of MSA to provide reliable estimates of the contribution of the different Mediterranean rookeries to turtle aggregations in foraging grounds is limited.

For instance, the contribution of Greece P-Z (Table 3) is probably underestimated. In fact, flipper tagging of a large number of females nesting in Greece revealed that the study area is one of the few important foraging grounds for adult females nesting at Peloponnesus-Zakynthos nesting sites (Margaritoulis *et al*., 2003). This also suggests that the study area is important for juveniles from the same nesting sites, because in other areas adult loggerhead turtles frequent the same neritic areas where they recruited when still juveniles (Limpus, 1994; Limpus and Limpus, 2001). However, the contribution from Cyprus may be overestimated, since only two females nesting in Cyprus which were satellite-tracked (Broderick *et al*., 2007) or tagged with flipper tags (A. Broderick, pers. comm.) have been observed in this area. Therefore, the present MSA results (Table 3) should be considered with caution. Certainly, they indicate important contributions from the few rookeries with specific haplotype frequencies, such as South Florida and Turkey. In particular, the contribution from Turkey is most probably underestimated by MSA; first, because the low haplotype structure (see above) favours rookeries with the predominant haplotype CC-A2 like Cyprus (Table 3), and second, because the Turkish rookeries are closer to the study area than the Atlantic ones.

Results therefore indicate for the first time and in contrast with previous hypotheses (Laurent *et al*., 1998) that the study area is not only an important foraging ground for turtles from Greece, but also for turtles from Turkey, the second largest nesting aggregate in the Mediterranean assessed so far (Margaritoulis *et al*., 2003). They probably settle there when they are juveniles shifting from the oceanic to the neritic stage, and then remain there as adults. If so, the reproductive migration to Turkish nesting sites would expose them to additional risks along the migratory routes (e.g. fishing, pollution).

Another sample from the same area (Tunisian continental shelf) (Table 4) showed a higher contribution from Greece than the present study (Table 3), although the samples did not differ significantly in haplotype frequencies, this may be explained by temporal fluctuations in the individual composition or actually by a higher contribution from Greece (see above). This is a further indication of the limited power of MSA to deal with low haplotype frequency differentiation among rookeries, gaps and rare haplotypes.

The MSA results for the sample from Egypt (Table 5) suggest a higher occurrence of turtles from Turkey in Egyptian waters than in the study area, which indicates a distribution gradient related to different neritic areas.

For the first time, turtles with haplotype CC-A1, known only from Atlantic rookeries (Table 1), were captured by trawlers in the continental shelf between Tunisia and Italy. This finding has important implications that bring with them new biological and conservation questions.

In fact, Atlantic turtles are known to frequent the oceanic areas of the western and central Mediterranean in high numbers (Laurent *et al*, 1998; Carreras *et al*, 2006). Therefore, previous findings of a few CC-A1 turtles in shallow waters adjacent to oceanic foraging areas such as the Tyrrhenian Sea, the Ionian Sea (Maffucci *et al*., 2006) and the Sicily strait (Lau-

rent *et al*., 1998) could be explained by opportunistic behaviour. The lack of turtles with this haplotype among those caught by trawlers on some of the largest Mediterranean continental shelves (Laurent *et al*, 1998), probably due to limited sampling, led to the belief that Atlantic turtles frequent the Mediterranean only while they are in the oceanic stage and then leave the basin and settle in neritic foraging areas in the western Atlantic (e.g. Bowen *et al*., 2004).

The present results demonstrate that Atlantic loggerhead turtles frequent the shallow waters of the large continental shelf in the Mediterranean, which is known to be one of the few important neritic foraging areas for Mediterranean turtles. The four CC-A1 turtles discovered in this study were determined to be in the neritic stage as they were captured by bottom trawlers and because benthic organisms were found in the faeces of one individual that was kept under observation for a period of time (P. Casale, unpubl. data). Moreover, Atlantic turtles enter the Mediterranean at a small size (range: 29.7-65.0 cm CCLn-t; n=34; Laurent *et al*., 1998) but the CC-A1 individuals in the present sample were in the larger part of the range (50.3-69 cm CCLn-t; n=4), which is similar to the range of Atlantic turtles recruiting to the neritic habitats in the western Atlantic (46-64 cm CCLn-t; Bjorndal *et al*., 2000), although the sample size is too small to show any statistically significant differences. This indicates that Atlantic turtles use Mediterranean trophic resources not only in oceanic areas but also in the neritic ones. It is even possible that this is partially induced by the inwards current at the Gibraltar Strait that acts as a barrier for small turtles (Revelles *et al*., 2007). The pattern of ecological shift of Atlantic turtles between the Atlantic and Mediterranean requires further investigation to answer the following questions: For how long do they use Mediterranean neritic grounds? At which size do they leave? Do they show fidelity to these areas? Indeed, Atlantic turtles entering a distant semi-closed basin like the Mediterranean represent a good opportunity for studying the degree of behavioural and ecological flexibility of *Caretta caretta*.

The present results also demonstrate that Atlantic turtles are not only subject to captures by Mediterranean longliners (Laurent *et al*., 1998; Carreras *et al*., 2006) but also by trawlers, and this may represent an additional threat to their populations.

In conclusion, the north African continental shelf in the central Mediterranean appears to be an important neritic foraging ground for at least two Mediterranean rookery aggregations and probably more, which correspond to several MUs, and is also frequented by individuals from Atlantic populations. For this reason it represents a hot spot for sea turtle conservation at regional and interregional levels. Of particular concern is the high fishing effort of Italian and Tunisian trawlers, with a rough overall estimation of 10000 turtles captured per year (Casale *et al*., 2007b; Jribi *et al*., 2007), which probably affects several Mediterranean populations as well as some Atlantic ones.

Conservation actions at national and international levels in Tunisia, Italy and Libya, are necessary and urgent. A higher chance of success can probably be achieved if these actions are included in a wider Ecosystem Based Management approach for the conservation of the still high biodiversity of this marine area through international cooperation and agreements.

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