Studies on the Ecology and Conservation of Marine Turtles, with Particular Reference to the Mediterranean.

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Abstract

Studies on the ecology and conservation of marine turtles were undertaken with particular reference to the populations in the eastern Mediterranean:

1) The reproductive biology and threats to both the green turtle (*Chelonia mydas*) and the loggerhead turtle (*Caretta caretta*) nesting in Northern Cyprus (1992-1995) are described.

2) An assessment is made as to the effect of tagging of nesting marine turtles on their resultant behaviour and reproductive success.

3) An investigation is made into the efficacy of using Passive Integrated Transponders (PIT tags) as an alternative method of marking Mediterranean marine turtles.

4) Recent patterns of marine turtle strandings in the Mediterranean are described using data-sets from Italy, Greece, Turkey and Cyprus.

5) Results of a survey of the attitudes and knowledge of artisanal fishermen regarding turtles and their bycatch off the coasts of Turkey and Northern Cyprus are presented.

6) Results are given of a small study involving the identification of the dietary items present in the gut of loggerhead turtles in the eastern Mediterranean.

7) An investigation is made into the use of stable isotope analysis of marine turtle proteins to discern trophic patterns in marine turtles.

8) Concentrations and patterns of organochlorine contaminants in marine turtles from Mediterranean and Atlantic waters are described and discussed in relation to feeding ecology.

9) Concentration of heavy metals in Mediterranean marine turtle tissues are described.

10) Results are presented regarding the molecular profile of loggerhead turtles nesting in Northern Cyprus and comparison is made with populations nesting in Greece and Turkey.

11) Patterns of marine turtle mortality in British waters (1992-1996) are described.

Candidate's Declaration

I declare that the work recorded in this thesis is entirely my own, unless otherwise stated, and that it is of my own composition. I have not submitted this work for any other degree.

Brendan Godley
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General Introduction

Classification of marine turtles

There are generally held to be eight extant species of marine turtle, and their taxonomy and distribution has recently been reviewed (Pritchard, 1997). Seven of these belong to the family Cheloniidae, characterised by the possession of a hard carapace. These are the green (*Chelonia mydas*), black (*Chelonia agassizi*), flatback (*Natator depressus*), loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), olive ridley (*Lepidochelys olivacea*), and Kemp's ridley (*Lepidochelys kempi*), turtles. The leatherback turtle (*Dermochelys coriacea*) is the sole member of the family Dermochelyidae, and is distinguished by its leathery, unscaled, keeled carapace.

All species are thought to share a common life cycle, (reviewed by Erhart 1995; Miller, 1997; Musick and Limpus, 1997), with only relatively minor interspecific variations. Migration of adults occurs from foraging sites to breeding areas where males and females come together to mate, having reached sexual maturity at an estimated 30-50 years old. Males then return to the foraging areas and the females move onto nesting areas. Females emerge from the sea and dig nests on sandy beaches, lay several large clutches of eggs (up to 100-150) which incubate in the sand for approximately two months. Hatchlings then emerge at the sand surface and crawl to the sea. Following this, hatchlings spend the next 20 or so years in the surface foraging zone of the open ocean, before migrating to demersal neritic foraging areas. Once mature, they then begin cyclic migrations between foraging and mating grounds.

Species pertinent to this study

This thesis largely focused on the loggerhead and green turtles in the Mediterranean with lesser reference being made to Atlantic leatherback turtles.

Study sites

Most field work and sampling was carried out in Northern Cyprus. However, through study visits and collaborative links, relevant samples and data were also obtained from Turkey, Greece, Italy and Britain.

Marine turtles in the Mediterranean and European Atlantic

Of the species of marine turtles, only three are found regularly in the Mediterranean and the European Atlantic. These are the loggerhead, the green and the leatherback turtle (Brongersma 1972; Groombridge 1990). Only the loggerhead and green turtle are known to nest in the Mediterranean. The green and leatherback turtles are globally classified by the IUCN (World Conservation Union) as "Endangered", and the loggerhead as "Vulnerable" (Groombridge 1990). All marine turtle species are listed in Appendix 1 of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Within the Mediterranean, the populations of both loggerhead and green turtles are recommended to be considered as "Endangered" (Groombridge 1990)

Threats to Marine Turtles

Worldwide, marine turtle populations are generally in decline (Eckert 1995; Limpus 1995). Throughout the Mediterranean and the rest of the world threats to marine turtles are escalating (Stancyk 1995; Arianoutsou 1988; Groombridge 1990; Hutchinson and Simmonds 1992; Lutcavage *et al.* 1997). These include:

1) habitat alteration and loss
2) both direct exploitation and incidental catch in fisheries
3) both marine and land based pollution

All three hazards are frequently cited as severe threats to marine turtles. However, quantification of the relevance of the specific threats in these categories is scant. Aspects of this thesis contribute to the understanding of parts 2) and 3) above.

Aims of this thesis

The aims of this thesis were pluralistic and constitute several different research fields, involving a wide range of techniques and both domestic and international collaboration. Each of the chapters in the thesis has been designed as an independent piece of research and as such, can be read in isolation without need of any cross reference. This approach was followed in the publication of the work. Each chapter attempts to make a discrete contribution to the better understanding of marine turtle ecology and behaviour, so that adequate conservation of these species might be more easily approached.

Chapter one describes the basic reproductive biology of both green and loggerhead turtles nesting in Northern Cyprus between 1992 and 1995. This involves a description of the main nesting sites, the temporal patterns of nesting, adult female morphometrics, nesting behaviour, hatching parameters, fates of nests and threats to the current status of the two species in Northern Cyprus.

As part of the ongoing research and monitoring activities in Northern Cyprus, tagging has been used as a tool to mark individual turtles. **Chapter two** describes an investigation undertaken into the possible quantitative effects that the tagging process might exert on resultant behaviour and reproductive success.

Given possible conservation and aesthetic objections against flipper tagging, the **third chapter** describes a preliminary investigation into the use of Passive Integrated Transponders (PITs) as a possible method of individual identification.

In the **fourth chapter,** the focus shifts to a wider context and patterns of mortality in marine turtles in the Mediterranean region are examined through the collation and analysis of stranding information. Data from Italy, Greece, Turkey and Cyprus spanning several years are used to gain information regarding basic ecology of marine turtles in the region.

Given the likely threat of incidental catch in marine fisheries, **chapter five** constitutes an analysis of results of a questionnaire based survey of fishermen from the Mediterranean coast of Turkey and Northern Cyprus. Data were collected regarding the knowledge and attitudes of fishermen to marine turtles and were used to generate a realistic estimate of the levels of incidental catch in the artisanal fisheries of the region.

The **sixth chapter** describes the results of a small study involving the identification of the dietary items present in the gut of loggerhead turtles in the eastern Mediterranean.

The **seventh chapter** describes the novel investigation into the use of stable isotope analysis of marine turtle proteins to discern trophic patterns in marine turtles.

Concentrations and patterns of organochlorine contaminants in marine turtles from Mediterranean and Atlantic waters are described and discussed in relation to feeding ecology in **chapter eight.**

Chapter 9 describes the concentration of heavy metals in the tissues of Mediterranean marine turtles.

In **chapter 10**, results are presented regarding the molecular profile of loggerhead turtles nesting in Northern Cyprus and comparison is made with populations nesting in Greece and Turkey.

Patterns of marine turtle mortality in British waters (1992-1996) are described in combination with contaminant levels determined in tissues of a small number of leatherback turtles in **chapter 11**.

The final section is a brief general discussion of the relevance of this contribution to the knowledge of the species, with an appreciation of priorities highlighted for further research.

All chapters (except the general discussion) have been submitted for publication and appear in the formats required for submission to the respective journals.

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Chapter 1

Reproductive Ecology of Marine Turtles Nesting in Northern Cyprus.

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Abstract:

The reproductive ecology of marine turtles in northern Cyprus, during the nesting seasons of 1992-1995, is described. Nesting was monitored on 88 beaches. *C.caretta* was found to be more widely distributed, nesting on 84 beaches, compared to the 56 on which *C.mydas* nested. Up to 461 *C.mydas* and 519 *C.caretta* nests were recorded in any one season. These may constitute up to 30% and 10%, of the estimated Mediterranean nesting populations of these species, respectively. Data are presented regarding spatial and temporal distribution of nesting, reproductive parameters, fate of nests and threats faced. Ecological parameters are discussed in a Mediterranean and global context. The main threats to these populations were found to be: potential beach development for recreational use, sand extraction, incidental catch in fisheries, pollution and nest depredation by foxes and dogs.

Introduction

In the Mediterranean, two species of marine turtle, *Chelonia mydas* (the green turtle) and *Caretta caretta* (the loggerhead turtle) have been recorded as nesting. Both species are protected under C.I.T.E.S. (Convention for the International Trade in Endangered Species) and classified as 'endangered' and 'vulnerable' respectively by the I.U.C.N. (International Union for the Conservation of Nature and Natural Resources). In a review of the status, distribution and conservation of these populations (Groombridge 1990), it was estimated that, in this region, as few as 300-400 *C. mydas* and 2000 *C.caretta* females might nest annually. It was also recommended that both species should be treated as 'endangered' in the Mediterranean.

By 1990, the major nesting beaches identified for *C.caretta* were in Greece and Turkey, with smaller numbers recorded in Cyprus, Libya, Tunisia, Israel and Italy. Distribution of *C. mydas* nesting was found to be much more localised, the only substantial nesting areas being Turkey and Cyprus, with a few nests also recorded in Israel. A 1995 survey of the Libyan coast. has revealed the possibility of greater populations of *C.caretta* than previously recorded (pers. comm. MEDASSET, UK). Additional recent surveys have added Egypt and Syria to the list of minor nesting areas for *C.caretta* (Kasparek 1993, 1995).

Whereas the populations in southern Cyprus have been studied for many years, (Demetropoulos & Hadjichristophorou 1989, 1995), where 75 *C. caretta* and 25 *C. mydas* females are thought to nest annually (Groombridge & Whitmore 1989), the populations in northern Cyprus have only recently been researched. In 1988, the first published survey of marine turtles in northern Cyprus was carried out (Groombridge 1988; Groombridge & Whitmore 1989). During this 28 day study, 218 nests were recorded, resulting in an estimate of the annual nesting population of 25-50 *C.mydas* and 50-75 *C.caretta.* However, subsequent field surveys by local conservation volunteers suggested greater numbers than these (pers. comm. I.E. Bell, Hon. Exec. Sec., Society for Protection of Turtles in Northern Cyprus). Since 1992, these populations have been monitored annually (Broderick & Godley 1993, 1994, 1995; Godley & Broderick 1992, 1994, 1995). This paper describes some of the results of this work.

Study area and Methodology

The island of Cyprus lies in the eastern Mediterranean close to the southern coast of Turkey. This study was carried out around the coastline of northern Cyprus, between 1992 and 1995, from late May until October each year, except in 1992 when work was undertaken slightly later in the season. Work was carried out by volunteers from the University of Glasgow, in conjunction with local volunteers and officials. Data were collected in two main ways: Firstly, an intensive study site was monitored at the main nesting beaches of Alagadi (fig. 1). In addition, the rest of the coastline was surveyed every 3-6 days, depending upon logistics and resources, with a tendency towards reducing these survey intervals as the project has progressed.

Nesting Data

Throughout the nesting season, the two beaches at Alagadi were surveyed nightly. When an adult female or fresh track was encountered, species was identified from track morphology. *C. mydas* makes a symmetrical crawl track, whereas that of *C. caretta* is markedly asymmetrical. If the female was not present, the activity was categorised as one of the following:

a) "successful nesting activity" was recorded when a crawl track visibly lead to an area of disturbed sand where digging and covering had occurred. With *C. mydas,* nesting activity involves a great deal of covering up by the turtle which leaves an associated pit. In comparison, very little sand disturbance occurs when *C. caretta* nests.

b) "false crawl attempt" was recorded when some digging had been undertaken, but successful nesting had not occurred.

c) "false crawl U-turn" was recorded when a turtle made no nesting attempt, but simply crawled onto the beach and then returned to the sea.

If the female was present, activity was observed. For successful nesting activities, at the onset of laying, curved carapace measurements, of both length and width, were taken. Turtles were examined for distinguishing features and any existing tags. After laying was completed, a small numbered plastic nest tag was placed in the nest above the egg chamber. If the adult was not tagged, plastic tags (Jumbo tags or Supertags, Dalton Supplies Ltd., UK) were placed through the trailing edge of both fore flippers in the position recommended by

Limpus (1992). Both the colours of, and numbers on the tags differed each year. Repetition was avoided, so as to ease identification of older tags which became worn. Positions of all activities on the beach were triangulated, to posts at 50m intervals at the back of the beach, using 50-100m tape measures. Tracks were subsequently raked over to avoid double counting. Daytime surveys of other nesting beaches were carried out according to the same protocol as activities at Alagadi, when the female was not observed.

Predated nests and other threats to marine turtles

Throughout the season, any evidence of nest predation was recorded. This was usually signalled by the presence of egg shells scattered around the remnants of an excavated egg chamber. Signs of predators, such as fox or dog tracks were also recorded. In addition, any other threats discovered were described and investigated.

Hatching data

At Alagadi, beaches were surveyed at dawn for signs of hatching from mid July until mid October. This was signalled by the presence of numerous small tracks, creating a mottled effect over the dry sand, caused by hatchlings which had previously crawled to the sea. Hatchling tracks were then traced back to the epicentre of activity. Any predator disturbance was noted. If there were less than 20 hatchling tracks apparent, the nest was left undisturbed and caged for protection. Once complete hatching was thought to have occurred, position of the activity with respect to beach markers was recorded and the nest was then excavated by hand. Care was needed at this point, as live hatchlings could be found in the sand column. Any live hatchlings were released or retained for release the following night. From hatchlings and embryos found, the species which laid the clutch could be identified. From nest contents, percentage hatching could be estimated. Nest tags aided in correlating laying and hatching dates and hence the calculation of incubation periods. All nest debris was removed from the beach to avoid causing confusion or attracting predators. As with adult tracks, hatchling tracks were raked over to avoid subsequent double counting. The extensive surveying was conducted according to a similar protocol. The only differences being, that surveying was not daily and, due to high predation levels, all hatched nests were excavated on the same day as they were discovered.

Results

Spatial Distribution of Nesting

Over the four years, marine turtle nesting has been recorded on 88 beaches, constituting some 40km, around the coast of northern Cyprus. These varied in length from 50m to in excess of 3km. Detailed descriptions of individual beaches can be found in Godley and Broderick (1992). *C. mydas* nested on 56 of these beaches and *C. caretta* on 84. Only 4 beaches were used exclusively by *C.mydas* whereas 32 beaches had only *C.caretta* nesting on them. Figures 1 and 2 illustrate the distribution of the main nesting sites of *C. mydas* and *C. caretta*, respectively. Few beaches held more than 20 nests in any season, 7 in the case of *C.caretta* and 11 in the case of *C.mydas*. The main nesting site at Alagadi was the site of over 50 nests of each species in all nesting seasons. This is also the most densely nested site, since the two beaches here measure less than 3km, in total.

Table 1 gives the number of nests recorded in each of the four years of this study, 1994 being the most prolific year to date for both species, with 461 *C.mydas* nests and 519 *C.caretta* nests recorded. In addition, an approximate estimate of numbers of nesting females per season is given, with 154 *C.mydas* and 173 *C.caretta* females thought to have nested in 1994. These figures are generated by dividing the number of nests by a factor of 3, the estimated mean of number of nests laid per female in any season (Groombridge 1990). There were no nests which were not identified to species in 1994 and 1995 due to increased surveying frequency.

Temporal Distribution of Nesting and Hatching

Figures 3 and 4 illustrate the temporal distribution of nesting and hatching, throughout northern Cyprus in 1995, of *C.mydas* and *C.caretta*, respectively. Table 2 gives the dates of the first and last nests laid and hatched for each species in each of 1993, 1994 and 1995. Most nesting is in June and July with hatching occurring mainly in August and September, however, considerable variation exists between years.

Size of Nesting Females

Table 3 shows the sizes of nesting females measured at Alagadi, between 1992 and 1995. Where a female nested more than once in a season, and variation existed in the measurements taken, the mean size was entered into the data set. Each female is thus represented only once in any one year. The annual mean curved carapace length of nesting *C.mydas* females ranged from 88.9 to 95.6cm (absolute range 78.0-105.7cm) and annual mean widths ranged from 80.7 to 84.8cm (absolute range 63.0-96.7cm). The range of annual mean lengths of the smaller *C.caretta* were between 71.1 and 77.9cm (absolute range 65.0-86.5cm) and annual mean widths ranged from 63.8 to 68.2cm (absolute range 54.5-75cm).Overall mean values for the four years of the study are given to ease comparison with data published by other workers.

Nesting Behaviour

Over the 4 years of this study, 59 *C.mydas* and 84 *C.caretta* females, nesting on the main beaches at Alagadi, have been tagged. In 1994, 33% of the *C.mydas* and 17% *C.caretta* females which were tagged in 1992 returned to nest. In 1995, a further 50% of these *C.mydas* and 70% of the *C.caretta* females returned. Thus, 83% of all individuals tagged in 1992 have returned to nest to date. One *C.caretta* female tagged in 1993 returned to nest in 1994 and 1995. No other females tagged in 1993 or 1994 have yet returned.

Table 4 shows data derived from these tagging studies. Inter-nesting intervals; the number of days between the laying of subsequent clutches for individual females in a given season, and the incubation periods of nests are given for each species. Inter-nesting periods of *C.mydas* ranged from 10-16 days, with yearly means ranging from 12.7-13.1 days. The overall mean for *C.mydas* was 12.9 days. The inter-nesting periods of *C.caretta* ranged from 11-17 days, with annual means ranging from 13.0-14.0 days. The overall mean period for *C.caretta* was 13.4 days. Incubation periods were longer in *C.mydas,* with annual means ranging from 50.6-51.4 days, overall mean 51.1 days (absolute range 44 -59 days). The annual mean inter-nesting periods of *C.caretta* nests ranged from 47.9-48.1 days, overall mean 48.0 days (absolute range 42-60 days).

Hatching

The overall mean clutch sizes were 115.5 for *C.mydas* and 70.0 for *C.caretta*. Yearly mean clutch sizes of *C.mydas* ranged from 106.9-123.1 eggs (absolute range 23-199 eggs) with *C.caretta* yearly mean clutch sizes ranging from 60.1-75.7 eggs (absolute range 12-126). Of the nests which hatched, percentage success was high for both species, the overall mean hatching success for *C.mydas* was 84.2% (absolute range 9-100%) for *C.mydas* nests. The overall mean hatching success for *C.caretta* nests was 79.1% (absolute range 2.5-100). Table 5 shows mean clutch sizes and mean percentage hatching success of nests from throughout northern Cyprus. Hatching success is given as the percentage of total eggs in the clutch which hatched.

Fate of Nests

The fate of nests laid in 1994 is shown in table 6 for each of the two species and those which were not identified to species at hatching or predation. If no embryo or hatchling remains were found in hatched nests, accurate species identification was not always possible, hence many nests were recorded as unidentified. However, of nests laid, 42% were recorded as hatched, 27% totally predated, 9% at least partly hatched with evidence of predation, therefore, for 22% of nests, fate was not determined.

