Ocean and Coastal Management 153 (2018) 70–75

Contents lists available at ScienceDirect

Ocean and Coastal Management

journal homepage: www.elsevier.com/locate/ocecoaman

Local differentiation in the origin of stranded loggerhead turtles, *Caretta caretta*, within an eastern Turkey foraging area

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ARTICLE INFO

Keywords:
Mixed stock analysis
Mediterranean
Caretta caretta, foraging ground
mtDNA

ABSTRACT

The eastern Mediterranean Sea is frequently visited by nesting and foraging loggerhead turtles and is also a nursery zone although the origin of these foraging animals has not yet been assessed. In order to estimate the natal origin of eastern Turkey foraging individuals we analysed a long fragment of the mtDNA control region from 135 loggerhead turtles and we performed a Bayesian mixed stock analysis to estimate the contributions from rookeries in the Mediterranean to the foraging grounds studied. A total of 5 haplotypes were identified but they were not homogeneously distributed across the sampling geographical range thus suggesting an east-west differentiation. The mixed stock analysis revealed that the turtles from the eastern feeding ground come mostly from the western nesting populations of Turkey (49%), while those from the western feeding ground come from Cypriot stocks (62%). These results show that anthropogenic activities on this area may have an impact on different populations depending on were this activities are located and overall pose threat to the survival of the western Turkish and Cypriot nesting beaches.

1. Introduction

The loggerhead turtle is a highly migratory animal (Bolten, 2003; Plotkin, 2003) with complex life cycle involving series of ontogenetic habitat shifts (Bolten, 2003; McClellan and Read, 2007). However, recent findings of Casale et al. (2008) suggested a relaxed model with general plasticity of habitat use. Thus, in the Mediterranean the proximity of different habitats of allow loggerhead turtles to feed upon benthic preys very early. This complex life history covers different geographical regions and habitats around the world. It is, therefore, vital to understand the links among different life stages to provide effective conservation strategies for the conservation of species (Rees et al., 2016). Assessing the natal origin of the sea turtles in foraging grounds are one of the key information for the conservation of sea turtles. In this sense many studies has been done in the Mediterranean (Carreras et al., 2006; Garofalo et al., 2013; Clusa et al., 2014; Karaa et al., 2016; Rees et al., 2017) but little is known about the composition of eastern Mediterranean foraging areas. The Mediterranean loggerhead turtles have been described as a Regional Management Unit (Wallace et al., 2010) that is considered to be at low risk but under high threat (Wallace et al., 2011). It has recently been listed under the IUCN criteria as Least Concern, but with the caveat of being conservation dependent (Casale, 2015). Today, the largest sea turtle nesting aggregations occur in Greece, Turkey, Cyprus, Syria and Libya (Casale and Margaritoulis, 2010). Turkish populations host almost one third of nesting abundance in the Mediterranean (Casale and Margaritoulis, 2010) and they are genetically differentiated from other Mediterranean populations (Shamblin et al., 2014). Within Turkey, the eastern Mediterranean coast has low numbers of loggerhead turtle (*Caretta caretta*) nests while concentrate the main nesting activity of green sea turtles (*Chelonia mydas*) in the Mediterranean (Turkozan and Kaska, 2010). Despite this low loggerhead nesting abundance, this part of the coast has been identified as a foraging ground for both loggerhead and green sea turtles (Oruç 2001) and thus the loggerhead individuals using this area may potentially originate in distant nesting areas. Dispersal simulations using particle modelling have predicted that the neighbouring Levantine zone was a nursery zone for the Mediterranean sea turtles while individuals born in Turkey nesting populations dispersed

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https://doi.org/10.1016/j.ocecoaman.2017.12.011
Received 11 July 2017; Received in revised form 9 December 2017; Accepted 9 December 2017

to Aegean and Levantine zones (Casale and Mariani, 2014). Although the predicted importance of Levantine foraging grounds, there is a lack of studies in terms of natal origin of turtles in foraging grounds, as only the south east Levantine area has been considered in previous studies (Clusa et al., 2014). Thus, the north east Levantine area remains being an important gap due to the proximity of the abundant nesting areas of Turkey. The use of mixed stock analysis initially designed to assess the stock origin of fish mixed aggregation (Grant et al., 1980), has proved to be useful for identifying the contribution of each rookery to foraging grounds in Mediterranean loggerhead turtles (Carreras et al., 2006; Clusa et al., 2014). Since bycatch of the sea turtles in foraging grounds is one of the most important factors (Casale, 2011) the information on the composition of the fishing ground is essential for the impact assessment. The aim of this study is to fulfil this gap of knowledge by analysing an important loggerhead foraging ground in eastern Turkey and to provide a baseline data from a less known life stage of the species for the conservation of the loggerhead turtles in Turkey.

2. Material and methods

2.1. Sample collection

Samples were obtained from 135 loggerhead sea turtles stranded on the seven locations namely Aykap (Ayaş and Kapızlı) (AYP), Davultepe (DTP), Limpoz (Liman and Pozcu) (LMP), Akyatan (AKY) and Kazanlı (KZL) between the years 2009 and 2012 (Table 1, Fig. 1). Tissue samples were preserved in 96% ethanol until DNA extraction. Furthermore, curved carapace length (CCL) of the strandings were measured in cm from the notch of nuchal scute to the outermost projection of supracaudals.

