Molecular Ecology (1998) **7**, 1529–1542

Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean

L. LAURENT,*†‡ P. CASALE,§ M. N. BRADAI,¶ B. J. GODLEY,** G. GEROSA§ and A. C. BRODERICK,** W. SCHROTH,†† B. SCHIERWATER,†† A. M. LEVY,‡‡ D. FREGGI,§§ E. M. ABD EL-MAWLA,¶¶ D. A. HADOUD,*** H. E. GOMATI,*** M. DOMINGO,††† M. HADJICHRISTOPHOROU,‡‡‡ L. KORNARAKY,§§§ F. DEMIRAYAK¶¶¶ and CH. GAUTIER‡

*Bio-Interface, 27 bd du 11 novembre 1918, BP 2132, 69603 Villeurbanne Cedex, France, †WWF International Mediterranean Programme, 57 via Garigliano, 00198 Rome, Italy, ‡UMR 5558 Biometrie, Génétique et Biologie des populations, Université Lyon I, 43, Bd du 11 novembre 1918, 69622 Villeurbanne, France, §Chelon, Marine Turtle Conservation and Research Program, Tethys Research Institute, Viale Val Padana 134/B, 00141 Roma, Italy, ¶Institut National des Sciences et Technologies de la Mer, Centre de Sfax, B.P. 1035, Sfax 3018, Tunisia, **Marine Turtle Research Group, Division of Environmental and Evolutionary Biology, Graham Kerr Building, University of Glasgow, Glasgow, G12 8QQ, UK, ††Abteilung Ökologie & Evolution, J.W. Goethe-Universität, 60054 Frankfurt, Germany, ‡‡Veterinary Surgeon, 16a Yaacov Cohen Street, Ramat-Hasharon, Israel, §§Dipartemento di Biologia Animale e dell'Uomo, Università di Roma 'La Sapienza', Via dell' Università, 32, 00185 Roma, Italy, ¶¶National Institute of Oceanography and Fisheries, Anfoushi, Qaied Bay, Alexandria, Egypt, ***Marine Biology Research Centre of Tajura, P.O. Box 30830 Tajura, Libya, †††Histologia y Anatomia Ptologica, Universitad Autonoma de Barcelona, Bellatera 08913, Spain, ‡‡‡Ministry of Agriculture and Natural Resources, Department of Fisheries, Nicosia, Cyprus, §§§Sea Turtle Protection Society of Greece, 35 Solomou Street, 10682 Athens, Greece, ¶¶¶Dogal Hayati Koruma Dernegi, P.K. 18, Bebek 80810 Istanbul, Turkey*

Abstract

Based on an extensive sampling regime from both nesting populations and bycatch, frequency analyses of mitochondrial (mt) DNA control region haplotypes in the Mediterranean were used to assess the genetic structure and stock composition of the loggerhead sea turtle, *Caretta caretta*, in different marine fisheries. The analyses show the following. (i) In drifting longline fisheries working in Mediterranean pelagic habitats 53–55% of turtles caught originated from the Mediterranean stock; (ii) In bottom-trawl fisheries all turtle bycatch is derived from this regional stock; (iii) This regional stock contribution to fishery bycatch suggests that the population size of the Mediterranean loggerhead nesting population is significantly larger than previously thought. This is consistent with a recent holistic estimate based on the discovery of a large rookery in Libya. (iv) Present impact of fishery-related mortality on the Mediterranean nesting population is probably incompatible with its long-term conservation. Sea turtle conservation regulations are urgently needed for the Mediterranean fisheries. (v) The significant divergence of mtDNA haplotype frequencies of the Turkish loggerhead colonies define this nesting population as a particularly important management unit. Large immature and adult stages from this management unit seem to be harvested predominantly by Egyptian fisheries. (vi) Combined with other data, our findings suggest that all the nesting populations in the Mediterranean should be considered as management units sharing immature pelagic habitats throughout the Mediterranean (and possibly the eastern Atlantic), with distinct and more localized benthic feeding habitats in the eastern basin used by large immatures and adults. (vii) Between the strict oceanic pelagic and the benthic stages, immature turtles appear to live through an intermediate neritic stage, in which they switch between pelagic and benthic foods.

Keywords: conservation genetics, DNA markers, loggerhead turtle *Caretta caretta*, mitochondrial DNA control region, population dynamics, population structure

Received 17 December 1997; revision received 19 March 1998; accepted 3 June 1998

Correspondence: L. Laurent. *Correspondence address. Fax: +33 4 78 93 87 80; E-mail: bioinsight@asi.fr

Introduction

Mark and recapture studies (i.e. tag returns) of marine turtles have revealed links between feeding grounds and nesting areas, which are often separated by great distances (Carr 1975; Pritchard 1976; Carr *et al*. 1978; Limpus *et al*. 1992, 1994a,b), and have highlighted the international nature of marine turtle conservation issues (Limpus *et al*. 1992). However, due to generally small sample sizes, mark and recapture data have been ill-suited to the quantification of links between nesting populations and fishery areas. Tagging programs cannot be carried out at all nesting locations nor is it possible to mark all individuals within a population. In addition, chronic tag loss further confounds such studies (Limpus 1992). This prevents an understanding of the impacts that fishery-related mortality has on population dynamics of the species, making the formulation of sound conservation and management strategies difficult.

Regardless of the different scenarios suggested for the future development of the Mediterranean countries (i.e. Blue Plan; Grenon & Batisse 1988), pressure on all marine turtle life-history stages will probably increase in this region. In addition to coastal urbanization, tourism and pollution, human-induced threats include incidental capture in marine fisheries. Fishery activity has become an increasing threat which is the most important mortality factor known in this region. Bottom trawl, longline, driftnet, and small coastal fisheries have a large bycatch of marine turtles causing substantial mortality (see Laurent *et al*. (1996) for synthesis). For example, in the western Mediterranean, Spanish longline fisheries interact with loggerhead turtles, *Caretta caretta*, (Caminas 1988), with an estimated annual capture rate of greater than 20 000 in 1991 and 1992, and an estimated mortality rate of 20% (Aguilar *et al*. 1995). For conservation management it would be highly desirable to have molecular markers at hand that allow the identification of incidentally caught sea turtles with respect to their affiliation to a specific nesting site population.

Studies of mitochondrial DNA genealogies by random RFLP and PCR analyses have revealed breeding population-specific polymorphisms in marine turtles (Bowen *et al*. 1989, 1992, 1993, 1994; Laurent *et al*. 1993; Allard *et al*. 1994; Broderick *et al*. 1994; Norman *et al*. 1994; Bass *et al*. 1996; Encalada *et al*. 1996; Schroth *et al*. 1996). By analysing mtDNA haplotype frequencies of marine turtle samples collected both in nesting and marine areas, specific polymorphisms have permitted the identification of the stock affiliation of turtles caught at sea (Laurent *et al*. 1993; Bowen *et al*. 1995, 1996; Sears *et al*. 1995). When populations can be identified by genotype frequency differences, quantitative population contributions can be estimated by maximum likelihood methods (Pella & Milner 1987). However, to be reliable, such an approach fundamentally requires a large mixture and widespread baseline samples, as well as large differences in genotype frequencies among potential candidate rookeries (Pella & Milner 1987; Xu *et al*. 1994). Obtaining large numbers of samples from widespread locations is particularly labour intensive in marine turtle studies.

Preliminary DNA studies have shown that loggerhead rookeries sampled in the Mediterranean, i.e. in Greece and Cyprus, share common haplotypes with those of the western Atlantic, but differ in haplotype frequencies (Bowen *et al*. 1993; Laurent *et al*. 1993). On the basis of such findings, analysis of the frequencies of cytochrome *b* haplotypes in immature samples from the western Mediterranean indicated that numerous individuals caught in this fishery area originated from the USA and elsewhere in the Atlantic (Laurent *et al*. 1993). This is the first evidence for the suggested transatlantic developmental loggerhead migration (Carr 1987). The molecular findings also confirmed an earlier speculation that entries of Atlantic loggerheads into the Mediterranean may be common (Argano & Baldari 1983; Carr 1987; Laurent 1990a; Bolten *et al*. 1992). However, no large nesting areas have yet been sampled for mtDNA haplotype analyses. This has prevented estimation of overall mtDNA haplotype frequencies in the Mediterranean, and therefore excluded an assessment of stock composition of marine turtle bycatch in Mediterranean fisheries.