Discussion

Spatial Distribution of nesting

Figures 1 and 2 illustrate the widespread nature of the nesting beaches for both species around the coastline of northern Cyprus. In addition, many of the beaches play host to the nesting of both species. There are few beaches in the Mediterranean that have both species nesting in significant numbers. The Alagadi beaches however, which constitute the most important nesting site in northern Cyprus, have approximately equal numbers of nests laid of each of the two species. Although all but 4 of the beaches on which *C.mydas* are found to nest also have nests of *C.caretta*, 32 beaches have only *C.caretta* nesting on them. This may be due to the nature of these beaches, an idea supported by the fact that many lie in the more sheltered areas, such as those of Famagusta Bay (on the east coast). This affects both the slope, depth and qualities of the sand, possibly making these beaches unsuitable for the deeper nests of *C.mydas*.

Groombridge (1990) estimated there to be 300-400 *C.mydas* and 2000 *C.caretta* females nesting annually in the Mediterranean. Assuming these estimates are reliable, the northern Cyprus population estimates, calculated by the same methods, suggest that in the region of, 30-40% of the total *C.mydas* population and 5-10% of the *C.caretta* population of the Mediterranean nest in this region. Data collected in 1992 are thought to have yielded underestimates of nesting numbers, as some beaches were not discovered until subsequent seasons and extensive surveying did not begin on all the major beaches until mid-June.

The estimate of population size and its accuracy will be discussed in greater depth in a separate publication in preparation. However, it should be noted that there will certainly be some variation in the mean number of clutches laid by populations of nesting turtles both between species, between years and between sites. It is therefore very important to research the study population to obtain this information so that more accurate estimates of population size can be generated.

Temporal Distribution of nesting and hatching

There appears to be wide variation in the seasons of the two species of marine turtles cited in the literature for the Mediterranean. In the 1994 season, Margaritoulis & Dimopoulos (1994) recorded the first nesting activity in Zakynthos, Greece, on the 28th May, with the last on the 3rd of September. In the south of Cyprus, both species generally nest from mid June until mid August (Demetropoulos & Hadjichristophorou 1989), however some variation has been noted (Demetropoulos & Hadjichristophorou 1995). At Kazanli, Turkey, *C.mydas* has been recorded nesting from June until early August (Coley & Smart 1992). Clearly there are geographical and, as the data from this study indicate, annual variations in the marine turtle nesting season in the Mediterranean. In northern Cyprus, for example, nesting in 1993 started later than in 1994 and 1995, possibly due to the atypically cool weather that prevailed in May.

Size of Nesting Females

Most studies of *C.caretta* outside the Mediterranean find even larger nesting females, ranging from 90 to 100cm mean length (Dodd 1988). Measurements for *C.caretta* female mean carapace lengths ranging from 71.0 to 78.0 cm, are smaller in northern Cyprus than those recorded in Greece. Sutherland (1985) quotes a mean carapace length for nesting *C.caretta* on Zakynthos, Greece beaches as 81.2cm. Margaritoulis (1989) gives the mean curved carapace length for *C.caretta,* at Kiparissia Bay, Greece as 83.1cm. In Turkey, however, Kaska (1993) reported mean curved carapace measurements of 73.8cm length for *C.caretta* at Kizilot and Baran & Kasparek (1989) gave an overall mean curved carapace length for the Turkish Mediterranean coast of 75.6cm. A statistical comparison using a t-test was made to compare these female sizes. *C.caretta* females nesting in Cyprus have significantly smaller curved carapace lengths than those nesting in Greece, ($t=16.29$, $p < 0.001$, d.f.=148) and those nesting in Turkey ($t=3.52$, $p< 0.001$, d.f.=157) according to data from Margaritoulis (1989) and Baran and Kasparek (1989). These statistics point to a possibility of sub populations within the Mediterranean.

As with *C.caretta*, *C.mydas* individuals in the Mediterranean are notably smaller than those found in other regions (Erhart 1982). Mean curved carapace lengths ranging from 88 to 96cm for *C.mydas* nesting in northern Çyprus are similar to those found in Turkey, the only other major nesting site of this species in the Mediterranean. Gerosa *et al.* (1995), in a study conducted on Akyatan beach in Turkey, recorded mean curved carapace lengths of *C.mydas* as 92.1cm. Coley & Smart (1992) cite mean curved carapace length of *C.mydas* of 96cm at Kazanli, Turkey. These data were however, based on a sample size of only 4 individuals. Baran and Kasparek (1989), however quote a mean curved carapace length for *C.mydas* in Turkey as a whole as 90.1cm, significantly smaller than the overall mean found in northern Cyprus ($t=2.31$, $p< 0.05$, d.f.=109) .

Statistical analyses are not used to compare the differences found between years, due to the possible confounding effect of pseudoreplication. Returning females were present in more than one year and would thus be over represented. Mean sizes for individual years are given to illustrate variation between years. In addition to the possibility of different sub populations nesting in different geographical areas, marked inter-annual variation at one nesting site may also indicate the possibility of temporally separated demes. Only results from genetic studies will help elucidate whether this is the case.

Specifics of Nesting Behaviour

The mean inter-nesting period for marine turtles around the world is given as between 9-15 days, with each female laying 2-10 clutches in a given year (Erhart 1982). For *C. mydas* on Ascension island, this period is cited as 14 days, and as 12.1 days in Tortuguero, Costa Rica (Mortimer & Carr 1987). No other relevant data have been published for mean inter-nesting periods of *C.mydas* nesting in the Mediterranean. It is interesting to note that *C.mydas* tends to have a shorter inter-nesting interval than *C.caretta*, even though it produces larger clutches, however larger sample sizes are needed before any conclusions are drawn from these data. Margaritoulis (1989) gives the inter-nesting period for *C.caretta* in Greece as 15.2 days. This is longer than the 13-14 days recorded in Cyprus. The possible reasons why inter-nesting intervals are shorter in Cyprus might be due to; the smaller clutch size in Cyprus, a difference in levels of nutritional availability or be due to the higher prevailing temperatures which could possibly increase metabolism and therefore the rate of egg production.

The general range of incubation periods for marine turtle nests world-wide is quoted in the literature as 50-70 days (Hirth 1980). Margaritoulis (1989) quotes a mean of 55.5 days for *C.caretta* in Peloponnesus, Greece. Peters & Verhoeven (1992) cite 55 days for *C.caretta* in the Göksu Delta, Turkey. For *C. mydas* nesting in Turkey, a mean incubation period of 54 days has been reported at Akyatan, one of its most southerly sites in Turkey (Gerosa *et al.* 1995). The shorter incubation periods of 50-51 days for *C.mydas* and 47-48 days for *C.caretta* could be due to the warmer climate found in Cyprus compared to other sites where marine turtle nesting studies have been undertaken in the Mediterranean. Other studies have shown that an increase in temperature decreases the incubation period (Billett *et al.* 1992).

Hatching

The mean clutch size varies greatly from place to place. World-wide, mean clutch sizes for *C.mydas* vary from between 81 to 147 eggs and 101 to 126 eggs for *C.caretta* (Hirth 1980).

In Turkey, the mean clutch size of *C.caretta* was 91.7 eggs in the Göksu Delta (Peters & Verhoeven 1992), whereas at Patara mean clutch was found to be 70 eggs (Kaska 1993). In Greece, a mean clutch size of 117.7 eggs was recorded in Peloponnesus (Margaritoulis 1989). *C.mydas* in Akyatan, Turkey, had a mean clutch size of 123 eggs in 1994 (Gerosa *et al.* 1995). The mean clutch sizes for *C.caretta* nesting in Northern Cyprus of 60 to 76 eggs are much lower than those recorded elsewhere in the Mediterranean, except at Patara. Although there are few data available to compare other nesting populations of *C.mydas* in the Mediterranean the results found in this study of 106 to 123 eggs are well within the range of those found elsewhere in the world and also by Gerosa *et al.* (1995) in Turkey.

The hatching success of *C.caretta* in northern Cyprus of 75-83% compares favourably to that found elsewhere in the Mediterranean. Margaritoulis & Dimopoulos (1994) give a mean hatching success for *C.caretta* in Zakynthos, Greece, as 67.7%. Whereas, Margaritoulis *et al.* (1994) give a percentage of 73.4% for *C.caretta* at Kiparissia Bay, Greece. In the Göksu Delta, Turkey, Peters & Verhoeven (1992) give the mean hatching success as 77% for *C.caretta*. For *C.mydas*, values of 80-85% in each of the four years of this study, are higher than those found for *C.caretta*. This may, in part, be due to the more stable conditions of temperature and moisture that would be expected in the deeper nests of *C.mydas*.

Fate of Nests

Due to the extensive nature of much of this study, it was not possible to mark every nest to give it an absolute identity. Thus, if no remains were found at hatching or predation, then firm species identification by positional data alone, was not always possible. For this reason it is not possible to directly compare the fate of nests of each species separately. This is since those recorded as, "species unknown", are either *C.caretta* or *C.mydas* nests. From the data shown in table 6, it can be seen that predation was a significant problem in 1994, with 36% of nests affected. This was similar in all years. However, 42 % of nests did hatch with 9% hatched and predated, either during or after hatching, so increasing the total hatched to 51%. It is thought that this is an underestimate as it is easier to miss the tracks of a set of hatchlings than those of a nesting adult whilst operating a 3 day surveying regime. Thus, the 22% of nests with no assigned fate should be considered a maximum number, with some of these likely to have hatched, but not have been recorded. The other nests which should possibly be considered as contributing part to this statistic are; infertile, infected, clutches parasitised by insects and their larvae, those inundated or washed away by tides or those laid in situations with other unfavourable nest conditions.

Threats to N. Cyprus Turtle Populations

Impact of recreational use of beaches

In northern Cyprus, many of the problems associated with recreational use of the beaches have not yet reached the proportions they have in places such as Zakynthos (Arianoutsou 1988; Warren & Antonopoulou, 1990) and southern Turkey (Baran and Kasparek 1989). Tourism is still at a relatively low level, but is increasing. Most nesting beaches have no associated development, very little human usage and often, are several kilometres away from the nearest village or surfaced road. Some beaches on the east coast, near Gazimagusa (Famagusta), and on the north coast, near Girne (Kyrenia), have been heavily developed for tourism. This has resulted in the degradation of the coastline with respect to marine turtle nesting and hatching. However, these beaches are in the minority and nesting still does occur at a low level. Much of current beach usage is a result of recreational use by local people.

The major sites where this could have a significant effect on nesting turtles are at the two most prolific nesting beaches, at Alagadi. These are also public bathing beaches. This is where the largest number of turtles and nests come into contact with human activity and the possible associated detrimental effects. In the summer of 1994, the Department of Environmental Protection declared these beaches closed between 8pm and 8am, throughout the marine turtle reproductive season. This has been successfully policed and enforced in both the 1994 and 1995 seasons. In addition, parts of the beach are now cordoned off to the public during the day in an effort to protect nests. Fires and vehicles are also prohibited. One small restaurant has been built behind one of the beaches. This is only open during the day and it's negative impact on sea turtle reproductive success is likely to be minimal. Hand-in-hand with this increased official involvement with the management of these two beaches, has come a more effective beach cleaning regime and public awareness campaign.

Unfortunately, the directive to protect the turtles at these beaches is not yet enshrined in local legislation.

Sand extraction

Sand extraction on a small scale has been found to be a considerable problem at many turtle nesting beaches in northern Cyprus. In 1993, approximately 100 tonnes of sand were being removed from behind the Alagadi beaches on a daily basis. On occasion, vehicles were removing sand from as close as 50m above the high water mark. Successful lobbying has resulted in a cessation of these activities at Alagadi. Unfortunately the beach is now more stony in these areas, as sand blown to the back of the beach now has no dunes to confine it. Parts are no longer suitable for turtles to nest.

Incidental catch in fisheries

There is no established turtle fishery or evidence of any trade in turtle parts in northern Cyprus. Turtles appear to be killed by some fishermen following damage to nets by accidental entrapment. There are also a few records of turtles being shot by spear-fishermen. Each year approximately 10-30 stranded turtles of various sizes are discovered. Many have traumatic lesions. The majority are dead, however 1-2 each year are found alive, entangled in debris such as discarded fishing net, and subsequently released.

Pollution

A vast amount of marine litter is washed on to the beaches of northern Cyprus. Much of this appears to be of south-eastern Mediterranean origin, with a large proportion being plastic and medical waste (Broderick 1994). This is not only potentially damaging to nesting and hatching turtles, but is aesthetically displeasing to local people and tourists using beaches. The north coast of the island is particularly prone to litter deposition, due to prevailing currents (pers. comm. Professor Ilkay Salihoglu, Middle Eastern Technical University). Local authorities have made attempts to clear some beaches, however resources are lacking and these efforts will have to be ongoing to minimise possible negative influences.

Predation by foxes and dogs

Although adult turtles on Mediterranean beaches face little predation threat, many animals prey upon their eggs and hatchlings. No harvest of eggs by man has been observed. The terrestrial predators are red foxes *(Vulpes vulpes)*, feral and domestic dogs, ghost crabs (*Ocypode cursor*) and scavenging birds such as hooded crows and magpies (*Corvus corone cornix* and *Pica pica*). In northern Cyprus, all of the above have been found to depredate turtle nests and hatchlings, the main predators being foxes and dogs. This is similar to findings on beaches in the south of Cyprus, where foxes can be responsible for disturbing up to 70% of nests (Demetropoulos & Hadjichristophorou 1989). The majority of predation in northern Cyprus occurs either during late incubation or during the hatching period. Very little predation is associated with laying. Although 9% of recorded predation by canids was associated with signs of hatching, it is likely that this is an underestimate, with more nests assigned as predated without hatching, being partly hatched. However, disturbance caused by the initial predation and secondary scavenging by birds and crabs will mask prior hatchling tracks.

The possible solutions to this significant problem of predation have been comprehensively reviewed (Stancyk 1982). Control in this case is problematic because the nesting is diffuse, with at least 88 beaches being used throughout a lengthy coastline. In 1994, a pilot screening programme was instituted, using wire and bamboo screens to cover the nests, allowing hatchlings out, but acting as a barrier to predators. This was met with a degree of success. The main difficulty was accurately determining the exact position of the clutch and therefore, where to best place screens, especially in the case of *C.mydas* nests which can be extensively covered. Other problems encountered were; screens being disrupted by nesting females and bamboo screens being destroyed by predators.

General Discussion

In four years, an excellent baseline of biological knowledge of marine turtles in northern Cyprus has been established. Using these data, a proposal has been made to the local authorities in northern Cyprus recommending the protection of the 10 main nesting beaches, in particular those which hold the majority of *C.mydas* nesting. In addition we recommend that conservation efforts and protection of nests be concentrated on these beaches which have been shown to hold over 70% of these nests. The size of these populations and the relative lack of direct threats to them demonstrate northern Cyprus to be a critical habitat for Mediterranean marine turtles, especially *C. mydas*.

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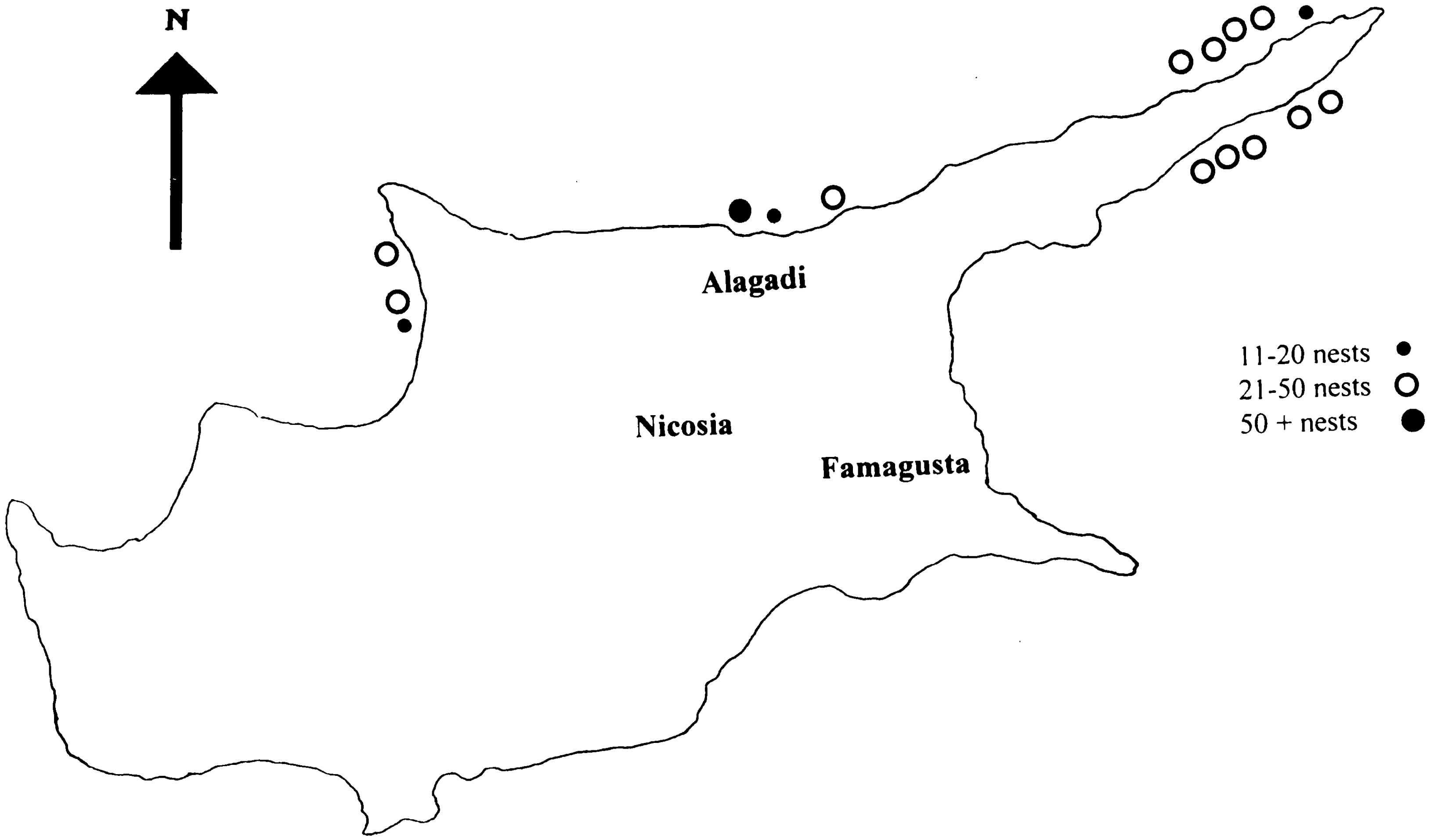

Figure 1. Main nesting sites of *C.mydas* in northern Cyprus, with an index of nest abundance at each site.