2.2. Laboratory procedures

Total DNA was isolated from skin and muscle tissues of stranded turtles with a modified version of the standard phenol-chloroform protocol (Hillis and Moritz, 1990). A fragment of 862 base-pair (bp) of the mtDNA d-loop region was amplified by polymerase chain reaction (PCR Mastercycler Personel, Eppendorf, Germany) using the primer pair LCM15382 (5′- GCT TAA CCC TAA AGC ATT GG -3′) and H950 (5′- GTC TCG GAT TTA GGG GTT TG -3′) (Abreu-Grobois et al., 2006). The PCR protocol was carried out according to Yilmaz et al. (2011), PCR products were visualized in agarose gel and purified with the GenElute PCR Clean-Up Kit, (Sigma, Germany). Purified PCR products were sequenced in both forward and reverse directions using a 3730xl capillary system automatic sequencer (Macrogen Inc., S. Korea). Sequences were aligned by eye using the program BioEdit ver 7.0.9 (Hall, 1999) and compared with previously described haplotypes recorded in the Archie Carr Center for Sea Turtle Research database (http://accstr.ufl.edu/) and GenBank (http://ncbi.nlm.nih.gov). Haplotype diversity (h) and nucleotide diversity (p) (Nei, 1987) were calculated for each sampling location using the program DNAsp 5.10 (Rozas et al., 2003).

2.3. Stock composition

BAYES software (Pella and Masuda, 2001) with MMC (Markov-Chain Monte Carlo) method was used to carry out mixed stock analysis (MSA). This analysis estimates the proportion of individuals in the stock coming from different rookeries. We used a baseline that includes all populations from the Atlantic ocean and the Mediterranean sea (Shamblin et al., 2014). Estimates on the size of each rookery (mean number of nests per year) were included in the Bayesian approach as a weighting factor as suggested by previous studies (Bass et al., 2004; Clusa et al., 2014). Furthermore, we explored two possible sources of genetic subdivision within our data, size and sampling location. Individuals were clustered in two size classes considering the minimum size at maturation of 70 cm CCL and each location was analysed separatedly. Pairwise genetic distances among groups (F_{ST}) were calculated using Arlequin 3.52 (Excoffier and Lischer, 2010) and significant genetic differentiation was assessed across the different groupings. Furthermore, we carried out principal coordinate analysis (Fig. 2) based on genetic distances (F_{ST}) among the different sampling localities to define geographical subdivision. Finally, partial MSAs were performed when significant genetic subdivision was found in our data following the same procedure described above for the complete dataset.

3. Results

The mean CCL of the stranded turtles was 65.4 ± 072 (range = 13.5–81) cm. There was no bias between the size and location (Man Whitney *U* test, $p > .05$). A total of 5 haplotypes were identified in 135 stranded turtles one of them was novel (CCA2.14) and another one (CCA44.1) previously recorded from Atlantic foraging area but reported for the first time in the Mediterranean. The remaining haplotypes (CCA2.1, CCA3.1 and CCA53.1) have been previously recorded from the Mediterranean (Carreras et al., 2007; Garofalo et al., 2009; Yilmaz et al. 2011). The most frequent haplotypes were CCA2.1 (83.7%) and CCA3.1 (14.1%). The haplotype and nucleotide diversity were 0.281 (0.000–0.338) and 0.00035 (0.0000–0.0006) respectively (Table 1). When we performed a MSA considering all the dataset we found that all the individuals from our feeding ground originated in Mediterranean nesting beaches (Supplement 1) with the exception of some contribution from the Atlantic population of Cay Sal, Bahamas (CSL) (Supplement 2), that has low sample size and presented only common haplotypes (Shamblin et al., 2014). Such cases of strange contributions from distant and low variable nesting populations have been previously reported as being artifacts (Engstrom et al., 2002; Godley et al., 2010). For this reason, we removed the Atlantic populations from our baseline and we used as a baseline only the 13 Mediterranean rookeries described in the literature (Garofalo et al., 2009; Yilmaz et al. 2011; Saied et al., 2012; Clusa et al., 2013; Carreras et al., 2014) as done also in other studies of foraging areas in the Mediterranean (Rees et al., 2017). When using the regional baseline, most of the turtles were predicted to be originated in Turkey (TKW = 42%, TKE = 34%) with some contribution of other Levantine populations (Supplement 3) and with no major differences when using the population size as a weighting factor with the exception of some reduction of

Table 1
Distribution of haplotypes occurring in Turkish foraging grounds. KZL; Kazanlı, AKY: Akyatan, LMP: Limpoz, DTP: Davultepe and AYP: Aykap, h: haplotype diversity, n: nucleotide diversity.

		CCA2.1	CCA2.14	CCA3.1	CCA44.1	CCA53.1	Total	h	n
EAST	KZL	65		16		1	82	0.338	0.0004
	AKY	8	1				9	0.222	0.0005
	LMP	3		1			4	0.500	0.0006
WEST	AYP	2					2	0.000	0.000
	DTP	35		2	1		38	0.152	0.0002
	Total	113	1	19	1	1	135		