In this study, we characterize the first complete sequence of the mtDNA control region of a reptile, the loggerhead sea turtle, and use frequency analyses of mtDNA control region haplotypes to assess population genetic structure and stock composition in different fisheries. We seek to correlate molecular stock identification with fishery interaction and the size and reproductive status of individuals. This integrated approach will be crucial for understanding population dynamics of loggerheads, so contributing to our limited knowledge of their life history, and the assessment of the impacts of present fishing activities on marine turtle populations in the Mediterranean.

Materials and methods

Sample collection

Six nesting areas were sampled, one being a combination of three small groups of nesting beaches samples (Table 1 and Fig. 1). Drifting longline fisheries provided samples during summer from the western and eastern Mediterranean; oceanographic basins which are separated by the channel of Sicily (Fig. 1). Bottom-trawl fisheries working during winter, spring and autumn provided samples from Tunisia, Egypt and the Gulf of

Table 1 Haplotype distribution in nesting and marine area samples. A and C are mtDNA cytochrome *b* haplotypes

	Haplotype													
	A	A1	A2	A3	A4	A5	A6	A7	A8	C	C1	C2	C3	*N*
Nesting areas														
USA Georgia/Carolina										23				23
USA Florida		16		1						9				26
Greece		9	1											10
Cyprus		35												35
Turkey		19		13										32
Southeastern basin														15
Libya		7												
Israel		6												
Italy (Lampedusa)		2												
Fishery areas														
Drifting longlines fisheries														
Western Mediterranean	46									13				59
Eastern Mediterranean		32		3	1	2		1	1		10	2		52
Bottom trawl fisheries														
Tunisia		33				1								34
Egypt		18		2		2	1							23
Turkey		1												1
Small coastal fisheries														
Italy (Lampedusa)		13		1							3			17
Undetermined fishing gear or stranded individuals														
Italy (Lampedusa)		2									4		1	7
Italy (Adriatic Sea)		8				1								9
Italy (Tyrrhenian Sea)		2									1			3
Cyprus		2												2

Iskenderun, Turkey. Samples from small coastal fisheries were obtained from bottom-trawl, bottom-longline and trammel net fisheries working in summer around Lampedusa Island, Italy. Additional samples were obtained from individuals stranded or caught in undetermined fishing gear (Table 1). DNA samples were derived either from eggs or dead hatchlings found in nests after the completion of hatching, or from blood or muscle tissue. Blood sampling was undertaken according to the protocol of Owens & Ruiz (1980). Blood was preserved at a low temperature by a variety of methods and subsequently stored at – 70 °C. Muscle samples were either preserved in 70–90% alcohol or frozen. The size of individuals was measured according to the Standard Curved Carapace Length method (Pritchard *et al*. 1983). Sex and reproductive status were recorded when possible according to the following procedure. Males were determined by external morphology. Large individuals of ≥ 70 cm, the minimum nesting female size in Greece (Margaritoulis 1982), with tail length from carapace tip to tail tip greater than 15 cm were assumed to be males. When tail length was greater than 20 cm the individual was assumed to be an adult male. Adult females were only determined when internal examination of mature follicles was possible, e.g. when slaughtered turtles were observed.

DNA extraction, PCR amplification and sequencing

Total DNA was extracted from blood by the rapid procedure of Fitzsimmons *et al*. (1995) and from other tissue samples by standard phenol–chloroform protocol. American, Greek and Cyprus nesting samples as well as a fishery sample from the western basin were previously analysed by sequencing a 420 bp fragment encompassing the 3' end of the $tRNA^{Glu}$ gene and the 5' part of the cytochrome *b* gene, using *Hpa*II–RFLP (Laurent *et al*. 1993). This analysis distinguished two divergent maternal lineages A and C. Lineage A haplotype was shared by both American and Mediterranean nesting populations, while lineage C was only found in nesting rookeries of the USA. We then analysed control region polymorphisms of the following samples: Mediterranean nesting areas, fishery samples of the eastern basin, and American nesting individuals with hap-

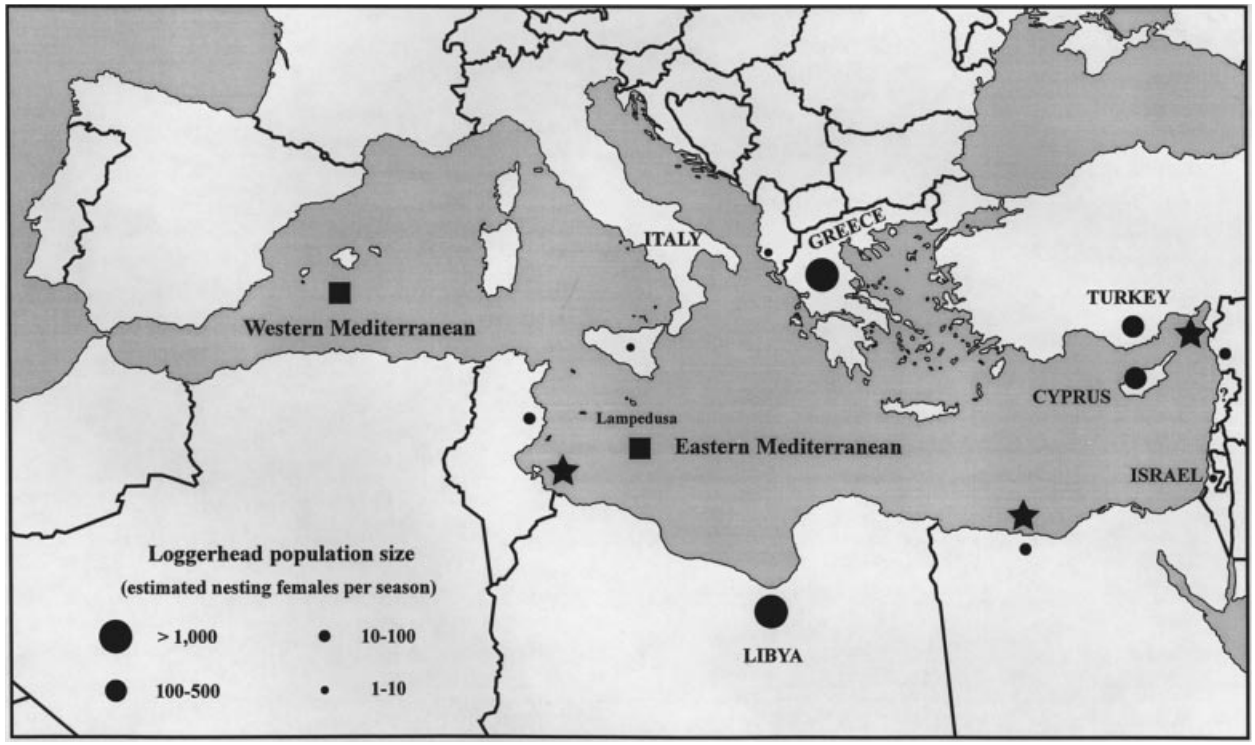


Fig. 1 Loggerhead turtle sample locations of nesting and fishery areas in the Mediterranean. The southeastern basin nesting area is a combination of small nesting beaches samples from Israel, Libya and Italy (Lampedusa Island) (Table 1). Estimates of loggerhead population sizes (nesting females per year) are compiled from a bibliography. Locations of drifting longline fishery bycatch are represented by solid squares, whereas bottom-trawl fishery bycatch are represented by solid stars.

lotype A, as the primary aims of our study were to differentiate Mediterranean from Atlantic stocks and to estimate population structure within the Mediterranean. PCR amplification of the 5' part (L strand) of the control region was obtained with primer L15926 (Kocher *et al*. 1989) and the HDCM1 primer (Allard *et al*. 1994). A H1074 primer was designed from sequence alignment within the annealing zone of primer L1091 (Kocher *et al*. 1989) in the 12S rRNA gene to amplify the 3' part, producing single-sized products with interindividual length polymorphism. Finally, primers HCD8 and LCD9 were designed for the investigation of a repetitive region, and L71 and H599 were designed to amplify variable 520–526 bp fragments (Fig. 2). The 5' to 3' sequences of the primers were as follows: HCD8, 5'-GCATCTGCAGTGCCGTGCTTTGTGATA-3'; LCD9, 5'-CACCTCGAGTACACCCAACTAACCAAAC-3'; L71, 5'-TGCCCAACAGAATAATATCCATAAT-3'; H599, 5'-TGCACGGCCAATCATTTTGAACGTAG-3'.