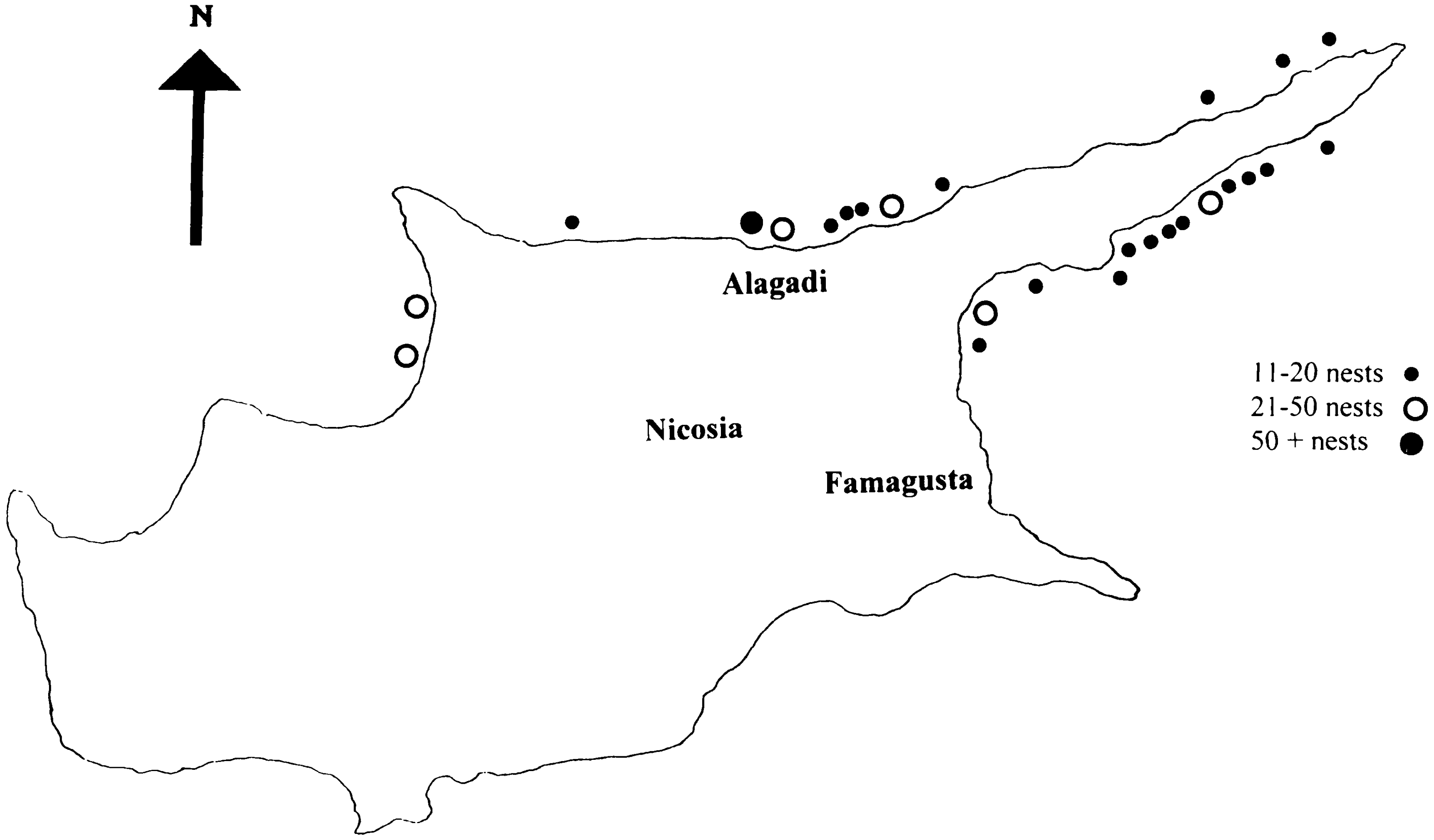

Figure 2. Main nesting sites of *C.caretta* in northern Cyprus, with an index of nest abundance at each site.

	No. of nests recorded			Population estimate		
Year	*Cm*	*Cc*	Un	*Cm*	*Cc*	Un
1992	81	65	55	27	22	18
1993	320	245	6	107	82	2
1994	461	519	0	154	173	0
1995	358	518	0	119	173	0

Table 1. The total number of nests recorded 1992-1995 in northern Cyprus with resultant population estimates, obtained by dividing the number of recorded nests by a factor of 3. Key: *Cm* - *Chelonia mydas*, *Cc* - *Caretta caretta*, Un - Unidentified species

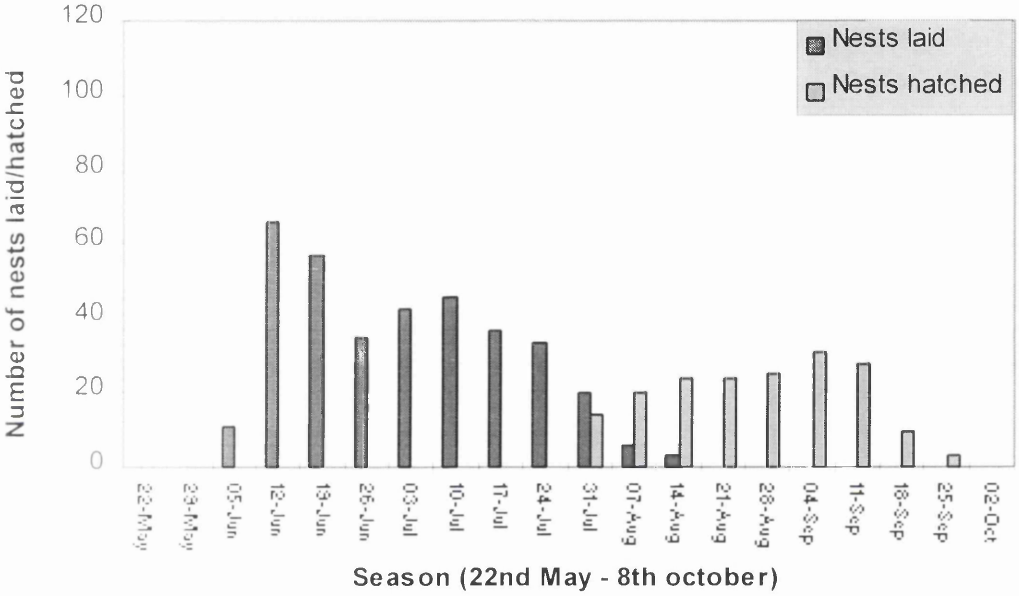

Figure 3. The Temporal distribution of nesting and hatching of *Chelonia mydas* in northern Cyprus, 1995. Weeks of the season run from 22nd May (week 1) until 8th October (week 20).

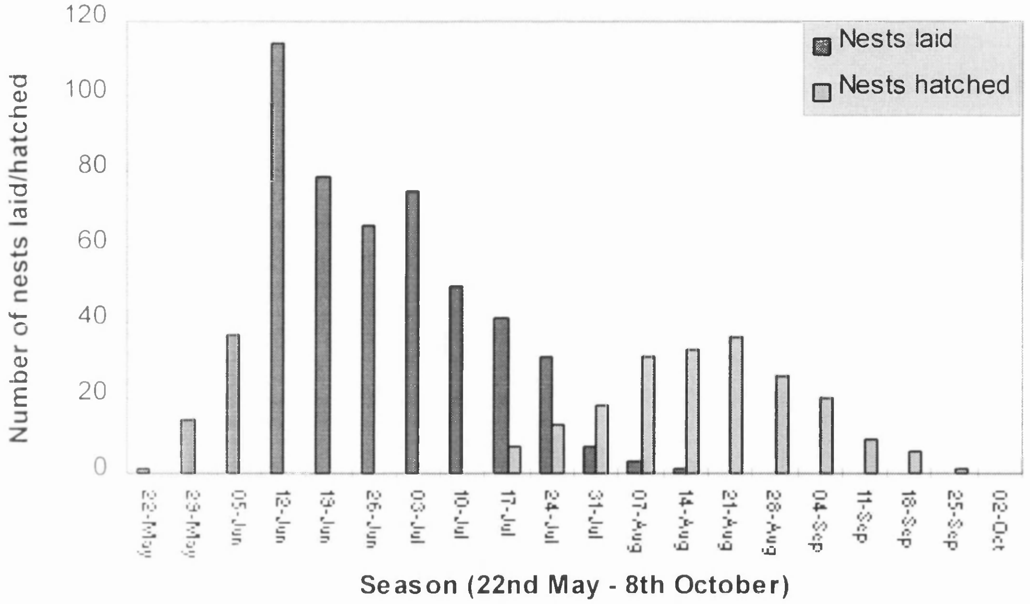

Figure 4. The Temporal distribution of nesting and hatching of *Caretta caretta* in northern Cyprus, 1995. Weeks of the season run from 22nd May (week 1) until 8th October (week 20).

	C.mydas		*C.caretta*	
Year	**Nesting**	**Hatching**	**Nesting**	**Hatching**
1993	16th June-25th Aug.	5th Aug.-8th Oct.	15th June-12th Aug.	3rd Aug.-8th Sept
1994	31st May-5th Aug.	29th July-24th Sept.	31st May-7th Aug.	18th July-24th Sept.
1995	6th June-9th Aug.	4th Aug.-25th Sept.	24th May-19th Aug.	20th July-1st Oct.

Table 2. The dates of the nesting and hatching seasons of *C.mydas* and *C.caretta* in northern Cyprus, 1993-1995.

	C.mydas		*C.caretta*	
Year	**Mean CCL±SE**	**Mean CCW±SE**	**Mean CCL±SE**	**Mean CCW±SE**
1992	92.1 ± 2.14, n=12 (78-99)	81.7 ± 1.61, n=12 (70-86.5)	71.1 ± 1.69, n=6 (66-76)	63.8 ± 1.67, n=6 (59-70)
1993	88.9 ± 1.21, n=16 (79-96)	80.9 ± 1.24, n=16 (74-89)	77.9 ± 1.83, n=8 (71-86.5)	68.2 ± 1.91,n=8 (63-77)
1994	95.6 ± 1.03, n=22 (86-105.7)	84.8 ± 1.50, n=22 (68-96.7)	72.5 ± 0.77, n=25 (66-81.7)	64.5 ± 0.73, n=25 (59-72)
1995	90.5 ± 1.44, n=19 (78.7-101.5)	80.7 ± 1.76, n=19 (63-95)	73.3 ± 0.78, n=39 (65-83)	65.5 ± 0.75, n=39 (54.5-75)
Overall mean	92.0 ± 0.74 n=69 (78-105.7)	82.2 ± 0.79 n=69 (63-96.7)	73.4 ± 0.53 n=78 (65-86.5)	65.3 ± 0.50 n=78 (54.5-77)

Table 3. The adult female curved carapace lengths (CCL) and widths (CCW) of *C.mydas* and *C.caretta* with standard errors, sample sizes and ranges, for each year of the study, 1992-1995.

	Inter-nesting period (days)		**Incubation period (days)**	
Year	*C.mydas*	*C.caretta*	*C.mydas*	*C.caretta*
1993	13.1 ± 0.42, n=15 (10-16)	-	50.6 ± 0.57, n=24 (45-54)	47.9 ± 0.62, n=17 (44-53)
1994	13.1 ± 0.32, n=18 (11-16)	14.0 ± 0.48, n=12 (12-17)	51.4 ± 0.53, n=45 (45-59)	47.9 ± 0.36, n=58 (43-55)
1995	12.7 ± 0.28, n=34 (10-16)	13.0 ± 0.32, n=20 (11-17)	51.0 ± 0.55, n=52 (44-59)	48.1 ± 0.55, n=40 (42-60)
Overall mean	12.9± 0.19 n=67 (10-16)	13.4 ± 0.27 n=32 (11-17)	51.1 ± 0.32 n= 121 (44-59)	48.0 ± 0.27 n=115 (42-60)

Table 4. Mean internesting periods and incubation periods with standard errors, sample sizes and ranges for *C.mydas* and *C.caretta* northern Cyprus for each year 1993-1995.

	Mean clutch size ±SE		**Mean hatching success (%) ±SE**	
Year	*C.mydas*	*C.caretta*	*C.mydas*	*C.caretta*
1992	112.6 ± 3.56, n=36	60.1 ± 2.89, n=30	85.3 ± 3.06, n=34	80.8 ± 4.20, n=32
1993	106.9 ±3.32, n=48	75.7 ± 3.92, n=34	83.9 ± 2.95, n=46	82.7 ± 3.37, n=34
1994	123.1 ± 3.04, n=127	70.4 ± 1.90, n=128	83.8 ± 1.78, n=125	81.6 ± 1.72, n=124
1995	112.3 ± 2.55, n=136	70.5 ± 1.98, n=131	84.4 ± 1.65, n=136	75.3 ± 1.88, n=131
Overall mean	115.5 ± 1.63 n=347	70.0 ± 1.21 n=323	84.2 ± 1.04 n=341	79.1 ± 1.16 n=321

Table 5. Clutch sizes and hatching success (%) of hatched nests for all beaches in northern Cyprus. 1992-1995.

Fate	***C.mydas***	***C.caretta***	***Unidentified***	**% of Total Nests**
Hatched	174 (38)	168 (32)	68	42
Predated	15 (3)	57 (11)	193	27
Hatched & Predated	28 (6)	28 (5)	36	9
Known Fate	217 (47)	253 (49)	297	78

Table 6. Fate of 461 *C.mydas* and 519 *C.caretta* nests laid in 1994, in northern Cyprus, numbers in brackets are percentage of total nests for each species.

Chapter 2

Assessing the Effect of Tagging Marine Turtles on Nesting Behaviour and Resultant Reproductive Success.

Submitted as:

Broderick A.C. and B.J. Godley. Assessing the effect of tagging marine turtles (*Chelonia mydas* and *Caretta caretta*) on nesting behaviour and resultant reproductive success. Animal Behaviour

Abstract

Green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles nesting on the island of Cyprus, in the eastern Mediterranean, were studied to determine the effects of flipper tagging on post-ovipositional behaviour and resultant reproductive success. Tagging was undertaken immediately following oviposition (61 green turtles; 111 loggerhead turtles). On 12 occasions, female loggerhead turtles immediately abandoned covering activities and proceeded directly to the sea. No green turtles abandoned nesting activity. In all other cases, where sufficient data were collected, the duration of three post-ovipositional phases of nesting behaviour: covering, camouflaging and descent, were compared between females that were tagged and those not tagged. No significant differences were recorded in the duration of any of the behaviours in either species. Comparisons were made of the durations of behaviours among females not tagged, tagged once or tagged twice. Descent times of loggerhead turtles tagged only once were found to be shorter. No such effect was recorded in green turtles. No effect of tagging on the likelihood of hatching or the resultant hatching success of nests of either species was demonstrated. In light of these results, the need to tag nesting marine turtles is further discussed.

Introduction

In the Mediterranean, green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles nest. The main nesting sites described for the loggerhead turtle are Greece, Turkey, Cyprus and Libya with the green turtle only being recorded in Turkey and Cyprus (Baran & Kasparek 1989, Broderick & Godley 1996, Laurent 1997, Margaritoulis 1989).

World-wide, most marine turtle research programmes involve the mark and recapture of nesting females and external flipper tags have been widely used as the method of choice in obtaining information on the life history of these species. Many different types of tags are used including plastic, monel and titanium (see Balazs (in press) for review). More recently, the use of Passive Integrated Transponders (PIT) has been explored (Fontaine et al. 1987, Parmenter 1993, McDonald & Dutton 1996, Godley et al. submitted). Loss of tags is considered a major problem in most species (Balazs 1982, Henwood 1986, Eckert & Eckert 1989, Limpus 1992, Alvarado et al. 1993, McDonald & Dutton 1994, Bjorndal et al. 1996). PIT tagging has been shown to have a lower rate of loss than that of conventional methods, possibly leading to less re-tagging, hence reduced interference as well as data of increased reliability and scientific value (McDonald & Dutton 1996, Parmenter 1993).

Few quantitative studies have examined the possible effect of human interference on nesting turtles. In a study of loggerhead turtles in Florida, Johnson et al. (1996) recorded a shorter duration of camouflaging of nests by females observed by tour groups. Similarly, Campbell (1994) observed a shortening of the covering stage when green turtles were subjected to flash photography whilst nesting in Costa Rica.

This is the first quantitative study to explore the effect of tagging on the subsequent behaviour of females and the resultant fate and success of nests.

Methodology

Study site

This study was conducted on Alagadi Beach, situated in northern Cyprus, in the eastern Mediterranean. This is one of the few sites in the region, where both species are found nesting in considerable numbers (Broderick & Godley 1996). There is very little habitation or artificial lighting in the vicinity of this 2 km beach, and it remains closed to the public from 20:00 until 08:00 throughout the nesting season, ensuring minimal anthropogenic disturbance of nesting turtles. Any visitors to the beach wishing to observe nesting females are accompanied by a researcher and follow strict guidelines after watching an informative video.

Data collection

Data were collected during patrols undertaken from 21:00 until 06:00 throughout the nesting seasons of 1993-1997. In northern Cyprus, most nesting occurs between late May and mid August (Broderick & Godley 1996). Turtles were found by walking at the waters edge without torch-light. Upon discovery, the stage of the nesting process being undertaken by the female was ascertained and the time recorded. All subsequent behavioural stages were timed and classified into the following: ascent of beach; digging body pit; digging egg chamber; oviposition; covering; camouflaging; descent of beach. A full and directly comparable description of these behaviours can be found in Johnson et al. (1996).

In this study, both green and loggerhead turtle females were tagged with plastic tags (Dalton Supplies Ltd., UK) on the trailing edge of the fore-flippers, in the position recommended by Limpus (1992). Females were only tagged if they successfully nested and attachment was undertaken during the covering phase, after the completion of oviposition. Where possible, females were tagged on both left and right fore-flippers. This was in an effort to reduce the possible confounding effects of tag loss upon ongoing ecological research. If a female had lost one tag, or it had become unreadable, a new tag was attached. Due to the short duration of the covering phase in loggerhead turtles, it was not always possible to double tag, even when no other tags were present. If a female already had two readable tags in place, no interference was necessary other than the examination of these tags and measurement of the nesting female, which took place in all cases.

In 1996, the number of successful green turtle nesting activities (n=8) was too small for inclusion in analyses and in 1997, all the individuals of this species were subjects of two additional studies which were thought to exert a possible confounding influence. In these studies, females were tagged with PIT tags (Godley et al. submitted) and had data-logging devices attached (Hochscheid et al. in prep.). None of these individuals were included in analyses. Data are therefore presented for loggerhead turtles nesting in the seasons 1993-1997 and green turtles nesting in the 1993-1995.

Results

The majority of data sets were found to deviate significantly from normality (Anderson Darling, $p<0.05$), therefore non-parametric statistical tests were used throughout and medians (with inter-quartile limits) are presented. Individuals were considered as tagged once, tagged twice or not tagged. No significant differences were found among years for the duration of any of the post-ovipositional behaviours in either species (Kruskal-Wallis, $p>0.05$: Table 1). Similarly, no significant differences were recorded in the median hatching success of nests among the years of this study for either species (Kruskal-Wallis, $p>0.05$: Table 1). Data were thus pooled for each species.

Green turtles

Descriptive statistics regarding the duration of post-ovipositional behaviours in green turtles subject to the three tagging treatments are given in table 2. In addition, data are given regarding the number of nests laid and both the number and percentage success of nests which hatched. No significant differences were recorded among the three groups of green turtles in the duration spent: covering the egg chamber (Kruskal-Wallis, $H=1.32$, $p=0.518$, d.f.=2); covering the body pit (Kruskal-Wallis; $H=2.38$, $p=0.304$, d.f.=2); or undertaking the descent of the beach (Kruskal-Wallis; $H=2.21$, $p=0.31$, d.f.=2). In addition, no significant differences were recorded when the total time spent on the beach after oviposition was examined among the three groups (Kruskal-Wallis, $H=2.94$, $p=0.231$, d.f.=2). No significant effects were recorded when the behaviours were compared between individuals which were tagged or not tagged (Mann-Whitney: covering, $W=2499$, $p=0.284$; camouflaging, $W=2440$, $p=0.14$; descent, $W=2724$, $p=0.5$; total time, $W=2078$, $p=0.091$).

The hatching success of nests laid by the three groups of green turtles was not found to vary significantly (Kruskal-Wallis, $H=1.71$, $p=0.425$, d.f.=2). There was no difference between the hatching success of nests of females tagged when compared to those not tagged (Mann-Whitney, $W=1717$ $p=0.284$). In addition, there was no difference in the proportion of nests of the females which hatched among the three groups (χ^2=0.682, p=0.711, d.f.=2) or between females that were tagged when compared to those who were not (χ^2=0.6482, p=0.421, d.f.=1)

Loggerhead turtles

On 12 occasions (<10% of tagging events; 6 in 1994, 3 in 1996, 3 in 1997) loggerhead turtles appeared to abandon nesting activity as a direct result of the tagging procedure and descended immediately to the sea. Of these individuals, three were not successfully tagged and it could not be ascertained if they revisited the site. Of the remaining nine individuals, five were subsequently recorded at the study site within the same season, with one individual emerging the following night and undertaking the full nesting process without laying eggs. Of the twelve nests laid by these individuals, four failed to hatch, two nests were transplanted (due to having been laid too close to the sea) and subsequently hatched, and the remaining six nests hatched naturally (median hatching success: 87.4%; range: 42.3-94.9%).

Duration of post-ovipositional behaviours in loggerhead turtles in the three tagging groups are presented in table 3 together with data regarding the number of nests laid, the number which hatched and the percentage hatching success. As was the case with green turtles, no significant differences were recorded in the duration of the time spent covering (Kruskal-Wallis. H=2.93, p=0.231, d.f.=2) or camouflaging (Kruskal-Wallis; H=3.7, p=0.158, d.f.=2) among the three groups of loggerhead females. There was however, a significant difference among the groups in the duration of the descent of the beach (Kruskal-Wallis; H=8.1, p=0.018, d.f.=2). Females tagged once descended the beach faster that either those tagged twice or those not tagged at all.

When the total duration of these post-ovipositional behaviours were examined, no significant differences were recorded among groups (Kruskal-Wallis, H=3.42, p=0.181, d.f.=2). When the data were pooled into females that were either tagged or not, no significant differences were recorded between groups for any of the three post-ovipositional behaviours or the sum of the durations of these stages (Mann-Whitney: covering, W=5902, p=0.128; camouflaging, W=7105, p=0.096; descent, W=6529, p=0.292.; total time, W=6021, p=0.952).

There were no significant differences in the hatching success of the clutches of loggerhead turtle females among the three tagging categories (Kruskal-Wallis, H=3.16, p=0.207, d.f.=2) or between females tagged or not tagged (Mann-Whitney, W=2625, p=0.079, d.f.=2). Additionally, no significant differences were demonstrated in the proportion of nests laid by females in the three categories which hatched (χ^2=0.309, p=0.857, d.f.=2) or in the two categories of tagged and not tagged (χ^2=0.064, p=0.801, d.f.=1).

Discussion

Personal observations of the authors would suggest that, although to a lesser extent in green turtles, there is usually some reaction by turtles to the tagging procedure, whether it be a limb withdrawal reflex or a sharp inhalation of breath. Anecdotally, it has been noted that the reaction in both species to PIT tagging is less than to flipper tagging (Godley et al. submitted). Evidence presented here demonstrates that, certainly in a small sample of loggerhead turtles, there is a clear negative reaction to the tagging process, consistent with that which would be expected resultant from experiencing a noxious stimulus. A large proportion of such identifiable individuals were recorded within the season as returning to the site (5/9) and a large proportion of these nests (6/10) hatched with a comparable median success rate (87.4%) to those of untagged turtles (93.5%; table 3) and subjected to "successful" tagging (90%; table 3). These factors combined suggest that there was little actual impact on the proximate reproductive success of these individuals.

Objectively collected data regarding behaviour and resultant reproductive success, of both green turtles and the remaining loggerhead turtles, subjected to rigorous statistical analyses suggest virtually no additional detrimental effect of tagging on the parameters measured. Tagging of green turtles has no apparent effect on the duration of subsequent nesting behaviours or on the resultant success of the nest (either as measured by the proportion of nests which hatched or median hatching success). In general, the data in loggerhead turtles are similar. However, loggerhead turtle females tagged once descended the beach significantly faster than either females which were not tagged or those tagged twice. It might be unexpected that females tagged twice did not show a difference compared with control (untagged) individuals. A possible explanation for this is that some females were tagged only once because, after the first tagging event, they quickly proceeded to undertake camouflaging before they could be tagged twice. It may be that these females demonstrated a milder form of individual sensitivity to tagging than the twelve individuals that abandoned the nesting activity. Although a statistically significant decrease in the duration of covering or camouflaging was not detected in these individuals, it may be that the behaviour of these individuals causes the duration of descent to be shorter in this group. Apart from the observation that a sub-sample of loggerhead females abandoned their nesting activity and this one result in loggerhead turtles, there would appear to be little effect of tagging female marine turtles on their subsequent behaviour. There were no demonstrable effects on the likelihood of hatching or level of success of the proximate clutch.

From an ethical stand-point, the negative impact of any invasive procedure such as tagging should be compared to the derived benefits:

1) Are conservationists unduly stressing their subject animals by tagging them? The stress response in marine turtles has been the subject of few studies (see Valverde et al. 1996). However, evidence presented here would suggest that, certainly a proportion of loggerhead turtles, may have been stressed by the tagging procedure. Animal welfare concerns should be a constant consideration in planning of research projects.

2) What is the long-term cost of tagging? Experiments in penguins subject to flipper tagging have shown this to be significant as a result of drag effects (Culik et al. 1993). Due to a combination of the facts that marine turtles are relatively large, slower moving organisms than small penguins, it might not be thought that drag effects would be substantial. However, several workers have noted the fact that tags are often subject to biofouling (Schmid in press, Balasz in press). Fouling organisms, once established, are likely to exert considerable drag effects. Additionally, it has been suggested that there is a possibility that tagging wounds may become infected (Witzell 1998). On Playa Grande, Costa Rica, a large number of leatherback turtles (*Dermochelys coriacea*) suffered from post-tagging infection in 1992 (Arauz pers. comm.). Although we are not aware of any documented cases, given that annually, many thousands of turtles are tagged globally, if this only occurs in a relatively small proportion of cases this impact may still not be acceptable. Possibly more importantly, a preliminary study, Nichols *et al.* (1998) has suggested that black turtles (*Chelonia mydas agassizii*) (n=2) may have become entangled in fishing nets by their plastic flipper tags. This was then partially confirmed by interviews

with fishermen and an aquarium study where only tagged turtles were observed to become entangled. It was suggested that turtles without such tags are better able to escape nets.

3) Does tagging really give best value for money? Besides welfare considerations, validity for tagging should be considered within the context of comparison with plausible alternatives in obtaining the information sought from tagging. Marking nesting marine turtles has been a crucial part of prior research leading to the current state of understanding of marine turtle biology. However, Witzell (1998) has recently questioned the validity of the current large number of tagging programmes world-wide, iterating an earlier call for caution (Mrosovsky 1983). Witzell (1998) suggests that with recent developments in two key areas (molecular genetics and satellite telemetry), resources currently given to tagging programmes in many cases may be better apportioned to techniques more likely to yield the answers to key questions.

Whilst dealing with research into the conservation and management of endangered species, it is healthy to consistently subject the validity and usefulness of any technique to objective retrospective analyses. This is especially true when relating to invasive procedures such as tagging. It is recommended that additional studies be undertaken by other tagging projects to objectively assess the quantitative impacts of these activities. In addition, increased effort should be afforded to the development of alternatives to existent methods, examples of steps already taken are PIT tagging and the use of photo-identification (McDonald & Dutton 1996). This is especially true in situations where there is a suggestion that the protocol may be causing increased mortality (Nichols et al. 1998). In situations such as these, the onus should be put on conclusively demonstrating the acceptable levels of safety of the technique rather than clearly demonstrating any negative effect.

In this study we have clearly demonstrated that tagging has some effect on the behaviour of at least a portion of loggerhead turtles, whereas green turtles subjected to the same treatment at the same site appeared unaffected. Although this effect does not appear to have any deleterious impact on subsequent reproductive success, it should be considered as part of the holistic rational regarding the justification of the use of an invasive procedure on endangered species.

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	Green turtles	*Loggerhead turtles*
Covering of egg chamber	H=0.23, p=0.889, d.f.=2	H=4.1, p=0.394, d.f.=4
Camouflaging	H=3.38, p=0.185, d.f.=2	H=4.41, p=0.354, d.f.=4
Descent of beach	H=4.88, p=0.092, d.f.=2	H=2.86, p=0.582, d.f.=4
Hatching Success	H=0.18, p=0.916, d.f.=2	H=8.92, p=0.058, d.f.=2

Table 1. Results of statistical comparisons (Kruskal-Wallis tests) of the duration of post-ovipositional behaviours and of hatching success among years (Green turtles: 1993-1995; Loggerhead turtles 1993-1997)

	Tagged once	*Tagged twice*	*Not tagged*	*Tagged*
Cover (min)	7 (5-10). n=13	8 (5.25-10). n=36	9 (7-10). n=59	8 (5-10). n=49
Camouflage (min)	45 (36.5-65) n=13	55 (37-70). n=35	55 (44-79), n=63	55 (37.5-67.5). n=48
Descent (min)	5 (4-9.5). n=13	5 (1-6). n=35	5 (1.25-6). n=60	5 (2.25-7), n=48
Hatch Success (%)	92.5 (79.5-97.0), n=10	89 (84-95). n=31	93.5 (83-96). n=48	90 (82-95.5), n=41
Number laid	13	39	69	52
Number hatched	10	31	50	41

Table 2. Median duration (inter-quartile range) of the three post-tagging behaviours of green turtles (1993-1995). In addition median hatching success (inter-quartile range) and the number of nests laid and the number which subsequently hatched are given.

	Tagged once	*Tagged twice*	*Not tagged*	*Tagged*
Cover (min)	4.5 (3-7.5), n=20	5 (3-9), n=66	6 (5-9), n=59	5 (3-8.25), n=86
Camouflage (min)	10 (6-15), n=23	11 (8-16.25), n=66	9.5 (6-14.75), n=60	11 (8-16), n=89
Descent (min)	1 (1-2), n=24	2 (1-4.25), n=66	2 (1-4), n=60	2 (1-4), n=90
Hatch Success (%)	88 (76-94), n=15	90.2 (69-94), n=38	81.3 (61-92), n=37	89 (73.5-94), n=53
Number laid	25	70	64	95
Number hatched	15	38	37	53

Table 3. Median duration (inter-quartile range) of the three post-tagging behaviours of loggerhead turtles (1993-1997). In addition median hatching success (inter-quartile range) and the number of nests laid and the number which subsequently hatched are given.

Chapter 3

The First Use of Passive Integrated Transponders (PITs) as a Tool in the Study of Mediterranean Marine Turtles.

Submitted as:

Godley, B.J., A.C. Broderick and S. Moraghan. The first use of Passive Integrated Transponders (PITs) as a tool in the study of Mediterranean marine turtles. Chelonian Conservation and Biology.

Introduction

A variety of methods have been utilised in the marking of free-ranging marine turtles to enable subsequent identification upon re-encounter. Information which can be derived as a result of such studies include data regarding movements, intra- and inter-seasonal nesting behaviour, estimation of population size and growth rates. These are parameters which must be accurately described to allow for successful conservation and management of these threatened and endangered species. The method most commonly used is flipper tagging, of which a variety of designs, materials and protocols have been used in different species due to factors such as tag strength, durability of markings and retention (Balazs, 1982; Henwood, 1986; Eckert and Eckert, 1989; Alvarado et al., 1993). Tag loss has been considered as a major confounding factor in many marine turtle studies (Limpus, 1992). In addition, recent concern has been expressed regarding the possible role certain tag types might have on the likelihood of bycatch in net-based fisheries (Nichols et al., 1998). Although most studies continue to use traditional flipper tags, a possible long-term alternative has been identified in the use of Passive Integrated Transponders (PITs) (Fontaine et al., 1987; Parmenter, 1993). After several years use, this technique has allowed estimation of tag loss and revision of previous over-estimates of nesting numbers (M^{c}Donald and Dutton, 1996).

Since 1992, an annual, intensive tagging programme of Mediterranean green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles has been undertaken at Alagadi beach, northern Cyprus (Broderick and Godley, 1996). Here, a combination of traditional stock tags (Jumbo tags and Supertags, Dalton Supplies Ltd., UK) have been used and 61 green turtles and 111 loggerhead turtles have been tagged on the trailing edge of both fore-flippers in the position recommended by Limpus (1992). Given the possible advantages of PIT tagging, an initiative has been developed using this technique and a test of its short-term usefulness is presented here. This study constitutes the first use of this technique in the Mediterranean.

Methodology

During the laying process all nesting female turtles at Alagadi, northern Cyprus, were checked for existing tags or tagging scars. In addition to conventional flipper tags, females nesting in 1997 were each tagged using PITs (Identichips, AnimalCare Ltd, UK). All manipulations were undertaken immediately following the cessation of laying, as the turtle began to cover the egg chamber. PITs were injected into the shoulder musculature approximately 5 cm from the carapace margin at the site suggested by M^{c}Donald and Dutton (1994). Bilateral implantation was undertaken whenever possible. However in a proportion of individuals, because of body position or a shortness of the period elapsing before large-scale flipper movements associated with nest camouflaging began, only unilateral tagging was possible. PITs were not considered successfully applied unless they were read correctly after injection. When females returned to lay a subsequent clutch later in the season, hand-held readers (AnimalCare Ltd., UK) were used to read PITs.

Results

A total of 17 loggerhead (13 bilaterally tagged; 4 unilaterally tagged) and 4 green turtle (4 bilaterally tagged) nesting females were marked using PITs. When females returned to lay subsequent clutches, it was possible to test whether the PITs were still readable. Of the 4 green turtles, 3 returned to nest and in all cases, both tags were read (100% re-identification). Of the 17 loggerhead turtles, 11 individual were re-encountered (8 bilaterally tagged; 3 unilaterally tagged). Of double tagged individuals, both tags were read in 7 cases, and only one was read in the other (100% re-identification). Of the three individuals singly tagged, only one was re-read (33% re-identification). In both cases where re-identification was not possible, the turtles were re-tagged. Overall, therefore, 82% of tagged loggerhead turtles would have been re-identified by PIT tagging alone. Some females (n=5) returned to nest more than one occasion after initial PIT tagging. If tags had been successfully read after one inter-nesting interval, they were always read again. Apart from slight haemorrhage, on no occasion were any deleterious post injection effects observed. All injection sites had healed well after one inter-nesting interval of 10-15 days, no wound infection or swellings were detected. In addition, qualitative observations of the behaviour of turtles during tagging, suggested that reaction to the PIT tagging process was less than that to traditional tagging. It may be that PIT tagging is less noxious to marine turtles than traditional methods

Discussion

It is apparent that in the short-term, PIT tagging yields high levels of re-identification (100% for green turtles; 82% for loggerhead turtles). Re-reading levels for individual PITs was very high (100% in green turtles; 84% in loggerhead turtles). It is likely that failure to re-read PITs was more due to the fact that they were actually lost through the implantation hole, soon after the time of application, before the wound had healed, rather than due to intrinsic failure of the system. This hypothesis is supported by the likelihood of failure of the methodology being greater in singly tagged loggerhead turtles, which had proved difficult to mark either due to the beginning of camouflaging movements or their body position during egg-laying. PIT loss could be reduced by the use of surgical glue to seal the wound after application.

Assuming long-term retention and function of PITs, over several years, the utilisation of such a technique will enable remigration patterns to be more accurately assessed since loss of conventional tags can confound such studies. In addition, given concern as to the possible long-term effects of some traditional tagging methods, both in marine turtles (Nichols et al., 1998) and other species (Culik et al., 1993) and qualitative observations regarding reactions to the tagging process (this study), PIT tagging would appear an extremely useful tool in the study of marine vertebrates. If use of this technique became widespread (with readers being made available to individuals who might have previously reported traditional tag returns), it may offer the numerous advantages of traditional tagging without concomitant deleterious effects. Major provisos on the usefulness of this technique are firstly, that all PITs used should be able to be read by all readers used and secondly, there is a need to standardise implantation sites in all species to ensure the greatest possible likelihood of data recovery by different operators. If these criteria are not met, the value of the technique beyond the proximate, local scale is severely reduced.

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Chapter 4

Recent Patterns of Marine Turtle Stranding in the Mediterranean.

Submitted as:

Godley, B.J., F. Bentivegna, A.C. Broderick and R.W Furness. Recent Patterns of Marine Turtle Stranding in the Mediterranean: What Can They Tell Us? Biological Conservation.

Abstract

The first results of the Mediterranean Marine Turtle Stranding Network are presented. Data regarding marine turtle stranding from Italy: 54 loggerhead (*Caretta caretta*) and 2 leatherback turtles (*Dermochelys coriacea*); Greece: 20 loggerhead turtles; Turkey: 15 loggerhead and 37 green turtles (*Chelonia mydas*); and Cyprus: 26 loggerhead and 75 green turtles. In Italy, two peaks in the timing of loggerhead turtles strandings were found in August and November. In Cyprus, although data were only collected between 28th May and 7th October, a peak of green turtle strandings was evident in early July. The size class distribution of loggerhead turtles in Italy were significantly different from those from the eastern Mediterranean, with loggerhead stranded in Italy being smaller. The species make up and size class distribution patterns of strandings for both species were similar in Cyprus and Turkey. Lesions present on many turtles highlight the harmful effect of turtle-fishery interactions in the region.

INTRODUCTION

Incidental catch

Marine turtle populations are generally in decline world-wide (Eckert, 1995; Limpus, 1995). Persistent direct exploitation for meat, shell, oil, skin and eggs has been implicated in many geographic areas. However, incidental catch in many fisheries has also been cited as a major cause of mortality, especially of adult and sub-adult individuals. Bottom trawl, longline, driftnet and small coastal fisheries, have a large bycatch of marine turtles causing substantial mortality (Hillstad *et al.*, 1995; Lutcavage *et al.*, 1997). In the USA, mortality due to the shrimp fishery has resulted in the mandatory use of Turtle Excluding Devices (TEDs) in shrimp trawlers (National Research Council, 1990). In the Mediterranean, several studies have been undertaken to investigate incidental catch and these have been recently reviewed (Laurent *et al.* 1996).

Marine turtles in the Mediterranean

Only three species of marine turtle are regularly found in the Mediterranean. These are the loggerhead (*Caretta caretta*), green (*Chelonia mydas*) and the leatherback turtle (*Dermochelys coriacea*) (Groombridge, 1990). Although loggerhead and green turtles breed within the region, leatherback turtles are thought to be non-breeding visitors and are only occasionally recorded (e.g. Taskavak *et al.* 1998).

About 2000 female loggerhead turtles nest annually (Groombridge, 1990), at sites widely distributed, primarily around the eastern Mediterranean basin. It is thought that most nesting occurs on the coast of Greece (Margaritoulis, 1982; 1988; Hays & Speakman, 1991), Turkey (Baran & Kasparek, 1989a; Erk'akan, 1993; Kaska, 1993; Baran & Turkozan, 1996), Cyprus (Demetropoulos & Hadjichristophorou, 1989; Broderick & Godley, 1996; MacLean *et al.*, 1998) and Libya (Schleich, 1987; Laurent *et al.*, 1997) with smaller numbers nesting in Syria (Kasparek, 1995), Egypt (Kasparek, 1993), Italy (Gramentz, 1989) and Israel (Kuller, 1995). Juveniles of this species are known to be widely distributed throughout the region, being incidentally caught in many marine fisheries quite distant from nesting beaches e.g. Croatia (Lazar & Tvrtkovic, 1995), Corsica (Delaguerre, 1987), Italy (Argano *et al.*, 1992), Spain (Caminas, 1988; Aguilar *et al.*, 1995), France (Laurent, 1991), Tunisia

(Laurent & Lescure, 1994) and Morocco (Laurent, 1990). In a recent, region-wide analysis of population genetics involving an extensive sampling regime from both fishery bycatch and nesting populations (Laurent *et al.*, in press), it was demonstrated that the Mediterranean stocks of this species represent both an endemic regional breeding population and a migratory feeding population from the western Atlantic.