PCR amplifications were performed in a 50 μL reaction volume containing 2 units of *Taq* polymerase, 1× PCR Buffer II (Perkin Elmer, Norwalk), 2.5 mM $MgCl_2$, 0.2 mM of dNTP, 0.2 μM each primersand 10–500 ng of genomic DNA. For each pair of primers an optimal PCR temperature profile was determined. For one typical example, primers HCD8 and LCD9, the amplification conditions were as follows: 30 cycles of 94 °C for 1 min, 65 °C for 1 min and extension at 72 °C for 1 min; a final extension reaction of 10 min at 72 °C was always added. Control reactions contained no template DNA. PCR product sizes were visualized by agarose gel electrophoresis and ethidium bromide staining; molecular weights were estimated by comparison to the Φ-x 174 marker.

For sequencing, double-stranded PCR products were purified by centrifugation with a QIAquick protocol (QIAGEN). Templates were cycle sequenced with each of the PCR primers using the Perkin Elmer GeneAmp PCR system 9600 and FS Dye Terminator ready mix reaction kit. Excess dye-labelled ddNTPs were removed from cycle sequencing reactions using Quick spin G50 columns (Boehringer Mannheim). The purified labelled extension products were analysed using Applied Biosystems automated DNA sequencers. Each sample was sequenced in both directions to eliminate ambiguous positions, leading to final sequences of 452 or 458 bp in length (Fig. 2). To assure accuracy, new haplotypes were amplified and sequenced twice.

Data analysis

Multiple sequence alignments were accomplished both by eye and using the Sea View editor (Galtier *et al*. 1996).

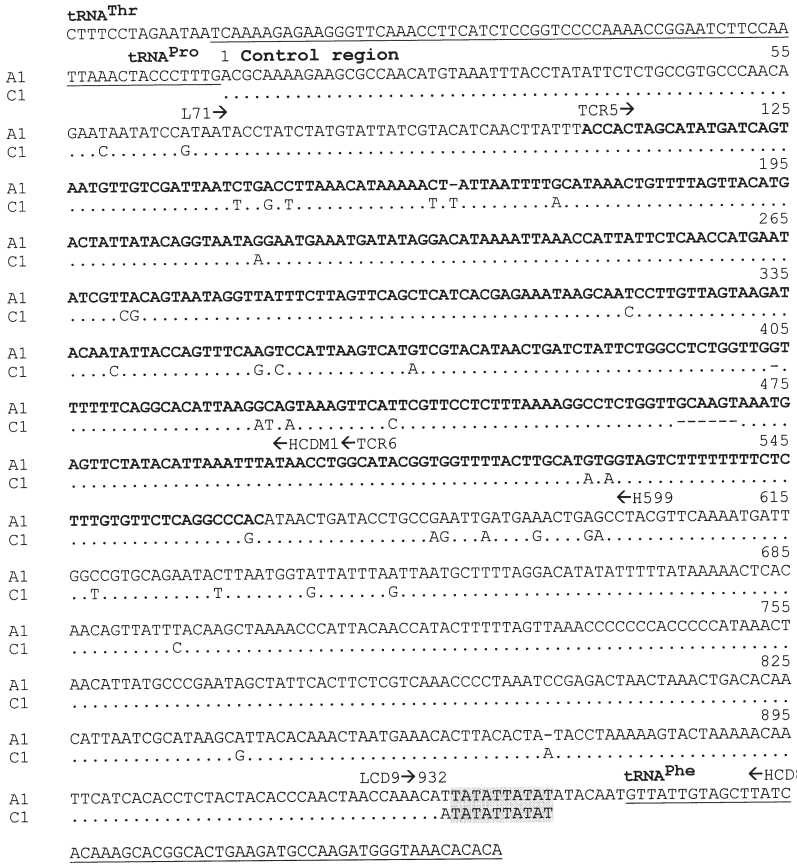


Fig. 2 Sequence of the loggerhead *Caretta caretta* control region and its flanking tRNA genes. The sequence shown is the light strand. A1 is a sequence haplotype of maternal lineage A, whereas C1 is a sequence haplotype of maternal lineage C. Sequences analysed with PCR primers L71/H599 are in bold. Dots below the top sequence indicate the same nucleotide, hyphens indicate insertions/deletions, and TATATTATAT represents the repetitive region shown with two arbitrary repeats. Primer-binding sites are shown above the sequence (see text for sequences). HCDM1 is from Allard *et al*. (1994); TCR5 and TCR6 are from Norman *et al*. (1994). GenBank Accession no. of sequence haplotypes A1 and C1 are L35254 and L35255. L35255 has an error, G in position 173 must be A (see Table 2).

Haplotype frequencies among nesting areas were compared by means of Fisher's exact test. Coefficients of differentiation Φ_{ST} (*F*-statistic analogue) were calculated using the program AMOVA (Excoffier *et al*. 1992). Molecular variance components and Φ-statistics were estimated with haplotype frequencies alone, i.e. haplotypes were considered as equidistant, and tested by a nonparametric permutational procedure under the null distribution of the variance components (and Φ–values) from 9999 random permutations of the original squared distance matrix. Pairwise Φ_{ST} enabled us to estimate the average gene flow per generation between nesting populations (*Nm*), which is the product of the effective population size *N* and the migration rate *m*, using the equilibrium relationship for haploid data: $F_{ST} = 1/(2Nm + 1)$.

Assessment of loggerhead stock composition in fishery bycatch was carried out on the basis of haplotype frequency differences between baseline (nesting areas) and mixture samples (fishery areas) by using maximum likelihood (ML) analysis (Pella & Milner 1987; Masuda *et al*. 1991; Xu *et al*. 1994). As the size of the baseline samples was relatively small with regard to mixture samples, we preferred using an unconditional approach with the program UCON (Masuda *et al*. 1991), which permits the baseline haplotype relative frequencies with the mixture samples to be adjusted (the conditional approach does not allow this adjustment). Precision of stock composition estimates for sampling error was determined by two procedures provided by UCON (Masuda *et al*. 1991). Standard deviations were calculated by the 'jacknife' option which only accounted for sampling error from the mixture, whereas 95% confidence limits were obtained by bootstrap resampling (500 times) of both baseline and mixture samples with replacement to generate a null random distribution of computed stock composition. Three stock composition analyses were performed. (i) USA vs. Mediterranean stock, by assuming that the Atlantic stock is only composed of Florida and Georgia/Carolina populations, which represent the largest regional nesting areas in the Atlantic by far, and are the closest to the Mediterranean. For this analysis we used a recent mtDNA survey of the American populations (SW/SE Florida and NEFL/NC) which is based on a larger sample size (Encalada *et al*. 1998). ML analysis was performed by analysing frequencies of haplotypes from the two distinct maternal lineages A and C. (ii) Atlantic vs. Mediterranean stock; individuals from pelagic habitats around the Azores, which were recently surveyed for mtDNA haplotype frequencies by Bolten *et al*. (1998), were used as a baseline sample for the Atlantic stock. This sample is

assumed to be representative for the pelagic immature developmental movement of all western Atlantic rookeries. The location is in the eastern part of the ocean current gyre systems, and is not influenced by the Canary current which may receive individuals endemic to Mediterranean swimming out of the Mediterranean. ML analysis was also performed with maternal lineage frequencies. (iii) Within-Mediterranean stocks by analysing control region haplotype frequencies; individuals in mixture samples with haplotypes not found in nesting areas were removed from the analysis.

Results

General organization and variability of the loggerhead control region

Figure 2 presents the first complete mtDNA control region sequence of a reptile. The loggerhead control region varies in length from ≈ 1230 to ≈ 1630 bp, and contains the main conserved domains; these are the three CSBs and the TAS putative sequences, and a repetitive region with a microsatellite TATAT element at the 3' end (Fig. 2). Unlike other vertebrates studied to date, loggerheads have an AT repetitive sequence.

Length of the repetitive region in 42 individuals ranged from 300 to 700 bp, with heteroplasmy in three cases, but this polymorphism did not enable nesting populations to be distinguished. However, these markers probably allow individual mtDNA fingerprint and population structure analysis at a local level, such as between adjacent nesting beaches. Similar uses of mtDNA control region variability have already been noted in other vertebrate species (Wilkinson & Chapman 1991; Wenink *et al*. 1994).