Green turtles are only found nesting in Turkey (Baran & Kasparek, 1989a; Coley & Smart, 1992; Yerli & Demirayak, 1996; Yerli & Canbolat, 1998), Cyprus (Demetropoulos & Hadjichristophorou, 1989; Broderick & Godley, 1996) and occasionally in Israel (Kuller, 1995). The estimated annual regional nesting population could be as low as 300-400 females (Groombridge, 1990). It is not known how these numbers compare to past population levels, but from anecdotal accounts of catch in the early part of this century (Sella, 1982), it would appear that the nesting population is likely to be low compared to historical levels. Groombridge (1990) recommended that both species should be treated as critically endangered within the Mediterranean. It is likely that juveniles of this species are more localised in distribution, than are juvenile loggerhead turtles. Although occasional records have been reported distant from nesting areas (Margaritoulis *et al.*, 1986), capture of green turtles in the western basin is very rare (L. Laurent pers. comm.).

Since the 1980s, the locations of major turtle nesting sites in the region have been identified, with ongoing monitoring and conservation projects undertaken at many of them. In comparison to the effort expended in researching nesting populations, relatively little effort has been channelled into studying distribution patterns, wider ecology and in particular, little is known regarding factors affecting mortality rates of these populations.

Marine Turtle Strandings

Through careful analysis of stranding information it may be possible to infer patterns regarding: differential distribution of species and size classes, factors affecting mortality and, over extended temporal scales, within-region trends of mortality may be assessed. Quantitative data regarding strandings in the Mediterranean region are, at best, scant when compared to data collated by the Sea Turtle Stranding and Salvage Network in the USA (e.g. Teas, 1993). It was recently proposed that a similar regional stranding network be established in the Mediterranean (Godley, 1995) and the findings presented in this paper represent the first results produced by this network.

METHODS

Data regarding the incidence of recent marine turtle strandings have been collected during the period 1991-1997 from four different regions (see fig 1): (1) the Gulf of Naples, Italy; (2) the island of Kos, Greece; (3) Icel, Turkey; and (4) northern Cyprus. In addition to recording the location, date, species and measurement of curved carapace length (CCL) cm, condition of stranded turtles was ascertained on an ordinal scale: 0 - Alive with no visible injuries; 1 - Alive and injured; 2 - Freshly dead; 3 - Moderately decomposed ; 4 - Severely decomposed: 5 - Dried carcass; 6 - Skeleton only. Where possible, specific carcass anomalies were recorded following the protocol of Teas (1993).

RESULTS

Location and species of stranded turtles

A breakdown of the number, species and locations of strandings is given in table 1. Apart from two leatherback turtles stranded in the Gulf of Naples, only loggerhead turtles stranded in the study regions in Greece and Italy during the period. In northern Cyprus and Turkey both green and loggerhead turtles stranded in considerable numbers, with the green turtles dominating at both sites. Although records from Cyprus were more numerous, there was no difference in the proportion of green and loggerhead turtles between these two sites (χ^2 =0.169, p=0.681, d.f.=1).

Temporal distribution

Italy

The temporal distribution of loggerhead turtle strandings, pooled for all years, is shown by month in figure 2a. There are two peaks of loggerhead turtle strandings. No strandings were recorded in December-February, between two and six individuals were discovered in each of the months March-July, with a pronounced peak of 10 individuals in August. Although only one individual was discovered in September, there was a marked peak in October and

November with 7 and 13 individuals being recorded, respectively. The two leatherback turtles found in this region both stranded in the autumn (1 in each of October and November).

Cyprus

Data regarding strandings in Cyprus were only collected between 28th May and 7th October (1992-1997) i.e. the turtle breeding season. The temporal distribution of green turtle strandings, pooled for all years, in 10 day periods is shown in figure 2b. Strandings occurred throughout the summer. A peak is observed at the start of the period, largely due to old carcasses being discovered on the first survey of the season. However, a marked peak in strandings is observed in July, with a gradual reduction in each 10 day period until the end of surveying on the 7th October. Because of the smaller sample size for loggerhead turtle stranding events (Figure 2c), no clear temporal pattern is evident.

Greece and Turkey

Owing to the opportunistic nature of sampling and the small sample sizes in these regions, no comment is made regarding temporal distribution in these areas.

Size class distribution

Loggerhead turtles

Figures 3a)-d) show the pooled size frequency distribution of the stranded loggerhead turtles in 5cm size classes discovered at the sites in Italy, Greece, Turkey and northern Cyprus, respectively. Investigation of the size-class distributions showed that most data sets were not normally distributed (Anderson-Darling $p<0.05$). Therefore for robust statistical treatment, non parametric tests were used throughout and medians were tested for differences among groups. Unfortunately, all relevant papers regarding incidental catch and mortality (see Table 2) cite mean values for carapace length. For comparison, mean curved carapace length for each site (with standard deviation) is also given in the relevant figure. The size class distribution in Italy was comparable to that found in a similar study carried out within the region (Meotti *et al.*, 1995). In addition, mean curved carapace length of Italian samples was very similar to that bycaught in fishery observer programmes in nearby Italian and Maltese fisheries (Table 2).

Median CCL differed among areas (Kruskall-Wallis $H_3=11.61$, $p<0.001$), with the size of loggerhead turtles stranded in Italy significantly smaller than those from Greece, Cyprus or Turkey. When data from the three sites in the eastern basin were combined (Fig 5a), it can be seen that the size distribution is very much biased towards large juvenile, and adult, sizes. If this pooled sample from the eastern Mediterranean is compared to that of the Italian sample, as an index of the loggerhead turtles being stranded in the western Mediterranean, there is a marked difference in median size (Mann-Whitney: $W=930.5$, $p=0.01$). Mean curved carapace length of loggerhead turtles stranded in the eastern Mediterranean samples (63-66cm) is very similar to those found in regional fisheries in Greece, Turkey and Egypt (Table 2).

Other studies have utilised a "large individual index" (Laurent *et al.*, in press) which assigns the number of likely adults as those with a curved carapace length greater than 70cm (the minimum size of nesting female at the largest loggerhead rookery in the Mediterranean, at Zakynthos in Greece; Margaritoulis, 1982).

In the eastern sites, 37% (23/61) of individuals had a CCL greater than 70cm whereas only 14% (7/50 of individuals measured) of the Italian individuals were larger than this threshold. This difference was highly significant ($\chi^2=7.83$, d.f.=1, $p=0.005$).

Green turtles

Figures 4a) and 4b) show the size frequency distribution of stranded green turtles in 5cm size classes in Cyprus and Turkey, respectively. There was no difference in median size of green turtles between the two sites (Mann-Whitney: $W=2112$, $p=0.895$). When data from both sites are combined (Fig 5b) it can be seen that the size class distribution of this species is skewed towards the smaller juvenile size classes, markedly different from the pooled eastern Mediterranean loggerhead turtle data set (Fig 5a). Taking the smallest nesting female size class as a minimum adult size (78cm, therefore 76-80cm CCL; Broderick and Godley 1996), it can be seen that very few adults are stranded (5% (4/75) in Cyprus; 11% in Turkey (4/37)). This proportion was not significantly different ($\chi^2=0.995$, d.f.=1, $p=0.329$).

Condition Score

Table 3 shows the breakdown of condition score of turtles stranded from Italy and Cyprus. It is evident that a large proportion of turtles stranded in Italy were encountered when still alive, so could be rehabilitated (Bentivegna *et al.*, 1993). In Cyprus, most turtles were dead when encountered, often in a very degraded state. This makes detailed comment on reasons for death difficult in some cases.

Carcass Anomalies

Table 4 illustrates the preponderance of major carcass anomalies recorded in the sub-sample of the turtles examined in both Italy and Cyprus. Anomalies are classified and compared to a large data set describing turtles recorded stranded on the Atlantic coast of the USA in 1992 (Teas 1993). Several important points emerge from these data:

1) A large proportion of loggerhead turtles examined in both the Italian sample and to a lesser extent the Cyprus strandings had been subject to prior bycatch in longline fisheries.

2) Incidental catch in longlines does not appear to be a problem in green turtles stranded in Cyprus.

3) Although green turtles stranded in Cyprus did not show any evidence of interaction with longlines, five turtles (all small individuals) were discovered entangled in discarded fishing nets.

4) A large proportion of the individuals of both species had cranial trauma. This, compounded with the discovery of individuals in which the skull was missing, and the high proportion of turtles which were mutilated (by severing of neck or removal of carapace) is highly suggestive of incidental capture and subsequent discarding by fishermen.

DISCUSSION

Marine turtle stranding information has yielded a number of insights into the biology of the loggerhead and green turtles in the Mediterranean and the threats they face: The wide distribution of the Mediterranean loggerhead is demonstrated as is the apparently restricted geographical range of the green turtle. The ecological reasons for these patterns are not yet known and are worthy of further investigation.

Detailed comment on the temporal distribution of strandings is difficult, as sampling may be biased by a number of factors such as prevailing currents, wind direction and strength as well as the size and species of the turtle. However, although it may be indicative of high levels of abundance or activity of turtles, the apparent peak in strandings July/August in both the Italian and Cyprus data sets may be related to peak fishing activity in summer. In a recent study, most fishermen questioned in Cyprus and Turkey indicated that they caught more turtles during summer months, also the time of peak fishing effort (Godley *et al.*, in press a). The second peak in the Italian data set during October and November could be due to the onset of cooler temperatures causing debilitated individuals to die or become increasingly debilitated, so increasing their likelihood of stranding (Morreale *et al.* 1992). These hypotheses would both require further testing.

Although only based on two individuals, the fact that the leatherback turtles were discovered in the autumn months is consistent with the pattern discovered by both the studies of Brongersma (1972) and Godley *et al.* (in press b) in the European Atlantic, thought to be correlated with individuals moving into northern waters when temperatures are at a peak in late summer. Another recent record of this species in the eastern Mediterranean was a capture in October 1997 in Turkey (Taskavak *et al.* 1998).

No turtles of less than 21 cm CCL were recorded stranding during this study. It might be expected that due to a combination of these individuals being less conspicuous and more likely to be consumed by marine and terrestrial scavenging animals would lead to relative under-observation. However, very few individuals less than 21 cm have been recorded during numerous incidental catch surveys (table 2), and any which have been caught have been in the western Mediterranean. It is uncommon to detect such small size classes except in pelagic areas (see Musick and Limpus, 1997 for review). Although these oceanic developmental habitats have been quite well identified for the loggerhead populations nesting in the south-eastern USA, they remain to be described for the Mediterranean population.

It has been hypothesised previously from stranding data (Baran & Kasparek 1989b; Godley *et al.* in press c) that the western Mediterranean is likely to be the early developmental habitat for loggerhead turtles, as relatively few juveniles are found in the eastern Mediterranean. The differential size-class distribution of loggerhead turtles among sites in this study further highlights this suspected difference between basins. If small juveniles were present in the eastern basin in large numbers, it is highly likely that they would be caught in fisheries and found stranded. Juvenile green turtles appear to be caught in large numbers in the eastern Mediterranean, and in other regions, where the same fishing methods are used, numerous loggerhead turtles are captured (table 2).

The size class distribution of green turtles stranded in the Mediterranean may reflect the population make-up in the region. However it is more likely that the lack of large individuals in the data set may be due to a lower likelihood of fishery interaction due to both the herbivorous nature of the green turtle and their large size. Herbivorous species are less likely to be caught as a result of taking long-line bait and opportunistically feeding on fish already caught in nets.

A large proportion of loggerhead turtles examined in both the Italian and the Cyprus sample had survived but had been injured in prior bycatch in longline fisheries. This is in concordance with previous studies in Italy (Meotti *et al.*, 1995) and Greece (Nantsou & Antipas. 1992). Incidental catch in longlines does not appear to be a problem in green turtles stranded in Cyprus (table 4). This is in broad agreement with a recent survey of fishery-turtle interaction in Cyprus (Godley *et al.*, in press a), in which fishermen cited nets as being the major mode of marine turtle bycatch. An additional five green turtles were discovered entangled in discarded fishing nets.

A major cause of concern in this region is the large proportion of the individuals of both species with apparent deliberately induced trauma, which is further suggestive of substantial incidental capture and subsequent discard by fishermen. This, compounded by the clear role of longlines. highlights the need for additional research into the role of incidental catch in the region.

This preliminary investigation has highlighted three targets for those endeavouring to conserve marine turtles in the Mediterranean: 1) The need for more research into the interaction between marine turtles and fisheries; 2) The need for a greater research effort to be channelled into understanding the distribution of juveniles and adults outwith nesting periods. 3) The need for wider scale recording and international collaboration to provide ecological information vital to the conservation of these species, through the analysis of stranding patterns.

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Specific area	*Period*	*C.caretta*	*C.mydas*	*D.coriacea*	*Total*
Gulf of Naples, Italy	1993-97	54	-	2	56
Kos, Greece	1993-94	20	-	-	20
Icel, Turkey*	1991-97	15	37	-	52
Northern Cyprus	1992-97	26	75	-	101
TOTAL		**115**	**112**	**2**	**229**

Table 1: Breakdown of the number of stranding reports for each species in each of the study areas. *Selected data (excluding nesting females thought to have been killed by jackals) are included with permission from the two University of Nijmegen technical reports (Peters & Verhoeven, 1992; van Pigglen, 1993).

Fishery type	*Location*	*Method*	*Sample Size*	*Size (CCL)*		*Literature source*
				range (cm)	**mean (cm)**	
All fishing gear	Algeria	Sampled	22	17-73	54.7	Laurent 1990
Drifting longlines	Spain 1990	All	472	27-76	47.4	Aguilar *et al.* 1995
Drifting longlines	Spain 1991	All	392	35-71	48.8	Aguilar *et al.* 1995
Trawling	France	Sampled	11	35-76	48.9	Laurent 1991, 1996
All fishing gear	France	Sampled	58	28-76	42.47	Laurent 1991, 1996
Trawling	Italy	Sampled	48	19-89	52.3	Argano (unpubl. data) cited in Laurent *et al*.1996
Drifting longlines	Italy	Sampled	656	18-95	55.4	
All fishing gear	Italy	Sampled	798	18-95	54.4	
Drifting longlines	Malta	Sampled	123	24-76	55	Gramentz (unpubl. data) cited in Laurent *et al.* 1996
All fishing gear	Malta	Sampled	186	20-76	52.3	
Trawling	Tunisia	All	15	43-90	73.4	Laurent & Lescure 1994
Trawling	Tunisia	Sampled	65	32-92	58.5	Laurent *et al.* 1996
All fishing gear	Tunisia	Sampled	147	18-92	56.5	Laurent *et al* 1996
Trawling	Greece	All	38	NQ	NQ	Margaritoulis *et al.* 1992
Trawling	Turkey	All	1	61	61	Laurent *et al* 1996
Trawling	Egypt	Sampled	16	49.4-83	64.7	Laurent *et al* 1996
All fishing gear	Egypt	Sampled	48	38-86.3	65.7	
All fishing gear	Egypt	Sampled	21	28-95.5	66.8	

Table 2. Size (CCL) of loggerhead turtles (*Caretta caretta*) captured by different fisheries in the Mediterranean. Method (Sampled: only a portion of individuals caught were measured; All: all individuals captured were measured; NQ: Not quantified)

Score	*Italy* Loggerhead (n=53)	*Italy* Leatherback (n=2)	*Cyprus* Loggerhead (n=26)	*Cyprus* Green(n=75)
0	15 (28%)	1	0	7 (9%)
1	14 (26%)		2 (8%)	0
2	11 (21%)	1	4 (15%)	13 (17%)
3	3 (6%)		5 (19%)	18 (24%)
4	10 (19%)		3 (12%)	10 (13%)
5	0		12 (46%)	27 (36%)

Table 3. Summary of data regarding condition score of marine turtles strandings (where available) collected as part of the study (Score: 0 - Alive with no visible injuries; 1 - Alive and injured; 2 - Freshly dead; 3 - Moderately decomposed ; 4 - Severely decomposed; 5 - Dried carcass; 6 - Skeleton only)

	All species USA 1992 (n=1560), Teas 1993	*Loggerhead Italy (n=30) This study*	*Loggerhead Cyprus (n=20) This study*	*Green Cyprus (n=49) This study*
Fishery related				
Hook in Gut	1.3%	33%	15%	0
Line in Gut	1.4%	7%	0	0
Entangled in fishing gear	2.1%	0	0	10%
Harpooned	not recorded	0	10%	0
Bullet wounds	0.3	0	0	2%
Skull injuries	2.5%	7%	45%	16%
Skull missing	3.6%	0	5%	16%
Other mutilation	1.8%	0	15%	25%
Boat strike	10.3%	0	5%	2%
Other trauma				
Carapace Damage	12.2%	17%	5%	2%
Plastron damage	2.1%	3%	0	0
Skull & flippers missing	7.8%	0	0	16%
Flippers missing	6.5%	23%	0	2%
Flipper damage	8.7%	10%	0	0
Pollution				
Plastic in guts	3.8%	0	0	10%
Oiling	0.2%	0	0	0
Entangled in non-fishing gear	0	0	5%	0

Table 4 Proportion of stranded individuals with different carcass anomalies based on nomenclature of Teas (1993).

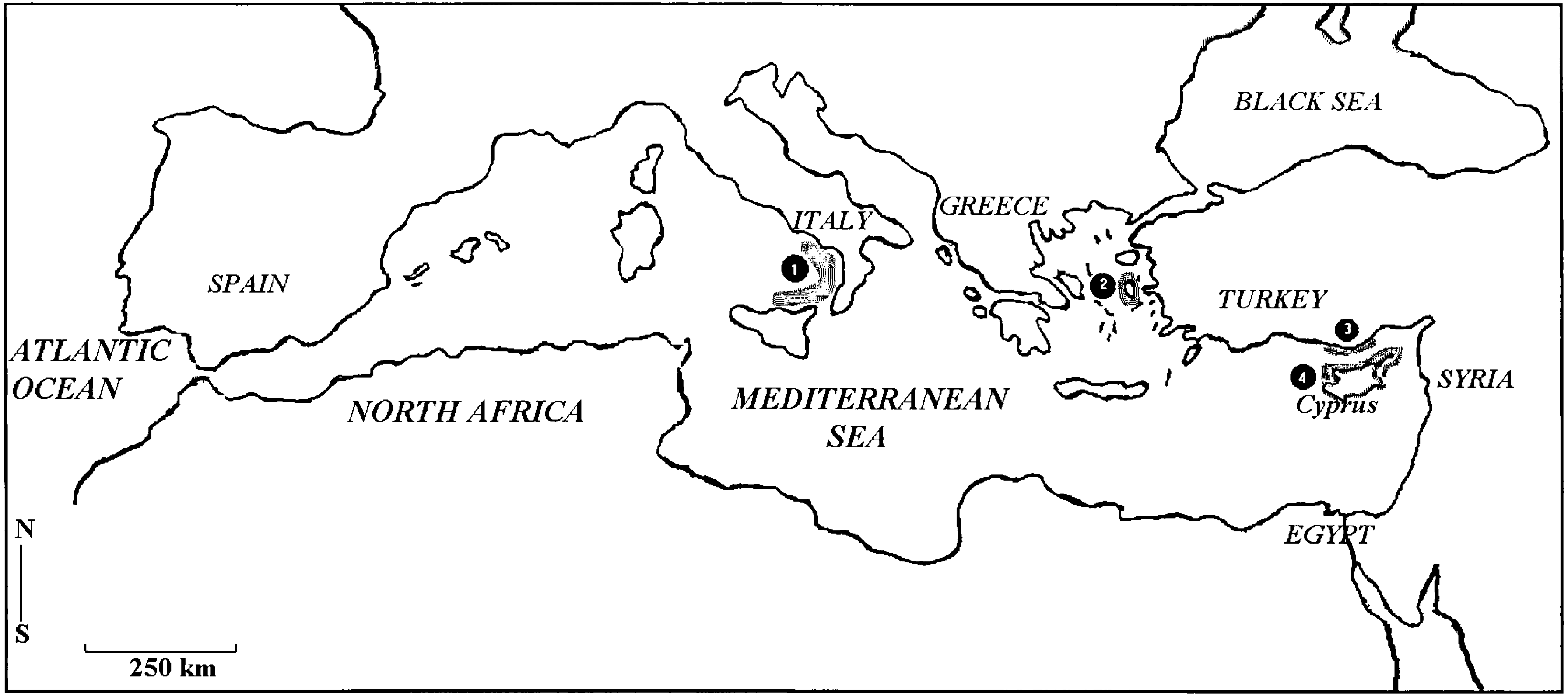
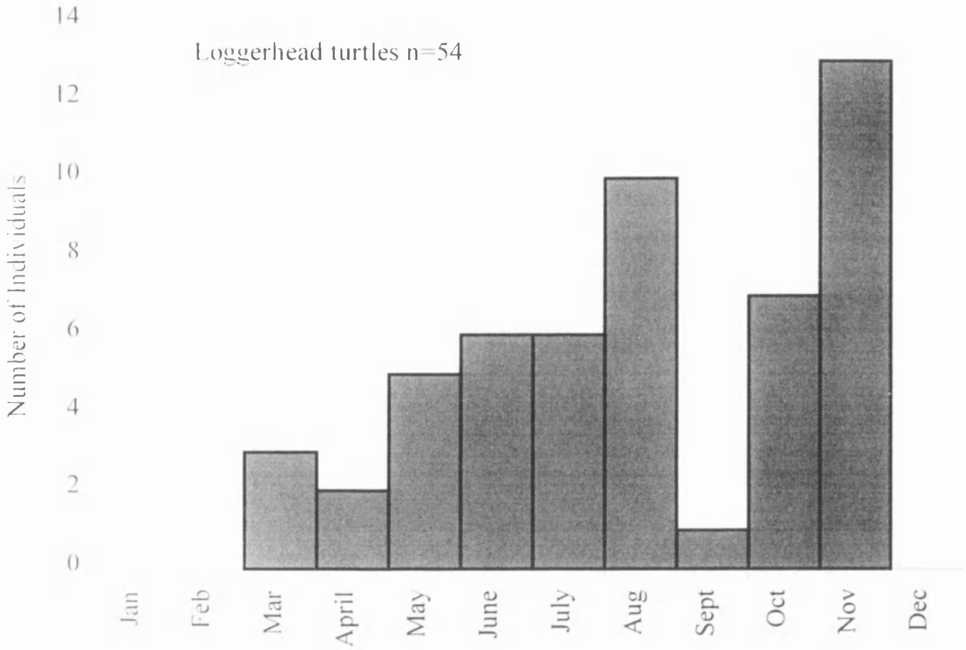


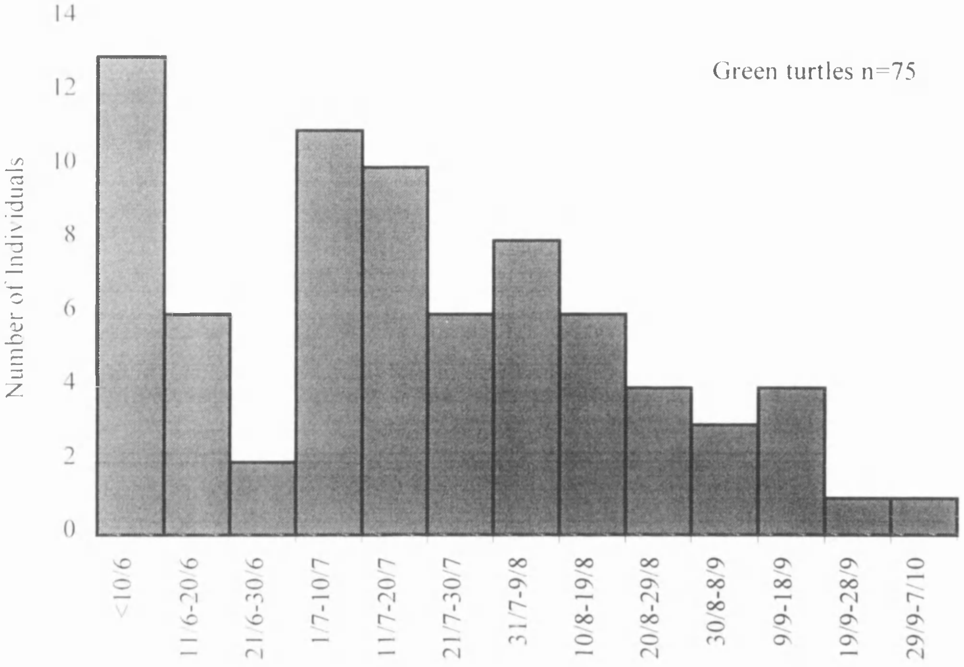
Figure 1. Map of Mediterranean Sea to illustrating sites for this study ((1) the Gulf of Naples, Italy; (2) the island of Kos, Greece; (3) Icel, Turkey; (4) northern Cyprus).

Figure 2. Temporal Distribution of Strandings in Italy (loggerhead turtles; Fig 2a) and Cyprus (green turtles: Fig 2b; loggerhead turtles: Fig 2c).

2a


Loggerhead turtles n=54
Number of Individuals
0
2
4
6
8
10
12
14
Jan
Feb
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Dec

2b


Green turtles n=75
Number of Individuals
14
12
10
8
6
4
2
0
<10/6
11/6-20/6
21/6-30/6
1/7-10/7
11/7-20/7
21/7-30/7
31/7-9/8
10/8-19/8
20/8-29/8
30/8-8/9
9/9-18/9
19/9-28/9
29/9-7/10

2c

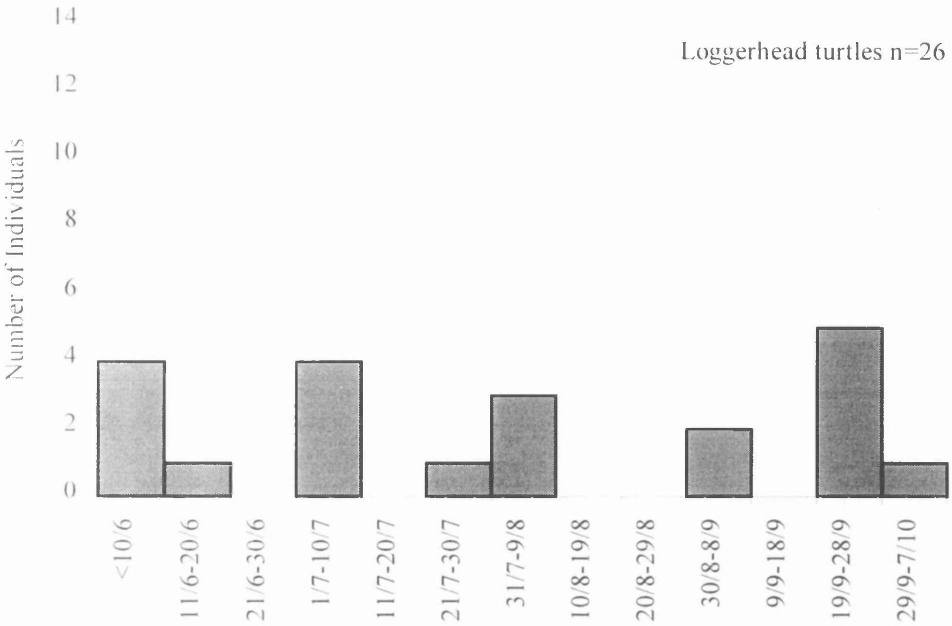
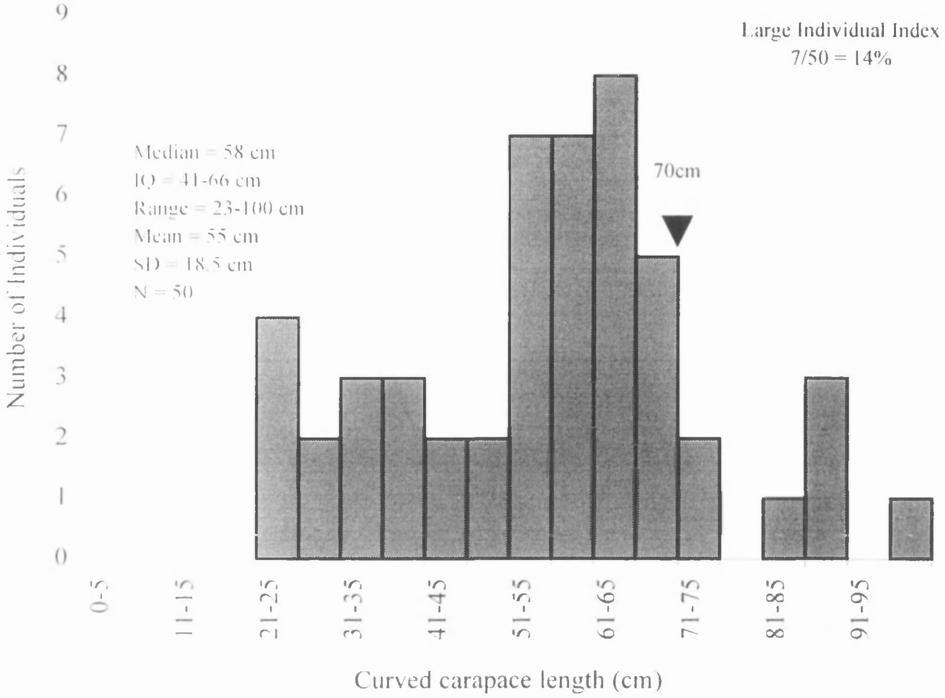
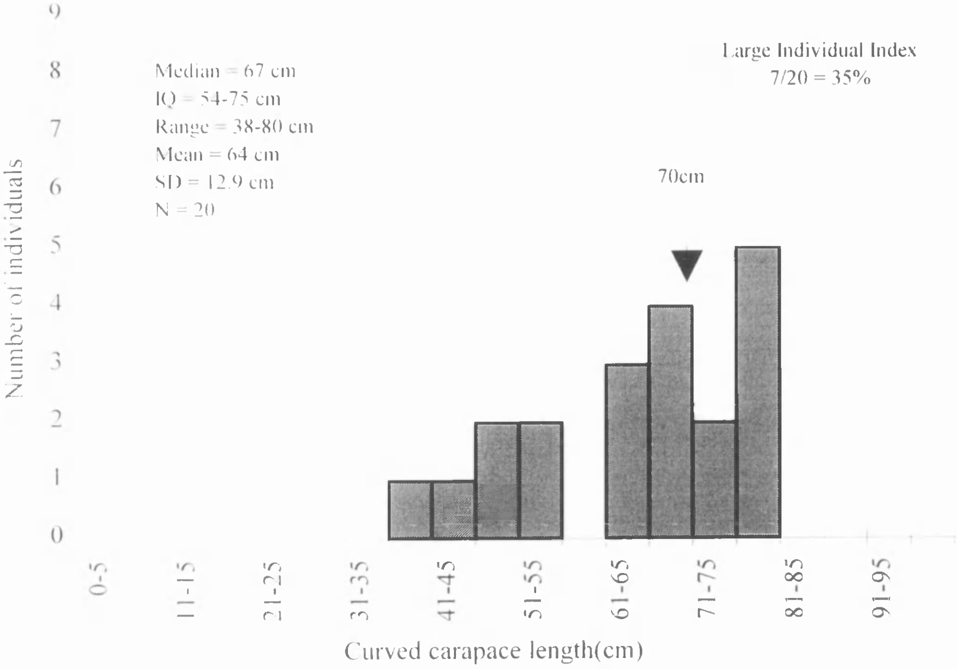

Loggerhead turtles n=26
Number of Individuals
14
12
10
8
6
4
2
0
<10/6
11/6-20/6
21/6-30/6
1/7-10/7
11/7-20/7
21/7-30/7
31/7-9/8
10/8-19/8
20/8-29/8
30/8-8/9
9/9-18/9
19/9-28/9
29/9-7/10

Figure 3. Size class distribution (5cm size classes)of loggerhead turtles stranded in Italy (Fig 3a), Greece (Fig 3b), Turkey (Fig 3c) and Cyprus. (Fig 3d). Descriptive statistics for data from each region are shown including "Large Individual Index".

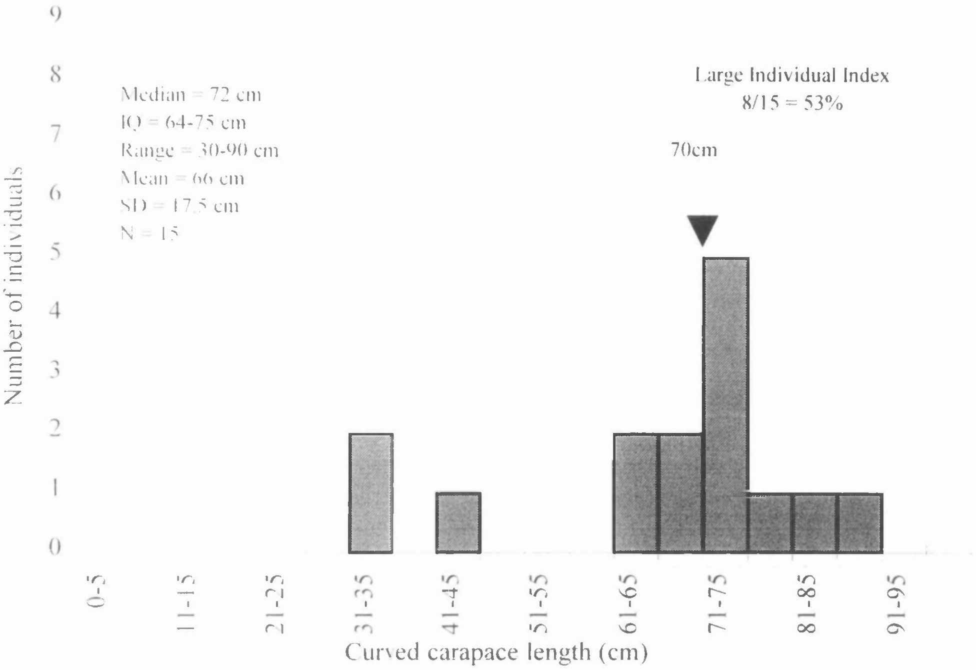
3a


Large Individual Index
7/50 = 14%
Median = 58 cm
IQ = 41-66 cm
Range = 23-100 cm
Mean = 55 cm
SD = 18.5 cm
N = 50
70cm
Number of Individuals
0
1
2
3
4
5
6
7
8
9
0-5
11-15
21-25
31-35
41-45
51-55
61-65
71-75
81-85
91-95
Curved carapace length (cm)

3b


Median = 67 cm
IQ = 54-75 cm
Range = 38-80 cm
Mean = 64 cm
SD = 12.9 cm
N = 20
Large Individual Index
7/20 = 35%
70cm
Number of individuals
0
1
2
3
4
5
6
7
8
9
0-5
11-15
21-25
31-35
41-45
51-55
61-65
71-75
81-85
91-95
Curved carapace length(cm)

3c


Median = 72 cm
IQ = 64-75 cm
Range = 30-90 cm
Mean = 66 cm
SD = 17.5 cm
N = 15
Large Individual Index
8/15 = 53%
70cm
Number of individuals
0
1
2
3
4
5
6
7
8
9
0-5
11-15
21-25
31-35
41-45
51-55
61-65
71-75
81-85
91-95
Curved carapace length (cm)

3d

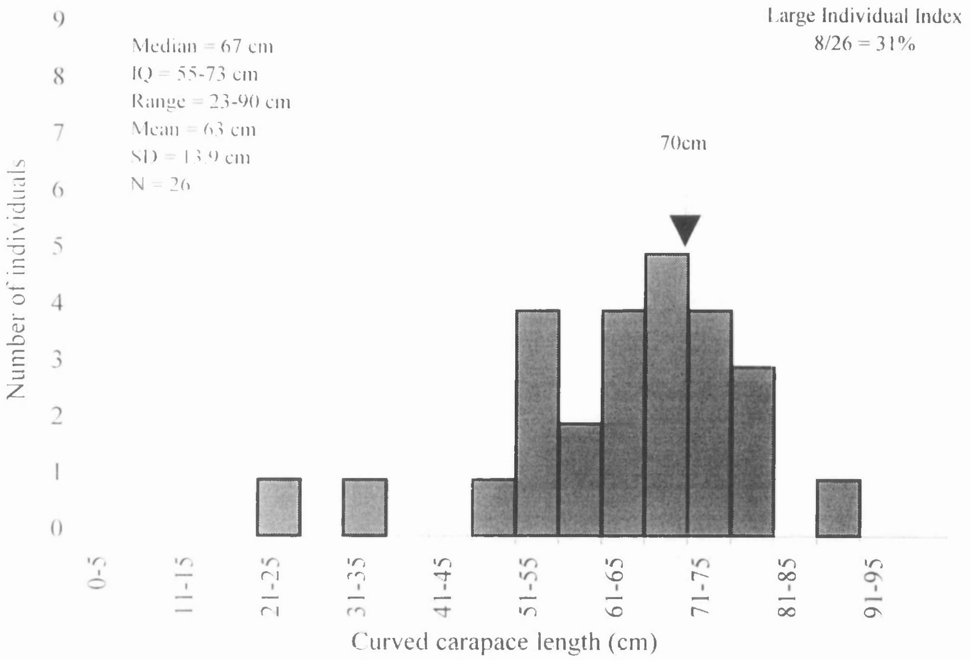
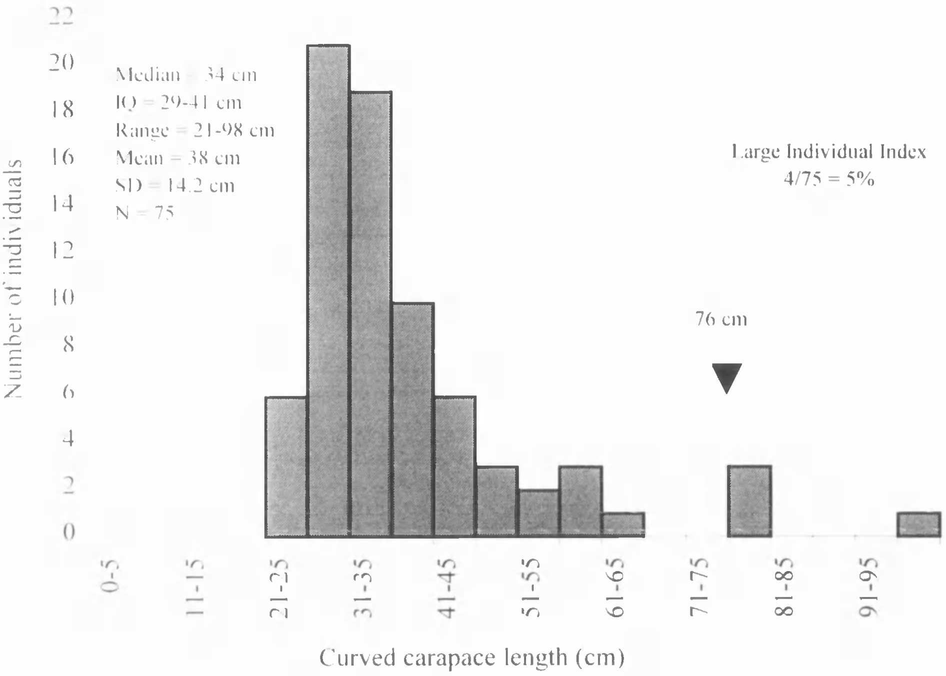

Median = 67 cm
IQ = 55-73 cm
Range = 23-90 cm
Mean = 63 cm
SD = 13.9 cm
N = 26
Large Individual Index
8/26 = 31%
70cm
Number of individuals
0
1
2
3
4
5
6
7
8
9
0-5
11-15
21-25
31-35
41-45
51-55
61-65
71-75
81-85
91-95
Curved carapace length (cm)

Figure 4. Size class distribution (5cm size classes) of loggerhead turtles stranded in Cyprus (Fig 4a) and Turkey (Fig 4b). Descriptive statistics for data from each region are shown including "Large Individual Index".