Sequence variation analysis distinguished the two divergent maternal lineages A and C previously revealed by cytochrome *b* sequence analysis (Laurent *et al*. 1993), and by random RFLP analysis (Bowen *et al*. 1993). The control region shows no short hypervariable sequences in the right domain near the repeated sequences, as is the case, for example, in shrews (Fumagalli 1995). Within-lineage variation was only detected in the middle third part of the control region (Fig. 2). Finally, this portion, which matches that of previous studies on marine turtles (Allard *et al*. 1994; Norman *et al*. 1994; Bowen *et al*. 1995; Bass *et al*. 1996; Encalada *et al*. 1996; Schroth *et al*. 1996), was chosen to generate sequences of 452 and 458 bp in length (Fig. 2), which are slightly longer than those in previous studies. Among 259 individuals, 35 polymorphic sites were observed defining 11 haplotypes (Table 2). Differences between haplotypes were either transitions (43 nucleotide sites) or deletions/insertions (nine sites). A collection of 33 individuals was surveyed for both cytochrome *b* and control region sequence polymorphism. Three cytochrome *b* haplotypes were found by examining a 420 bp fragment, while five were found for the control region (849 nucleotides screened). Both regions provided the same number of haplotypes per number of nucleotides. Within lineages (more closely related sequences), the observed divergence in the cytochrome *b*

Table 2 Polymorphic sites observed among 259 individuals at 459 positions. Haplotype A1 is the reference (– is deletion/insertion). Sequence numbering begins at the 5' end of the control region (Fig. 2)

Position	1	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5
	4	4	4	6	6	7	8	0	1	2	7	7	9	2	4	5	5	6	6	0	2	2	2	2	3	6	6	6	6	7	0	2	2	6
	2	5	7	1	3	3	8	6	4	9	1	2	8	0	0	4	6	5	9	4	2	4	5	7	7	5	6	7	8	9	0	0	6	8	3
Haplotype																																		
A1	C	A	C	C	-	G	G	A	G	A	T	A	G	T	T	A	T	T	G	G	A	G	C	G	T	G	C	A	A	G	T	C	G	G	A
A2																													G					
A3													A																					
A4				T				G																										
A5											G																							
A6									G						C																			
A7																				G										T				
A8							A																											
C1	T	G	T	T	T	A		A		C	G		C	C	G	C		A	-		A	T	A	C	-	-	-	-	-	-		A	A	G
C2	T	G	T	T	T	A		A		C	G		C	C	G	C		A	-		A	T	A	C	-	-	-	-	-	-	T	A	A	G
C3	T	G	T	T	T	A	A	A		C			C	C		C		A	-		A	T	A	C	-	-	-	-	-	-	T	A	A	G

gene ranged from 0 to 0.24% and was similar to that found in the control region. Among lineages, the observed divergence was between 0.95 and 1.19% for the cytochrome *b* gene vs. 4.61% for the control region. This indicates that the control region evolves about four times faster than the cytochrome *b* gene.

Population structure

An analysis of molecular variance based on variation partitioning into an American group (Georgia/Carolina and Florida samples), and a Mediterranean group (containing all other samples) (Table 1), attributed 41% (Φ_{CT} = 0.412; $P < 0.0001$) of the total genetic variation to among groups. This analysis shows a significant and substantial divergence between the American and the Mediterranean regional nesting areas, indicating two different stocks of loggerheads. Within the Mediterranean, 33% (Φ_{ST} = 0.331; $P < 0.0001$) of the total variation was accounted for by differences among nesting area samples, indicating a considerable population structuring. However, this is mainly a result of the Turkish area, which holds a nesting population genetically distinct from the others (Table 3). Indeed, the nesting areas of Greece, Cyprus and the southeastern basin were genetically indistinguishable (Table 3), and were combined in a subsequent analysis. Estimates of interpopulation matriarchal gene flow *Nm*, i.e. number of reproductively successful migrant individuals per generation, ranged from 0 to 2.3 between American and Mediterranean populations, whereas estimates among Mediterranean populations ranged from 0.75 to 2.02 (Table 3).

Stock composition

In the western and eastern Mediterranean drifting longline bycatch, the proportion of individuals with lineage C haplotypes (endemic to west Atlantic nesting colonies) was 22 and 23%, respectively. This is in marked contrast with Tunisian, Egyptian and Turkish bottom-trawl fishery samples where these haplotypes were not observed (Table 1). In the western basin contribution of Mediterranean stock to drifting longline fisheries was 53%, whereas contributions of the American populations were estimated at 45% from Florida and 2% from the Georgia/Carolina assemblage. In the eastern basin, Mediterranean stock was estimated at 51%, and 47% from Florida and 2% from the Georgia/Carolina assemblage (Table 4). In the bottom-trawl bycatch from Tunisia and Egypt, Mediterranean stock contribution was estimated at 100% (Table 4). In the second ML analysis the Mediterranean stock was compared with the whole Atlantic stock and its contribution in drifting longline fisheries was estimated at 55% in the western basin and 53% (± 12 SD) in the eastern basin. Contribution to bottom-trawl fisheries was 100% (Table 4). Based on these findings, we assumed that only Mediterranean populations contribute to bycatch from bottom-trawl fisheries. Comparisons of haplotype frequencies between Tunisian and Egyptian bottom-trawl fisheries samples showed a

Table 3 Pairwise haplotype frequency comparisons among nesting areas. Above the diagonal, Fisher's exact test and Φ_{ST} with significance; below the diagonal, *Nm*

Carolina	Georgia/Carolina	Florida	Greece	Cyprus	Turkey	Southeastern basin
Georgia/Carolina	–	$P < 0.0001$ 0.587 $P < 0.0001$	$P < 0.0001$ 0.940 $P < 0.0001$	$P < 0.0001$ 1.00 $P < 0.0001$	$P < 0.0001$ 0.720 $P < 0.0001$	$P < 0.0001$ 1.00 $P < 0.0001$
Florida	0.35	–	$P = 0.0332$ NS	$P = 0.0002$ 0.366 $P < 0.0001$	$P = 0.0003$ 0.178 $P < 0.0001$	$P = 0.0089$ 0.260 $P = 0.0027$
Greece	0.03		–	NS NS	$P = 0.0136$ 0.198 $P = 0.0237$	NS NS
Cyprus	0.00	0.87		–	$P < 0.0001$ 0.399 $P < 0.0001$	NS NS
Turkey	0.19	2.3	2.02	0.75	–	$P = 0.0025$ 0.302 $P < 0.0001$
Southeastern basin	0.00	1.42			1.15	–

Table 4 Stock composition in Mediterranean fishery areas based on UCON. SD was calculated using the jacknife method, whereas 95% confidence intervals (CI) were calculated with bootstrap resampling (see the Materials and methods)

	Drifting longline fisheries						Bottom trawl fisheries					
Stock analysis	Western Mediterranean	SD	95% CI	Eastern Mediterranean	SD	95% CI	Tunisia	SD	95% CI	Egypt	SD	95% CI
1. USA Georgia/Carolina	0.02	0.00	0.00–0.07	0.02	0.00	0.00–0.08	0.00	0.00	0.00–0.00	0.00	0.00	0.00–0.00
USA Florida	0.45	0.12	0.26–0.57	0.47	0.13	0.28–0.58	0.00	0.00	0.00–0.00	0.00	0.00	0.00–0.00
Mediterranean	0.53	0.12	0.36–0.73	0.51	0.13	0.34–0.71	1.00	0.00	1.00–1.00	1.00	0.00	1.00–1.00
2. Atlantic	0.45	0.11	0.26–0.65	0.47	0.12	0.24–0.70	0.00	0.00	0.00–0.00	0.00	0.00	0.00–0.00
Mediterranean	0.55	0.11	0.35–0.74	0.53	0.12	0.30–0.76	1.00	0.00	1.00–1.00	1.00	0.00	1.00–1.00
3. Turkey							0.00	0.00	0.00–0.20	0.25	0.17	0.00–0.62
Greece/Cyprus/Southeastern basin							1.00	0.00	0.80–1.00	0.75	0.17	0.38–1.00

slight but significant substructuring among samples (Φ_{ST} = 0.083; P = 0.0234), indicating that each fishery is impacting differently on different loggerhead populations. We conducted a third ML analysis, considering two stocks within the Mediterranean, the Turkish and the combined Greek, southeastern basin, and Cyprus one. No contribution was made by the Turkish stock to the distant Tunisian fishery; however, it contributed some 25% to the closer Egyptian fishery (Table 4).

These findings clearly indicate that the Mediterranean pelagic habitat, sampled by drifting longline fisheries, is utilized by distant populations both from within and outside the Mediterranean. This is in marked contrast to the benthic areas exploited by bottom-trawl fisheries that are only used by regional turtle populations.