4a



Median = 34 cm
IQ = 29-41 cm
Range = 21-98 cm
Mean = 38 cm
SD = 14.2 cm
N = 75
Large Individual Index
4/75 = 5%
76 cm
Number of individuals
0
2
4
6
8
10
12
14
16
18
20
22
0-5
11-15
21-25
31-35
41-45
51-55
61-65
71-75
81-85
91-95
Curved carapace length (cm)

4b

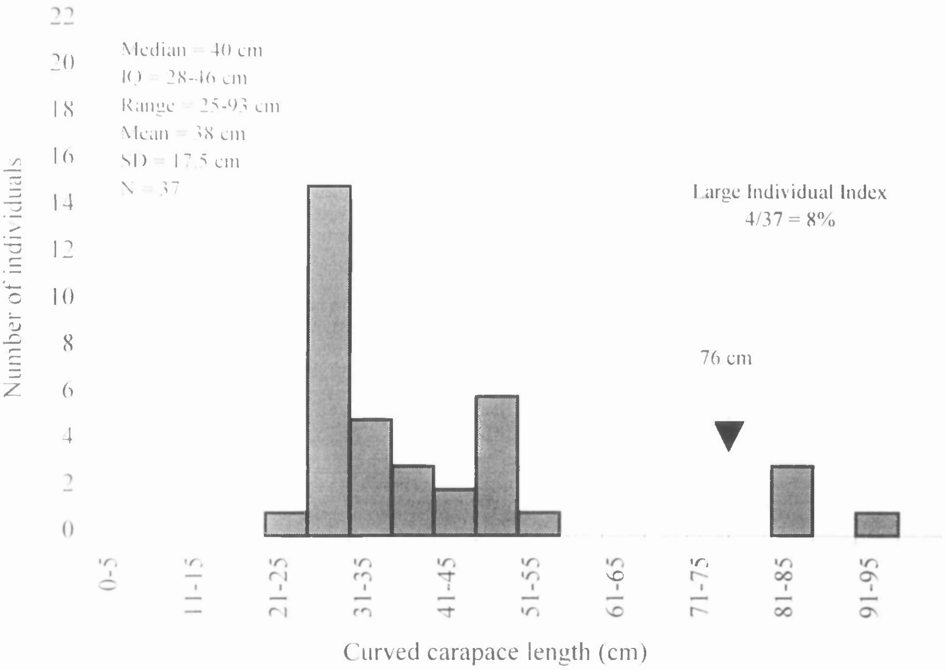
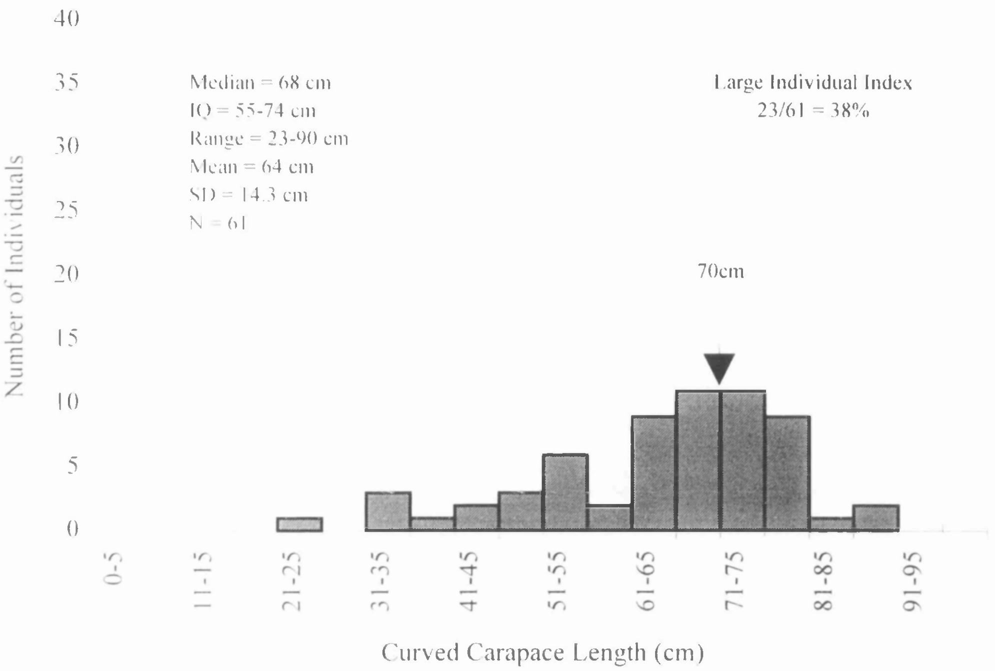
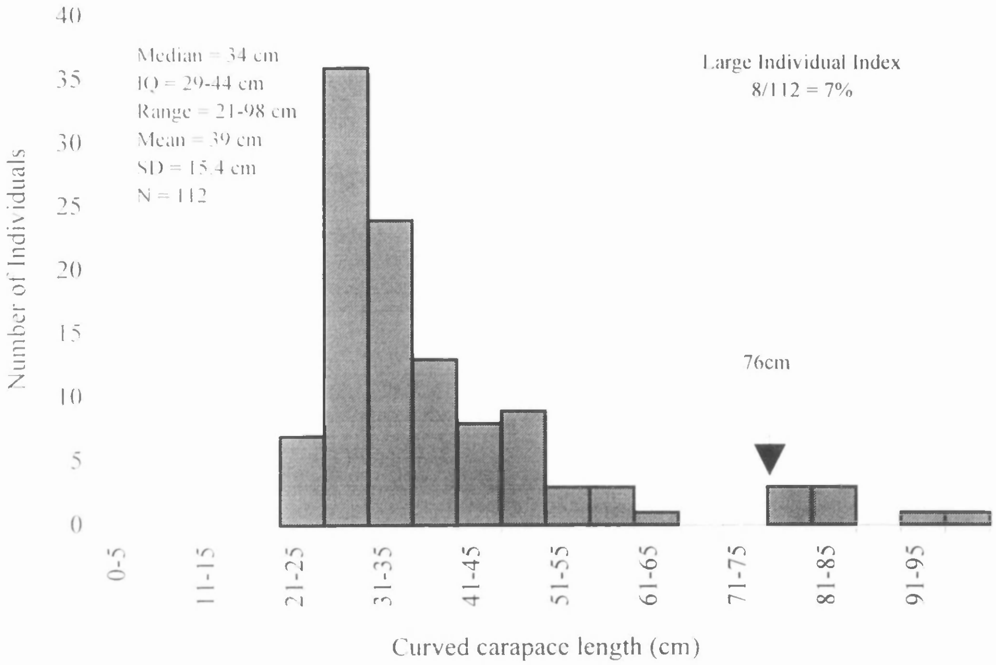

Median = 40 cm
IQ = 28-46 cm
Range = 25-93 cm
Mean = 38 cm
SD = 17.5 cm
N = 37
Large Individual Index
4/37 = 8%
76 cm
Number of individuals
0
2
4
6
8
10
12
14
16
18
20
22
0-5
11-15
21-25
31-35
41-45
51-55
61-65
71-75
81-85
91-95
Curved carapace length (cm)

Figure 5. Overall size class distribution (5cm size classes) of stranded loggerhead turtles (Fig 5a) and green turtles (Fig 5b) in the eastern Mediterranean. Descriptive statistics are shown including "Large Individual Index".

5a


Median = 68 cm
IQ = 55-74 cm
Range = 23-90 cm
Mean = 64 cm
SD = 14.3 cm
N = 61
Large Individual Index
23/61 = 38%
70cm
Number of Individuals
0
5
10
15
20
25
30
35
40
0-5
11-15
21-25
31-35
41-45
51-55
61-65
71-75
81-85
91-95
Curved Carapace Length (cm)

5b


40
35
30
25
20
15
10
5
0
Number of Individuals
Median = 34 cm
IQ = 29-44 cm
Range = 21-98 cm
Mean = 39 cm
SD = 15.4 cm
N = 112
Large Individual Index
8/112 = 7%
76cm
0-5
11-15
21-25
31-35
41-45
51-55
61-65
71-75
81-85
91-95
Curved carapace length (cm)

Chapter 5

Interaction Between Marine Turtles and Artisanal Fisheries in the Eastern Mediterranean.

In press as:

Godley, B.J., A.C. Gucu, A.C. Broderick, R.W, Furness and S.E. Solomon. Interaction between marine turtles and artisanal fisheries in the eastern Mediterranean: a probable cause for concern? Zoology in the Middle East.

Abstract

A survey of the levels of marine turtle bycatch in the small-boat based fisheries in northern Cyprus and the Turkish Mediterranean (Alanya to Mersin) was undertaken by questioning a sample of fishermen in all harbours in the survey region. Fishermen in Turkey caught an estimated 2.5 turtles/boat/year *versus* an estimated 4 turtles/boat/year in Cyprus. This yielded a likely minimum bycatch estimate of over 2000 marine turtles per year in the region. An estimated 10% of turtles were thought to be dead at the point of capture. Turtles were caught in both nets and on long-lines and were perceived as detrimental to the livelihood of fishermen either by damaging nets, spoiling catch or removing bait. Although few fishermen admitted to deliberately killing turtles, a larger proportion thought others did so. Given the likelihood that a proportion of sea turtles caught will be green turtles (*Chelonia mydas*), and due to the highly endangered status of this species in this region, interaction with these fisheries is thought to constitute a real threat to marine turtles in the eastern Mediterranean.

Introduction

Marine turtles in the Mediterranean

Of the seven or eight extant marine turtle species (PRITCHARD 1997), only three are found regularly in the Mediterranean. These are the loggerhead (*Caretta caretta*), green (*Chelonia mydas*) and the leatherback (*Dermochelys coriacea*) turtles (GROOMBRIDGE 1990). Within the region, loggerhead and green turtles both nest regularly. It is not known how current nesting numbers compare to past population levels, but from anecdotal accounts it would appear that they are now considerably lower and GROOMBRIDGE (1990) recommended that both species should be treated as critically endangered within the region. Due to the relative paucity of reliable demographic information regarding most life history stages, numbers of nesting females are used as an index of the relative abundance of the two species.

Loggerhead turtle

This species is by far the more widely distributed within the Mediterranean. Significant nesting occurs on the coast of Greece (MARGARITOULIS 1982, 1989), Turkey (BARAN & KASPAREK 1989a; BARAN & TURKOZAN 1996; ERK'AKAN 1993; YERLI & DEMIRAYAK 1996) and Cyprus (BRODERICK & GODLEY 1996; DEMETROPOULOS & HADJICHRISTOPHOROU 1989). It is likely that a sizeable population nests on the coast of Libya (LAURENT et al. 1997) and low levels of nesting occur in Tunisia, Egypt, Syria, Israel and Italy. Juveniles are thought to be widely distributed within the region, however it has been hypothesised that small juvenile phases are not present in large numbers in the eastern basin (BARAN & KASPAREK 1989b; GODLEY et al. in press a). A recent molecular resolution of stocks of this species in the Mediterranean (LAURENT et al. in press) has demonstrated that although substantial numbers from Atlantic breeding populations share foraging grounds within the region, the Mediterranean breeding stock is functionally independent.

Green turtle

Green turtles are now only found nesting in Turkey (BARAN & KASPAREK 1989a; COLEY & SMART 1992; GLEN et al. 1997; YERLI & CANBOLAT 1998), Cyprus (BRODERICK & GODLEY 1996; DEMETROPOULOS & HADJICHRISTOPHOROU 1989) and occasionally in Israel (KULLER 1995). It is likely that juveniles of this species are more localised in distribution to the eastern basin, where they are often caught in fisheries and recorded stranded (BARAN & KASPAREK 1989b; GODLEY et al. in press a; LAURENT et al. 1996; MARGARITOULIS et al. 1986). Capture in the western basin is exceptional (pers. comm. L. Laurent). The estimated annual female nesting population of green turtles could be as low as between 300-400 in the Mediterranean (GROOMBRIDGE 1990). It is highly likely that this population should be considered functionally independent from that of the wider Atlantic (BOWEN et al. 1992).

Incidental catch

World-wide. marine turtle populations are generally in decline (ECKERT 1995; LIMPUS 1995). Persistent direct exploitation for meat, shell, oil, skin and eggs have been implicated in many regions. However, incidental catch in many fisheries has been suggested as major causes of mortality, especially of adult and sub-adult individuals (HILLSTAD et al. 1995; LUTCAVAGE et al. 1997). In the waters off the USA, mortality due to the shrimp fishery has resulted in the mandatory use of Turtle Excluding Devices (TEDs) in shrimp trawlers (HENWOOD & STUNTZ 1987; NATIONAL RESEARCH COUNCIL 1990).

Incidental catch in the Mediterranean

Incidental catch of marine turtles in fisheries is a well established problem, and has been considered the major threat to marine turtles in the region in a recent review (LAURENT 1997: table 1). Turtles interact with most types of fishing gear. Large numbers (mostly loggerhead turtles) are caught in pelagic longlines, during bottom trawling, and drift netting. Proximate mortality appears high in the Italian drift net fishery and the Spanish long-line fishery, however low levels of direct mortality (possibly approaching zero) are reported in trawling. In should be noted that no studies have quantified any delayed mortality from

Mediterranean trawling activity. Although generally lower numbers are caught in the less industrialised fisheries, mortality rates of animals caught appears to be considerably higher.

Although there are no recent records of marine turtle bycatch from Cyprus, turtle harvest in Turkey in the recent past has been significant. Until the early 1980s relatively detailed records (by weight) of both turtles and dolphins caught by the Turkish marine fisheries were published (**see table 2**). No turtle or dolphin catch was recorded after 1984 or 1982, respectively. Although neither of these data sets are thought to be exhaustive, they do give an indication of the importance of the catch of both these taxa in the past. It is apparent that the largest proportion of both the total marine harvest and of dolphins caught is attributed to the Black Sea ports. However, although generally low in productivity, the Mediterranean region is where most turtles were caught (433 tons of turtles were caught between 1968 and 1984).

In the eastern Mediterranean, fishing strategies can be simplified and are generally considered as being undertaken by two classes of boats (GUCU & BINGEL 1994). Firstly, "small boats " - 4-10m in length carry out beach-seining, long lining and fishing with trammel and gill nets and secondly, "large boats" - over 10m in length carry out purse-seining and trawling. This study was conducted to ascertain the levels of incidental catch of marine turtles in the small boat, artisanal, fishery in northern Cyprus and on the Turkish Mediterranean coast.

Methods

The study was undertaken in August 1995, during the closed season for trawling and purse-seining. Data were only collected from "small-boat" fishermen. Incidental catch in the regional trawl fishery has been the subject of recent international study (LAURENT et al. 1996; ORUÇ et al. 1997). The study area constituted two separate regions (**figure 1**): the complete coastline of northern Cyprus and the area of the Turkish Mediterranean coastline (Mersin to Alanya).

Sample fishermen from every harbour in the study area were interviewed. Interviews were carried out in Turkish. All interviews in Turkey were carried out by BG and ACG. Those in Cyprus were carried out by BG and a member of staff from the local Department of Environmental Protection. Preceding each interview fishermen were urged to give full honest answers, as regardless of the outcome, no steps would be taken against them. Interviews were carried out in as standard a manner as possible, with questions asked in set order. Only data from one fisherman per vessel were included.

Port authorities in both regions were contacted to discover numbers of fishing vessels in each port. This was complicated by the fact that in Turkey, only numbers for each administrative region were available and these included vessels licensed to fish but inactive. In addition, vessels move freely between local harbours. In northern Cyprus, only numbers of licensees were available, and it was not possible to assign these to set harbours. Because of these factors, the total number of small vessels in each size class was generated by asking the fishermen at each harbour how many vessels were based there. It was thought that this would give by far the most accurate estimate of the number of active fishing boats in each location.

Fishermen were asked how many turtles they caught in a season, the proportion which died as a result, in which months fishing was carried out, in which months turtles were sighted at sea and what size class they were (small <30cm, medium 30-50cm, large 50-90cm, very large >90cm), in which month turtles were caught and what sizes they were. Following this, a series of anecdotal questions regarding general attitudes and opinions were asked. It was not possible for fishermen to accurately assign the sea turtles to species, although they were all aware of the fact that sea turtles were distinct from the Nile soft-shelled turtle (*Tryonyx triunguis*) which is also caught and called "yahudi-kaplubaga"(Jewish turtle) by the fishermen in the Mersin region. In addition, fishermen in Cyprus also described rare but regular sightings of a "large black turtle", most likely to be leatherback turtles. This study focuses on green and loggerhead sea turtles.

Results and Discussion

Estimation of current day incidental catch and mortality

Table 3 illustrates that the fishing fleet in the study area is considerable (179 boats in northern Cyprus; 531 in Turkey: Mersin-Alanya). Although the fleet in northern Cyprus is more obviously dominated by small vessels (96% *versus* 77% in Turkey), the overall majority of vessels are of the small size class (82%). All vessels were found to utilise a combination of long-lines and gill /trammel nets. A summary of the statistics regarding the estimated number of turtles caught per year is given in **Table 4**. The estimated catch rate in northern Cyprus (median, 4 turtles/year/boat) was significantly higher than that in Turkey (median, 2.5 turtles/year/boat; Mann-Whitney: W=1033, $p<0.01$). If these median estimates are taken as typical, the median estimate of incidental overall catch would be 2012 turtles (IQ range of estimates: 571-3875 turtles). Mortality levels estimated by fishermen are described in **Table 5**. There was an extremely high degree of variability in estimates among fishermen, ranging from 0-100% in both regions of the study range, however the interquartile ranges were much narrower. Median estimates of resultant mortality level were 10% in both Turkey and northern Cyprus (Mann-Whitney: W= 781, N.S.). Taking the median point estimate, the number of turtles killed by this fishery would be 202 annually (IQ range of estimates: 100-733). When excluding cases where fishermen recorded no turtle catch, there was no correlation between number of turtles caught and the mortality level (northern Cyprus: R_s=0.27, p=0.27, N.S.; Turkey: R_s=0.07, p=0.712, NS).