Inputs for population dynamics analysis

Stock compositions were related to size class data to assess the impact of fisheries on populations. Individuals caught by drifting longlines were small and medium immatures with a proportion of adults close to zero (Fig. 3a,b). In the western Mediterranean sample, individuals had a mean size of 47.4 cm (range: 27.6–69.0; SD = 10.35; N = 62) (Fig. 3a), and of 45.9 cm in the eastern Mediterranean (range: 33.0–75.5; SD = 7.45; N = 53) (Fig. 3b). The proportion of large individuals (≥ 70 cm) captured was low. Only one large individual was collected; it had a short tail, but because it was not internally investigated it could not be sexed. On the other hand, loggerheads caught in bottom-trawl fisheries were medium and large immatures and adults (Fig. 3c,d). In the Tunisian sample, individuals had a mean size of 61.3 cm (range: 32.3–91.8; SD = 15.14; N = 80) with a proportion of large individuals of 35.0% (Fig. 3c). Of seven males detected, six were adults. Although no individuals were internally examined, and sexed as adult females, the total proportion of adults was 7.5%. In the Egyptian fishery, body mean size was 67.0 cm (range: 49.4–86.3; SD = 9.54; N = 27) and large individual index was 37.0% (Fig. 3d). Of the 10 large individuals (≥ 70 cm) collected (Fig. 3d), only six were sexed: four of these were adult males and two were adult females. The proportion of adults was 22.2%.

Individuals endemic to west Atlantic nesting colonies with haplotype C observed in the Mediterranean had a mean size of 48.6 cm (range: 29.7–65.0; SD = 8.31; N = 34), while the mean size for haplotype A individuals from both Mediterranean and Atlantic stocks was 51.4 cm (range: 23.0–90.0; SD = 14.78; N = 165). The difference is not significant (Kolmogorov–Smirnov two-sample test); however, no large individuals (≥ 70 cm) were found in lineage C. This represents a significant difference with the proportion of large individuals of lineage A (15.1%)

(Fisher's exact test, $P = 0.006$), indicating that individuals from western Atlantic nesting colonies entering the Mediterranean do not grow to a large size in the Mediterranean.

Discussion

Population structure

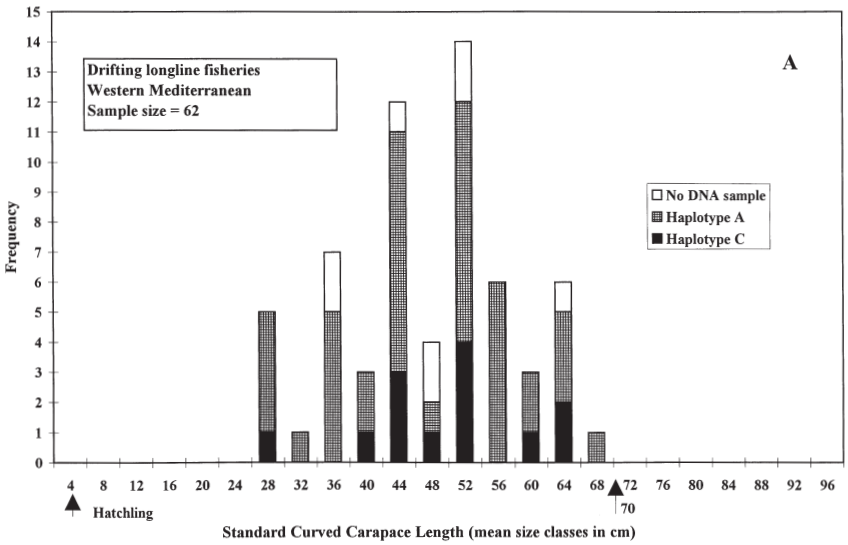
This study provides the first rigorous estimate of mtDNA haplotype frequencies of the Mediterranean loggerhead population. The data confirm previous findings (Bowen *et al*. 1993; Laurent *et al*. 1993) that the Mediterranean population constitutes an endemic breeding population and represents a functionally independent stock, which has diverged in mtDNA genotypes as a result of low levels of contemporary female-based gene flow with Atlantic populations. This is consistent with other studies on interregional loggerhead population structure (Bowen *et al*. 1993; Bowen *et al*. 1994). Furthermore, as no western haplotype C was found in the 25 large individuals (≥ 70 cm) (including at least nine identified adult males), Atlantic males seem not to breed in the Mediterranean, which causes a low male-based gene flow between the Atlantic and the Mediterranean. This highlights the functional independence of the Mediterranean loggerhead breeding population.

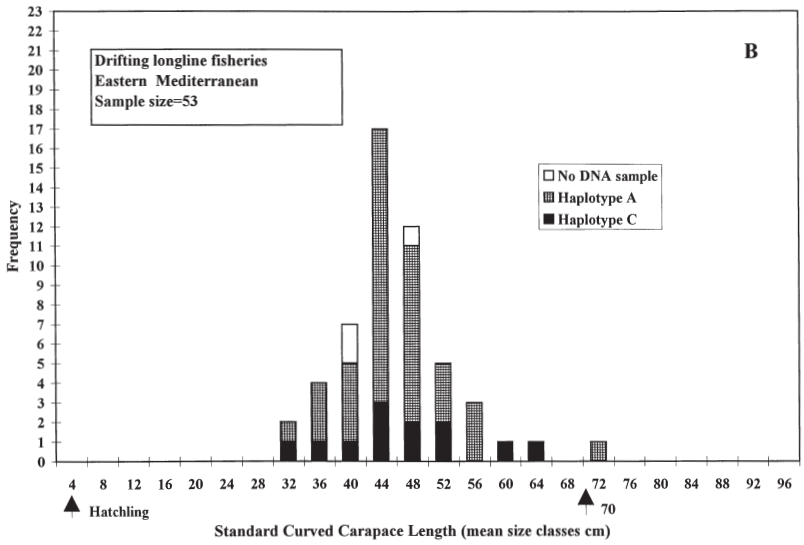
Our mtDNA data demonstrate that the Turkish colonies are genetically distinct from the others in the Mediterranean, indicating that Turkey holds an independent population unit. Lack of genetic divergence among the other nesting areas (Cyprus, Greece and the southeastern basin) does not necessarily imply panmixia because potential differences may have stayed undetected due to sample size limitations or lack of resolution of the marker. Indeed, some haplotypes observed in marine areas have not yet been assigned an origin (Table 1). This is the case, for example, for haplotype A5, which was found in both pelagic and benthic areas in five different individuals (Table 1). This haplotype was not observed in a recent mtDNA haplotype frequency survey of western Atlantic nesting areas, nor in another Greek sample from Peloponnesus (Encalada *et al*. 1998), nor from pelagic habitats in the eastern Atlantic (Bolten *et al*. 1998). This strongly suggests that haplotype A5 may be endemic to Libya, and is also consistent with a large rookery in Libya, not yet fully genetically investigated. The absence of this haplotype in the Libyan nesting area sample may be due to the small sample size ($n = 7$). Furthermore, the significant differences in the size of nesting females from the different nesting areas of Cyprus, Greece and Libya (Laurent *et al*. 1995; Broderick & Godley 1996) strongly support a population structuring. Together, these findings suggest that all the nesting areas possess independent population units. Within nesting areas it is likely that population differentiation among adjacent nesting sites occurs, as this was recently shown for different nesting sites in Turkey by means of analysis of both mitochondrial and nuclear markers (Schroth *et al*. 1996).

Stock composition in Mediterranean marine areas

Our ML analyses focused on the Mediterranean stock contribution in fishery bycatch and were based on the lineage C haplotype frequency in order to increase precision of contribution estimates for subsequent population dynamics analyses. The proportion of this haplotype was recently found to be 99.00% ($N = 105$) in Georgia/Carolina and 44.00% ($N = 50$) in south Florida (Encalada *et al*. 1998); western Atlantic colonies recently shown to be the primary sources of juveniles loggerhead in pelagic habitats of eastern Atlantic around the Azores and Madeira (Bolten *et al*. 1998). In these two eastern Atlantic zones, located in the current path of transatlantic developmental loggerhead migration before reaching Gibraltar strait, the proportion of the lineage C haplotype was found to be 49.36% ($N = 79$) and 51.90% ($N = 52$), respectively. In contrast, in the Mediterranean nesting aggregate the frequency of this haplotype is equal to zero ($N = 92$) (Table 1). This was subsequently confirmed in Mediterranean benthic habitats ($N = 58$) (Table 1). On the basis of this high haplotype frequency difference, contribution of the transatlantic developmental migration to the pelagic habitat of the western and eastern Mediterranean was estimated to be 45 and 47%, respectively (Table 4), which is lower than previously believed (Carr 1987), but confirms a previous hypothesis (Laurent 1990b).

When assuming in our first stock composition analysis that the Atlantic stock is only composed of northwestern Atlantic rookeries we did not take into consideration the other haplotypes, and other Atlantic colonies which may make very small contributions to Mediterranean pelagic habitats. For example, some of the haplotypes found in pelagic habitats may be from Mexico or other USA rookeries, e.g. haplotypes A4 and A7 (Table 1). However, haplotypes J and C originally found and considered as endemic to Mexico and to Florida, respectively (Bolten *et al*. 1998; Encalada *et al*. 1998), were also observed in Greek and Turkish nesting areas, respectively (haplotype A2, A3; Table 1). This shows that small haplotype frequencies should be used with caution in mixture samples. If both Mediterranean and Atlantic nesting areas could be surveyed completely for control region haplotypes, more detailed stock composition analyses could be conducted. This would be carried out by using small haplotype frequencies and without removing informative individuals with haplotypes not found in nesting areas from the mixtures samples.





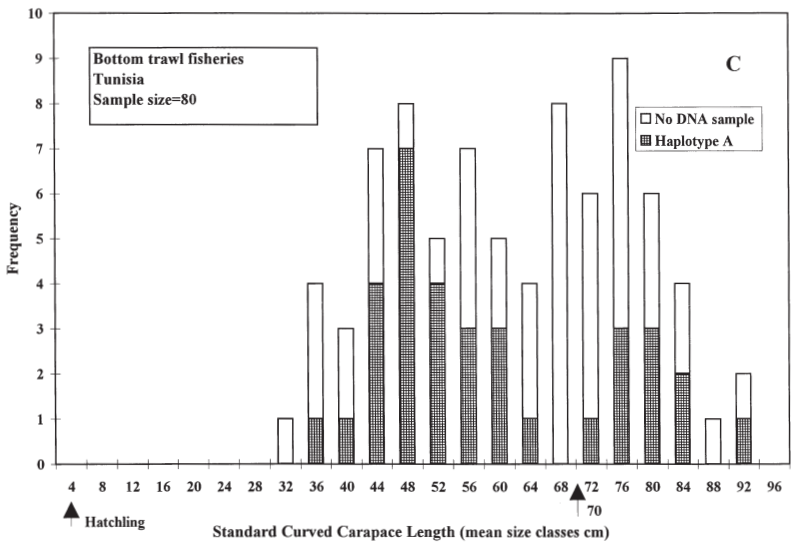


Fig. 3 Size class distribution of loggerhead turtles in fishery bycatch. Key: 70 cm = smallest nesting female in Greece; 4 cm = hatchling size in the Mediterranean.

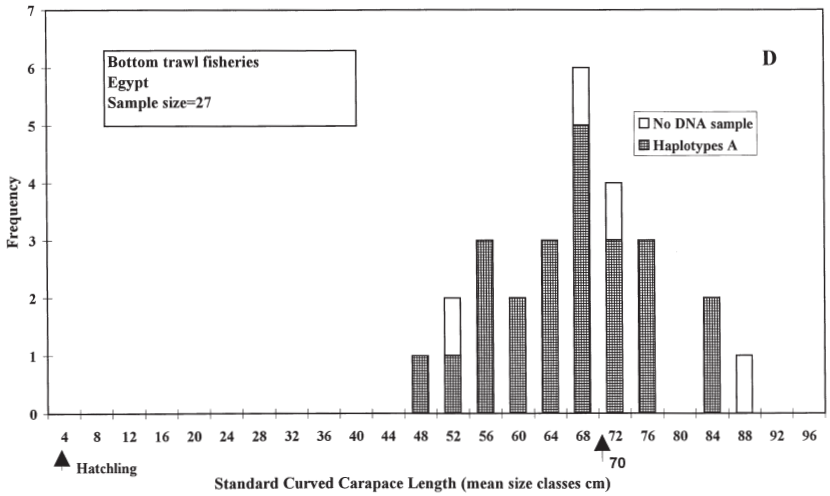


Fig. 3 *Continued.*

Population management

Our stock composition assessment demonstrates that all captures from bottom-trawl fisheries and more than half of the captures from drifting longline fisheries derive from the Mediterranean stock. This totally modifies our estimation of the Mediterranean loggerhead nesting population size. Indeed, these populations were thought to be small and most of the individuals caught by fisheries in the western Mediterranean were considered to originate from the Atlantic (Carr 1987; Groombridge 1990). This regional stock contribution to fishery bycatch is consistent with a recent holistic estimate based on more detailed investigations of previously neglected nesting areas, e.g. in northern Cyprus (Broderick & Godley 1996), and especially the recent discovery of a large loggerhead rookery in Libya (Laurent *et al*. 1995, 1997).

Fisheries-related mortality of loggerhead turtles in the Mediterranean is probably substantial. Although the effect of these fisheries on turtle population dynamics needs to be accurately quantified using demographic models, our stock composition assessment is explicit in itself. If long-term conservation of Mediterranean loggerhead turtle populations is to be ensured, sea turtle conservation regulations are urgently needed for the Mediterranean fisheries. For the Mediterranean marine environment, one of the most important conservation issues is to manage fishing activity in a way to obtain sustainable exploitation of resources on the one hand, and environmental protection of nontarget species on the other hand. There is an urgent need for the European Community and the wider Mediterranean community as a whole to make policies for resource management, and for the environment, more coherent.

The particular mtDNA haplotype divergence of the Turkish loggerhead population defines this nesting population as a management unit (MU) (Moritz 1994), and our stock composition analysis suggests that large immature and adult stages of this MU might be predominantly harvested by Egyptian fisheries. Our DNA data do not allow MUs for the other nesting areas to be defined, although the existence of such MUs is indicated by the significant differences in the size of nesting females. It would therefore be unwise to base a policy on the monitoring of one nesting site only, i.e. Zakynthos (Greece). Each Mediterranean nesting population should be managed separately at all life-history stages by considering small immature stages of these populations as sharing pelagic habitats throughout the Mediterranean (and possibly the eastern Atlantic), while large immatures and adults have distinct and more localized benthic feeding habitats in the eastern basin. These habitats need to be accurately identified in further studies using both mtDNA and nuclear markers.

Ecological shift in the loggerhead life cycle

The present work has shown that loggerheads from the Atlantic stock enter Mediterranean pelagic habitats, but do not recruit to benthic habitats in the region (at least during autumn, winter and spring). The simplest interpretation is that an ecological shift in the loggerhead life cycle occurs during an immature stage which occupies both habitats (see the Results). The life cycle of the loggerhead has been considered as having two immature life-history phases: an early nursery oceanic pelagic phase of hatchlings, posthatchlings and small immature stages, followed by a benthic subadult phase reaching maturity in shallow coastal waters (Carr 1982, 1987; Limpus *et al*. 1994a). Our data give an insight into this shift and suggest that it may be induced by a strong behavioural component (in addition to ecological factors), and that the

limited dispersal behaviour of females (Table 3) is already determined at the immature pelagic phase. Such findings strengthen the evidence regarding the major role of natal philopatry in the determination of female dispersal.

Hatchlings and posthatchlings in the Mediterranean are only rarely observed in neritic waters, indicating that they effectively enter an oceanic phase after leaving nesting beaches. Larger individuals are caught by longline fisheries in western and eastern Mediterranean pelagic areas (De Metrio *et al*. 1983; Caminas 1988; Aguilar *et al*. 1995; Panou *et al*. 1996). Capture per unit effort of longline fishing is higher during summer in the western Mediterranean (Caminas 1988; Caminas & De La Serna 1995) with only a low incidence of loggerhead bycatch in this area during winter (Caminas & De La Serna 1995). Immature stages are known to enter shallow coastal waters off France during summer, where they are captured by bottom trawlers and coastal trammel nets (Delaugerre 1987; Laurent 1991; Laurent *et al*. 1996). Larger loggerheads are caught in the eastern basin (Laurent *et al*. 1996) by bottom-trawl fisheries, at least during winter, as is the case in Tunisia (Laurent *et al*. 1990, 1996; Bradai 1992), in Lakonikos bay (Greece) (Margaritoulis *et al*. 1992), in Iskenderun Bay (Turkey) (Laurent *et al*. 1996; Oruç *et al*. 1997), in Egypt (Laurent *et al*. 1996), and probably in the northern Adriatic (Lazar & Tvrtkovic 1995). Dietary analysis has indicated that loggerheads caught during winter in South Tunisian waters actively feed on benthic invertebrates, mostly gastropods, hermit crabs, holothurians, lamellibranchs and sponges (Laurent & Lescure 1994). Together, these data suggest that immature turtles do not recruit to the benthic phase in one step from the strict oceanic pelagic phase, but progressively from a later flexible neritic stage. In this phase, individuals would feed on pelagic prey most of the time, but would also be able to facultatively switch to feeding on benthic prey in coastal waters. Support for this hypothesis also comes from the record of four tagged immatures originally caught by longlines and subsequently, 1 year later, captured by small coastal fisheries in French Mediterranean waters (Argano *et al*. 1992; R. Argano *et al*. unpublished data). Captures of Atlantic loggerheads by bottom fisheries around Lampedusa (Table 1) could also be explained by such a behavioural flexibility.

Acknowledgements

This research was part of the WWF project 9E0103 undertaken within the framework of the WWF International Mediterranean Programme directed by Paolo Guglielmi, and was cofunded by the French Ministry of Environment (contract DGAD/EGPN/94022), the Regional Activity Centre for Specially Protected Areas (MAP/UNEP), Greenpeace Mediterranean Sea Project and the Institute of Ecology University Paris VI. The authors would like to thank Magnus Sylven (WWF International), Mohamed Saied and Chedly Rais (RAC/SPA), Mario Damato (Greenpeace), Jean Clobert (Institute of Ecology) as well as Jean Pierre Gasc (Muséum National d'Histoires Naturelles) for their constant support. We also gratefully recognize Brian Bowen for providing DNA samples from the American nesting rookeries. Many thanks to Mohamed Ktari, Mongi Moncef and the crew of the trawler Anoir (Tunisia), Xavier Pastor, F. Costa, Juan Xampeny, D. Rosell and Juan Marti (Spain) as well as Z. Kuller (Israel) for their help in sample collection. The laboratory work was performed at the 'Centre d'Analyse Moléculaire de la Biodiversité' University Lyon 1 and we thank Jérôme Briolay for his support. We are grateful to Frédéric Bedin, Bernard Mandrand and Alain Rajoharison (CNRS/Biomerieux laboratory) for assistance in sequencing. We thank Jerry Pella and Michele Masuda for technical assistance in ML analyses. We thank anonymous referees for helpful comments.

References

Aguilar R, Mas J, Pastor X (1995) Impact of Spanish swordfish longline fisheries on the loggerhead sea turtle *Caretta caretta* population in the western Mediterranean. In: *Proceedings of the Twelfth Annual Workshop on Sea Turtle Biology and Conservation*. (eds Richardson JI, Richardson TH), pp. 1–6. National Oceanographic and Atmospheric Administration technical memorandum NMFS-SEFSC-361. National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, Florida.

Allard MW, Myamoto MM, Bjorndal KA, Bolten AB, Bowen BW (1994) Support for natal homing in Green turtles from mitochondrial DNA sequences. *Copeia*, **1994**, 34–41.

Argano R, Baldari F (1983) Status of western Mediterranean sea turtles. Rapports et Procès-verbaux des réunions de la Commision Internationale pour l'Exploration Scientifique de la Mer Méditerranée. *Monaco*, **28**, 233–235.

Argano R, Basso R, Cocco M, Gerosa G (1992) New data on loggerhead (*Caretta caretta*) movements within Mediterranean. *Bollettino Del Museo Dell' Istituto Di Biologia Dell' Università Di Genova*, **56–57**, 137–163.

Bass AL, Good DA, Bjorndal KA, Richardson JI, Hillis ZM, Horrocks JA, Bowen BW (1996) Testing models of female reproductive migratory behaviour and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. *Molecular Ecology*, **5**, 321–328.

Bolten AB, Bjorndal KA, Martins HR, Dellinger T, Biscoito MJ, Encalada S, Bowen B (1998) Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. *Ecological Applications*, **8**, 1–7.

Bolten AB, Martins HR, Bjorndal KA, Cocco M, Gerosa G (1992) *Caretta caretta* (loggerhead). Pelagic movement and growth. *Herpetological Review*, **23**, 116.

Bowen B, Abreu-Grobois FA, Balazs GH, Kamezaki N, Limpus CJ, Ferl RJ (1995) Trans-Pacific migrations of the loggerhead sea turtle demonstrated with mitochondrial DNA markers. *Proceedings of the National Academy of Sciences of the USA*, **92**, 3731–3734.

Bowen B, Kamezaki N, Limpus CJ, Hughes GR, Meylan AB, Avise JC (1994) Global phylogeography of the Loggerhead (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. *Evolution*, **48**, 1820–1828.

Bowen B, Meylan AB, Ross JP, Limpus CJ, Balazs GH, Avise JC (1992) Global population structure and natural history of the Green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. *Evolution*, **46**, 865–881.

Bowen BW, Avise JC, Richardson JI, Meylan AB, Margaritoulis D, Hopkins-Murphy S (1993) Population structure of the loggerhead *Caretta caretta* in the northwest Atlantic Ocean and Mediterranean Sea. *Conservation Biology*, **37**, 834–844.

Bowen BW, Bass LW, Garcia-Rodriguez A, Diez CE, Van Dam R, Bolten A, Bjorndal KA, Miyamoto MM, Ferl RJ (1996) Origin of hawksbill turtles in a Caribbean feeding area as indicated by genetic markers. *Ecological Applications*, **6**, 566–572.

Bowen BW, Meylan AB, Avise JC (1989) An odyssey of the green sea turtle: Ascension Island revisited. *Proceedings of the National Academy of Sciences of the USA*, **86**, 573–576.

Bradai MN (1992) Les captures accidentelles de *Caretta caretta* au chalut benthique dans le Golfe de Gabès. Rapports et Procès-verbaux des réunions de la Commision Internationale pour l'Exploration Scientifique de la Mer Méditerranée. *Monaco*, **33**, 285.

Broderick AC, Godley BJ (1996) Population and nesting ecology of the green turtle, *Chelonia mydas*, and the loggerhead turtle, *Caretta caretta*, in northern Cyprus. *Zoology in the Middle East*, **13**, 27–46.

Broderick DC, Moritz JD, Miller M, Guinea RJ, Prince Limpus CJ (1994) Genetic studies of the hawksbill turtle: evidence for multiple stocks. *Pacific Conservation Biology*, **1**, 123–131.

Caminas JA (1988) Incidental captures of *Caretta caretta* with surface long-lines in the western Mediterranean. Rapports et Procès-verbaux des réunions de la Commision Internationale pour l'Exploration Scientifique de la Mer Méditerranée. *Monaco*, **31**, 285.

Carr A (1975) The Ascension Island green turtle colony. *Copeia*, **1975**, 547–555.

Carr A (1982) Notes on the behavioural ecology of sea turtles. In: *Biology and Conservation of Sea Turtles* (ed. Bjorndal KA), pp. 19–23. Smithsonian Institution Press, Washington DC.

Carr A (1987) New perspectives on the pelagic stage of sea turtle development. *Conservation Biology*, **1**, 103–121.

Carr A, Carr MH, Meylan AB (1978) The ecology and migrations of sea turtles. 7. The west Caribbean green turtle colony. *Bulletin of American Museum of Natural History*, **162**, 1–46.

De Caminas JA, La Serna JM (1995) The loggerhead distribution in the western Mediterranean Sea as deduced from captures by the Spanish Long Line Fishery. In: *Scientia Herpetologica* (eds Llorente GA, Montori, A, Santos X, Carretero MA), pp. 316–323. Asociación Herpetológica Espanola, Barcelona.

De Metrio G, Petrosino G, Matarrese A, Tursi A, Montanaro C (1983) Importance of the fishery activities with drift lines on the populations of *Caretta caretta* and *Dermochelys coriacea* (Reptilia, Testudines), in the Gulf of Taranto. *Oebalia*, **9**, 43–53.

Delaugerre M (1987) Statut des Tortues marines de la Corse et de la Méditerranée. *Vie Milieu*, **37**, 243–264.

Encalada SE, Bjornda KA, Bolten AB, Zurita JC, Schoeder B, Possardt E, Sears CJ, Bowen BW (1998) Population structure of the loggerhead turtle (*Caretta caretta*) in the Atlantic and Mediterranean as interred from mitochondrial DNA control region sequences. *Marine Biology*, **130**, 567–575.

Encalada SE, Lahanas PN, Bjornda KA, Bolten AB, Miyamoto MM, Bowen BW (1996) Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: a mitochondrial DNA control region sequence assessment. *Molecular Ecology*, **S5**, 473–483.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Fitzsimmons NN, Moritz C, Moore SS (1995) Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Molecular Biology and Evolution*, **12**, 432–440.

Fumagalli L (1995) Variabilité inter- et intraspecifique de l'ADN mitochondrial chez les musaraignes du groupe Sorex araneus. Thèse, Université de Lausanne, Suisse.

Galtier N, Gouy M, Gautier C (1996) SEAVIEW and PHYLO-WIN: two graphic tools for sequence alignment and molecular phylogeny. *Computers Applied to Biological Sciences*, **12**, 543–548.

Grenon M, Batisse M (1988) *Futures for the Mediterranean Basin: The Blue Plan*. Oxford University Press, Oxford.

Groombridge B (1990) *Marine Turtles in the Mediterranean: Distribution, Population Status, Conservation*. European Council, Strasbourg.

Kocher TD, Thomas WK, Meyers A, Edwards SV, Paabo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA*, **86**, 6196–6200.

Laurent L (1990a) Les tortues marines en Algérie et au Maroc (Méditerranée). *Bulletin de La Société Herpétologique de France*, **55**, 1–23.

Laurent L (1990b) L'origine des tortues Caouannes *Caretta caretta* de Méditerranée occidentale. Rapports et Procès-verbaux des réunions de la Commision Internationale pour l'Exploration Scientifique de la Mer Méditerranée. *Monaco*, **32**, 240.

Laurent L (1991) Les Tortues marines des côtes françaises méditerranéennes continentales. *Faune de Provence (C.E.E.P.)*, **12**, 76–90.

Laurent L (1996) Synthèse historique de la présence de tortues marines sur les côtes de France (côtes méditerranéennes). Observatoire du Patrimoine Naturel. Groupe Tortues Marines. Direction de la Nature et des Paysages. Sous-direction de la chasse, de la faune et de la flore sauvages. Ministère Français de l'Environnement, Paris. Unpublished.

Laurent L, Abd El-Mawla EM, Bradai MN, Demirayak F, Oruç A (1996) Reducing sea turtle mortality induced by Mediterranean fisheries: trawling activity in Egypt, Tunisia and Turkey. WWF Project 9E0103. World Wide Fund for Nature International Mediterranean Programme, Rome.

Laurent L, Bradai MN, Hadoud DA, Gomati HE (1995) Marine Turtle nesting activity assessment on Libyan coasts. Phase 1: survey of the coasts between the Egyptian border and Sirte. Regional Activity Centre for Specially Protected Areas (UNEP), Tunis.

Laurent L, Bradai MN, Hadoud DA, Gomati HE (1997) Assessment of sea turtle nesting activity in Libya. *Marine Turtle Newsletter*, **76**, 2–6.

Laurent L, Lescure J (1994) L'hivernage des tortues Caouannes *Caretta caretta* dans le sud Tunisien. *Revue d'Ecologie (Terre et Vie)*, **49**, 63–86.

Laurent L, Lescure J, Excoffier L, Bowen B, Domingo M, Hadjichristophorou M, Kornaraky L, Trabuchet G (1993) Genetic studies of relationships between Mediterranean and Atlantic populations of loggerhead *Caretta caretta* with a mitochondrial marker. *Comptes Rendus de l'Académie Des Sciences, Paris*, **316**, 1233–1239.

Laurent L, Nouira S, Jeudy de Grissac A, Bradai MN (1990) Les Tortues marines de Tunisie: premières données. *Bulletin de La Société Herpétologique de France*, **53**, 1–17.

Lazar B, Tvrtkovic N (1995) Marine turtles in the eastern part of the Adriatic sea: preliminary research. *Natura Croatica*, **4**, 59–74.

Limpus CJ (1992) Estimation of tag loss in marine turtle research. *Wildlife Research*, **19**, 457–469.

Limpus CJ, Couper PJ, Read MA (1994a) The loggerhead *Caretta caretta* in Queensland: population structure in a warm temperature feeding area. *Memoirs of the Queensland Museum*, **37** (1), 195–204.

Limpus CJ, Couper PJ, Read MA (1994b) The green turtle, *Chelonia mydas*, in Queensland: population structure in a warm temperature feeding area. *Memoirs of the Queensland Museum*, **35** (1), 139–154.

Limpus CJ, Miller JD, Parmenter CJ, Reimer D, McLahan N, Webb R (1992) Migration of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles to and from eastern Australian rookeries. *Wildlife Research*, **19**, 347–358.

Margaritoulis D (1982) Observations on Loggerhead sea turtle *Caretta caretta* activity during three nesting seasons (1977–79) in Zakynthos, Greece. *Biological Conservation*, **24**, 193–204.

Margaritoulis D, Kousias N, Nicolopoulou G, Teneketzis K (1992) Incidental catch of sea turtles in Greece: the case of Lakonikos bay. In: *Proceedings of the Eleventh Annual Workshop on Sea Turtle Biology and Conservation* (eds Salmon M, Wyneken J), pp. 168–170. National Oceanographic and Atmospheric Administration technical memorandum NMFS-SEFSC-302. National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, Florida.

Masuda M, Nelson S, Pella J (1991) *User's Manual for GIRLSEM, GIRLSYM, and CONSQRT. Personal computer version. USA-DOC-NOAA-NMFS, Auke Bay Lab. Us-Canada Salmon Program, 11305 Glacier Highway, Juneau, Alaska*, 99801–8626.

Moritz C (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology*, **3**, 401–411.

Norman JA, Moritz C, Limpus CJ (1994) Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. *Molecular Ecology*, **3**, 363–373.

Oruç A, Demirayak F, Sat G (1997) *Fishery in the Eastern Mediterranean and its Impact on Sea Turtles*. Report for the WWF International and DHKD. Istanbul, Turkey.

Owens DW, Ruiz GW (1980) New methods of obtaining blood and cerebrospinal fluid from marine turtles. *Herpetologica*, **36**, 17–20.

Panou A, Tselentis L, Voutsinas N, Antypas G, Mourelatos C, Kaloupi S, Voutsinas V, Moschonas S (1996) Interaction between sea turtles and surface long line fisheries in the Ionian Sea, Greece. *7th International Congress on the Zoogeography and Ecology of Greece and Adjacent Regions*, in press.

Pella J, Milner GB (1987) Use of genetic marks in stock composition analysis. In: *Population Genetics and Fisheries Management* (eds Ryman N, Utter F), pp. 247–276. University of Washington Press, Seattle.

Pritchard P, Bacon P, Berry F, Carr A, Fletemeyer J, Gallagher R, Hopkins S, Lankford R, Marquez MR, Ogren L, Pringle W, Reichart H, Withman R (1983) *Manual of Sea Turtle Research and Conservation Techniques*, 2nd edn. Center for Environmental Education, Washington, DC.

Pritchard PCH (1976) Post-nesting movements of marine turtles (*Cheloniidae* and *Dermochelydae*) tagged in the Guianas. *Copeia*, **1976**, 749–754.

Schroth W, Streit B, Schierwater B (1996) Evolutionary handicap for turtles. *Nature*, **384**, 521–522.

Sears CJ, Bowen BW, Chapman RW, Galloway SB, Hopkins-Murphy SR, Woodley CM (1995) Demographic composition of the feeding population of juvenile loggerhead sea turtles (*Caretta caretta*) off Charleston, South Carolina: evidence from mitochondrial DNA markers. *Marine Biology*, **123**, 869–874.

Sella I (1982) Sea turtles in the eastern Mediterranean and the northern red Sea. In: *Biology and Conservation of Sea Turtles* (ed. Bjorndal KA), pp. 417–423. Smithsonian Institution Press, Washington DC.

Wenink PW, Baker AJ, Tilanus MGJ (1994) Mitochondrial control region sequences in two shorebird species, the Turnstone and the Dunlin, and their utility in population genetic studies. *Molecular Biology and Evolution*, **11** (1), 22–31.

Wilkinson GS, Chapman AM (1991) Length and sequence variation in evening bat D-Loop mtDNA. *Genetics*, **128**, 607–617.

Xu S, Kobak CJ, Smouse PE (1994) Constrained least squares estimation of mixed population stock composition from mtDNA haplotype frequency data. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 417–425.

Luc Laurent heads BIOINSIGHT, a consultancy specializing in biological conservation management. This research was also undertaken by numerous individuals working as part of different marine turtle projects including, among others: Paolo Casale and Guido Gerosa who conduct studies on marine turtle ecology and conservation in the Mediterranean; Mohamed Nejmedine Bradai who works on marine turtle ecology and conservation programs in Tunisia; and Brendan Godley and Annette Broderick who conduct both fundamental and applied research in marine turtle biology. Werner Schroth is a PhD student in the laboratory of Bernd Schierwater, in which molecular techniques are used to study the evolutionary ecology of marine animals. Alon Levy works with the Israeli Marine Mammal Society. Daniela Freggi is in charge of the Italian sea turtle research programme at Lampedusa Island.