The seasonality of fishing effort appears to be quite different between Turkey (**Fig 2a**) and northern Cyprus (**Fig 2b**). Although the effort in both fisheries is higher between May and September, there appears to be a far more marked decline in the proportion of fishermen who fish outwith summer months in Turkey. Although there is likely to be an effect of fishing effort on the number of sightings, it is possible that the temporal pattern of turtle sightings represents seasonality of either turtle visibility, abundance or activity levels. The seasonal pattern of when fishermen observe turtles at sea is very similar in both Turkey and Cyprus (**Fig. 2a and 2b**), with turtles only being observed between May and September, with peak numbers between June and August. When the size classes are plotted (**Fig 3**), it is evident that although most fishermen see large juveniles and adults, very few see turtles less than 30cm in carapace length. This could be due to a combination of difficulty in detecting small turtles and the fact that very small individuals are largely to be found in a pelagic developmental environment (MUSICK & LIMPUS 1997).

The temporal distribution of the catch of marine turtles as described by the fishermen in Cyprus (**Fig 2b**) is in general similar to that of sightings, with most activity taking place between May and September and peak catch occurring June-August. There are however, a few small differences in the distribution as described by Turkish fishermen (**Fig 2a**). Although a few Turkish fishermen say they catch turtles in March and April, none are recorded as having caught any in September.

Upon inspection of the size class distribution of what fisherman recorded as being caught (**Fig 4**) it can be seen that is biased more to the small and medium sizes (<50cm), markedly so in northern Cyprus. This suggests that the lack of small turtles in observations is due to difficulties in their detection. The size distribution in catch may mimic the natural makeup of the population or might represent an increased tendency for small turtles to be caught. There are two reasons why this might be the case: Firstly, large turtles of any species may be less likely to be caught during an interaction with fishing gear due to their size. Fishermen often report damage having been done to their nets and lines by entangled or hooked turtles which have escaped. This is more likely to occur in cases where larger, stronger individuals are caught. Secondly, a recent study utilising stable isotope analysis has suggested that green turtles in the region may not be obligate herbivores (GODLEY et al. in press b). Given the expected juvenile tendency to carnivory (BJORNDAL 1997), smaller turtles may be more likely to interact with long-line bait and captured fish in nets, increasing the bycatch of these size classes. If present, juvenile loggerhead turtles would also be expected to be caught in these fisheries as in other regions (**Table 1**). Evidence suggests that there is a paucity of these small individuals in the region, possibly due to some level of developmental emigration to the western basin (BARAN & KASPAREK 1989b; GODLEY et al. in press b.). This hypothesis has been supported by recent molecular data (LAURENT et al. in press.).

Wildlife-fishery interactions

It was the general opinion of fishermen in Turkey that both nets and longlines were involved in catching turtles: five (23%) thought nets more important, five (23%) thought longlines more important and 12 (55%) thought both methods equally important. However, in Cyprus, although 16 (50%) thought both methods were equally involved in turtle catch, a large proportion (15; 47%) thought nets were important, with only one (3%) citing long-lines alone as significant. A large proportion (in Turkey 17(77%); in northern Cyprus 31 (97%)) of fishermen considered that turtles damaged their livelihood in some way, either by damaging nets, spoiling catch or removing bait from hooks. No individuals in Turkey saw turtles as the most problematic wildlife species, other species were cited (sharks: 3 (14%); dolphins: 4 (18%), monk seals: 1(5%)), however this was not the case in Cyprus where 25 fishermen (78%) considered turtles to be a major problem with an additional three (9%) citing dolphins.

The status of fish and turtle stocks

When asked about the status of fishery stocks, 20 (91%) of the fishermen in Turkey thought these were declining. All of these fishermen considered general over-fishing was to blame (20; 100%), especially the trawl fishery (16; 80%). In addition a number thought pollution was also a reason (9; 45%). In Cyprus all fishermen who had an opinion (25; 78%) thought that fish stocks were, in general, declining due to trawler fisheries (22; 88%), pollution (19; 76%), general over-fishing (10; 40%) and weather changes (5; 20%). When asked the same question about turtles, of 16 respondents in Turkey, eight (50%) thought turtle populations to be decreasing, four (25%) thought them to be stable and four (25%) thought they were increasing. In Cyprus, opinion was relatively similar, with 12 fishermen (38%) stating they thought stocks to be decreasing, 13 (42%) thought them stable and only six (19%) thought them to be increasing.

Attitudes of fishermen to turtles

When asked if they ever killed turtles, no fishermen in Turkey responded positively although seven (32%) thought that other local fishermen did kill them. In Cyprus nine fishermen (28%) admitted that they killed turtles and 20 (63%) thought that other fishermen did so. All fishermen in both regions were aware that turtles were protected and in Turkey, 21 (95%) thought that this should be the case, whereas only 21 (66%) in Cyprus agreed with protection. When asked what they did with the turtles they caught or killed, the fishermen in Turkey cited the non-commercial use of shells, meat and blood and fat for medicinal purposes (including the treatment of asthma). In Cyprus, fishermen said that they occasionally used their meat and shell but mostly discarded the carcasses.

General Discussion

A questionnaire-based survey of incidental catch is not as powerful as one which uses onboard observers to generate a catch per unit effort. However, for fisheries with such diffuse effort, it is the only one which could realistically have been undertaken. Given that all fishermen knew of the protected status of turtles and the fact that the survey was conducted by a foreign researcher and a local authority figure, it is reasonable to expect the estimate of the numbers of turtles caught by fishermen to be a low one. It is likely that the incidental catch by artisanal fisheries in the eastern Mediterranean is substantial and certainly in excess of the two thousand individuals in the combined study areas.

The estimate of 10% mortality at time of capture, would mean that at least two hundred turtles die immediately as a result of interaction with these fisheries each year. For the same reasons as described above, it is also likely that this figure is a low estimate. In addition, it is plausible that additional mortality resultant from physical injuries and hypoxic damage occurs. From data given regarding the size class of catch and comparison with available marine turtle stranding data in the region (BARAN & KASPAREK 1989b; GODLEY et al. in press a) it is likely that a large proportion of the turtles caught are juvenile *C.mydas*. Given the highly endangered status of the Mediterranean population of this species, this interaction is a matter of concern.

Among the scientific community, there are grave concerns as to the future of Mediterranean fish stocks (BINGEL et al. 1993). This is mirrored in the popular opinion of its artisanal fishers collated here. There would appear to be a need for greater control of fisheries in the region. On many occasions, the authors (BG and ACG) have observed trawlers flouting existing regulations and fishing within several hundred meters of the shore.

Turtles undoubtedly harm artisanal fisheries, whether by spoiling catch, eating bait or damaging equipment and often die as a result, whether by drowning, due to injuries sustained or being killed by fishermen. Finding sound and sustainable solutions is problematic. Compensation schemes would appear inherently flawed in that they may encourage fishermen to actively catch turtles. Undoubtedly, there is some goodwill in the fishing communities in both regions which should be built upon through education.

Within the eastern Mediterranean, there is urgent need for additional research into the interaction between marine turtles and fisheries. Key regions of interest are the Turkish artisanal fisheries both east and west of the present study. In addition, there are no published accounts of the effects of fisheries of southern Cyprus, Syria, Lebanon and Israel. The work of LAURENT et al. (1996) suggests a significant bycatch of both turtle species by Egyptian fisheries. It is likely that fisheries in the countries constituting the eastern Mediterranean shores between Turkey and Egypt will also impact these populations, especially that of the green turtle which appears more localised in the region. There is an urgent need to discover foraging and developmental habitats for these species in this region so that pragmatic conservation steps can be taken towards an integrated management plans to preserve the Mediterranean populations of these species.

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Fishing gear	Fishing zone	Annual number of captures	Direct mortality %	N	References
Trammel nets					
Lobster	France	low	100	8	LAURENT 1991
Lobster	Corsica	low	93.3	15	DELAUGERRE 1987; LAURENT 1996
Fish	France	low	28.5	9	LAURENT 1996
Fish	Corsica	low	75.0	4	LAURENT 1996
Sole	France	low	53.1	128	LAURENT 1991
Gill nets					
	France	low	50.0	6	LAURENT 1991
	Italy	NQ	50.0	?	ARGANO *ET AL.* 1992
Drifting Longlines					
	Spain 1990	35,637	0.4	673*	AGUILAR et al. 1995
	Spain 1991	22,000-23,637	0.4	425*	AGUILAR et al. 1995
			24.4⊗	45	AGUILAR et al. 1995;
	Italy (Ionian Sea)	100-1,000	NQ		MAS 1996 DE METRIO & MEGALOFONOU 1988
	Malta	1,500-2,500	NQ		GRAMENTZ 1989
	Greece (Cephalonia)	50	NQ		PANOU et al. 1992
	Morocco	3,000	NQ		LAURENT 1990
	Algeria	300	NQ		LAURENT 1990
Drift-nets					
	Italy (Ionian Sea)	16,000	29.0	31*	DE METRIO & MEGALOFONOU 1988
	Italy (Ligurian and Tyrrhenian Seas)	low	0.0	5*	DI NATALE 1995
	Spain (1994)	117-354 #	3.3	30*	AGUILAR 1995
Bottom trawl					
	Greece (Peloponesus)	NQ	2.6	38	MARGARITOULIS et al. 1992
	Italy	1.000-1,500	NQ		ARGANO 1979
	Croatia	2,500	NQ		LAZAR & TVRTKOVIC 1995
	Tunisia	3,500-4,000	0.0	15*	LAURENT & LESCURE 1994
	Tunisia	2.000-2,500	0.0	1*	BRADAI 1992
	Turkey	high	0.5	138	ORUÇ et al. 1997
	Turkey	high	0.0	1*	LAURENT et al. 1996
	Egypt	high	NQ		LAURENT et al. 1996
	France	low	3.0	97	LAURENT 1991, 1996
	Corsica	low	3.8	26	DELAUGERRE 1987
	Spain	low	NQ		AGUILAR 1995

Tab. 1. Review of estimates of incidental capture of marine turtles and mortality level (%) in different Mediterranean fisheries (after LAURENT 1997; used with permission from the author).KEY: N: number of individuals sampled to estimate mortality; ⊗: delayed mortality; *: onboard observations; #:95% confidence limits; NQ - not quantified.

Region	Percentage of marine harvest (1992)	Turtle catch (1968-1984)		Dolphin catch (1968-1982)	
Mediterranean	11 %	423 tons	(85.8%)	43 tons	(0.1%)
Aegean	15 %	52 tons	(10.5%)	5 tons	(0.01%)
Marmaris	10 %	13 tons	(2.6%)	7 tons	(0.02%)
Black Sea	64 %	5 tons	(1.2%)	38 256	(99.9)
Total	100 %	493 tons	(100%)	38 310	(100%)

Tab. 2. The pattern of distribution of total marine harvest attributed to ports from the 4 divisions of the Turkish fishing fleet (for 1992) versus the overall proportion of each of the cumulative turtle catch in recent times (1968-1984). Data as recorded in the annual fishery statistics (TURKISH STATE INSTITUTE OF STATISTICS 1970, 1971, 1974, 1979, 1981a, 1981b, 1982, 1984, 1985, 1986, 1994). Total annual catch of dolphins from the same publications for the period (1968-1982) are also shown.

	Class	Cyprus	Turkey	Total
Number of vessels (% fleet)	small	171(96%)	409 (77%)	580 (82%)
	large	8 (4 %)	122 (23%)	130 (18%)
	total	179	531	710
Number sampled (%)	small	32 (19%)	22 (5%)	54 (9%)

Tab. 3. Summary statistics describing the fishing fleet in both regions.

	Cyprus (n=32)	Turkey (n=22)	Total
Median no./boat	4	2.5	-
Interquartile range	3.1-9.5	0.1-5.5	-
Absolute range	0-25	0-6	-
Median estimate of catch	684	1328	2012
Interquartile catch estimate	530-1625	41-2250	571-3875

Tab. 4. Data regarding the estimate of incidental catch of marine turtles.

	Cyprus (n=32)	Turkey (n=22)	Total
Median mortality level	10%	10%	-
Interquartile range	0-10%	7.5-50%	-
Absolute range	0-100%	0-100%	-
Median kill estimate	69	133	202
Interquartile range of kill estimate	0-69	100-664	100-733

Tab. 5. Data regarding mortality levels of marine turtles caught in fisheries

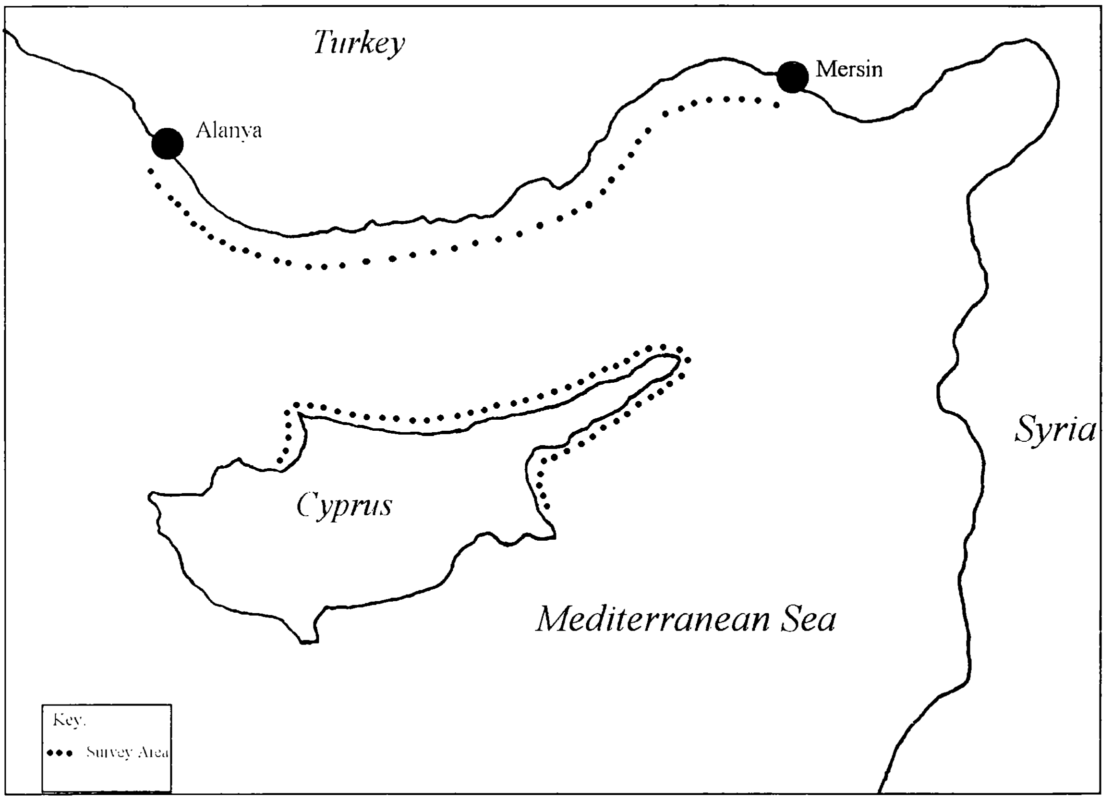

Fig. 1. Map of the eastern Mediterranean showing study sites.

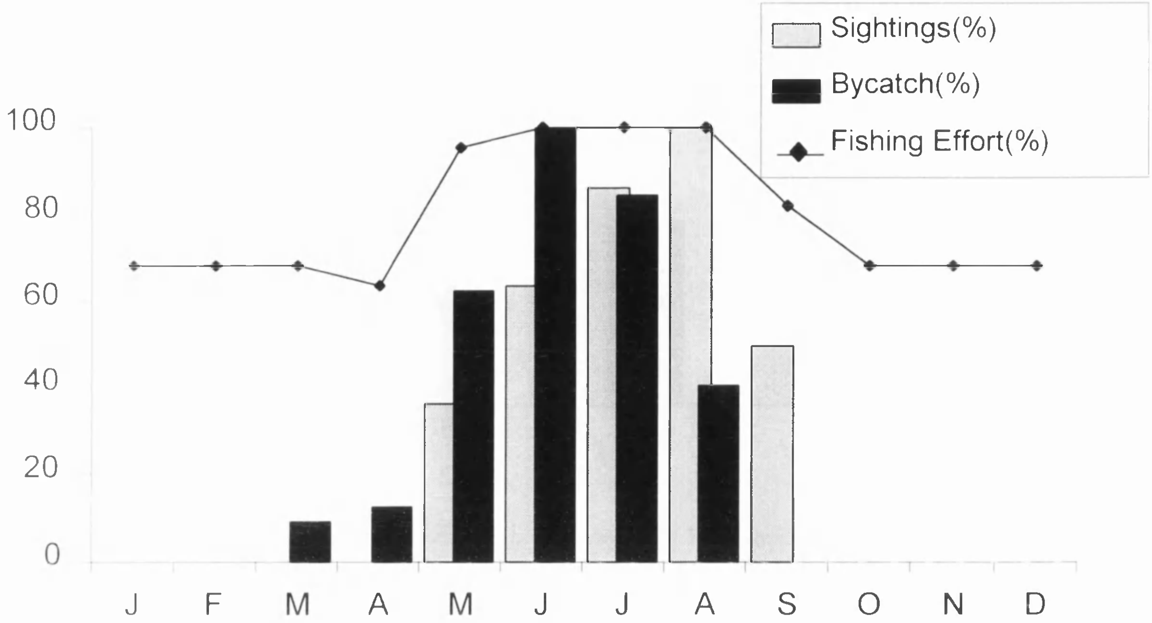

Fig. 2a. Temporal distribution of sea turtle sightings, turtle bycatch and fishing effort as recorded by fishermen questioned in Turkey (% of those surveyed).

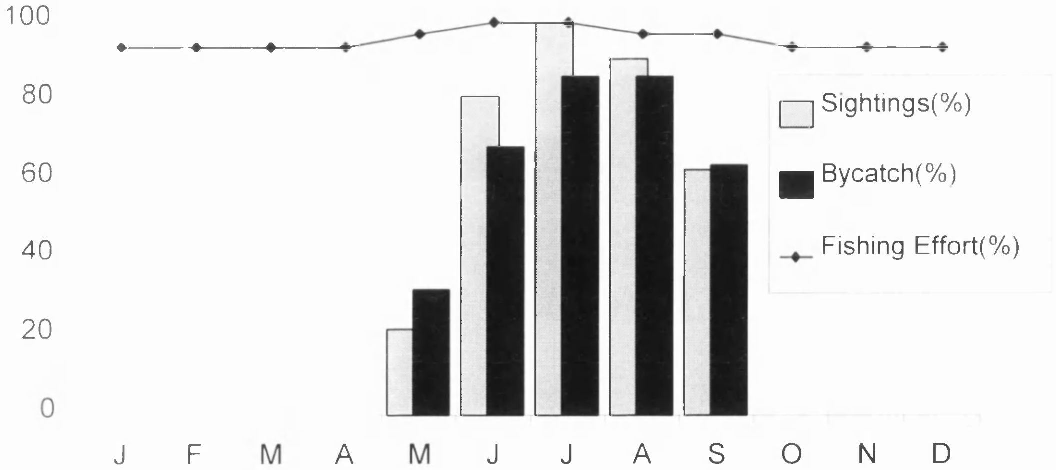

Fig. 2b. Temporal distribution of sea turtle sightings, turtle bycatch and fishing effort as recorded by fishermen questioned in northern Cyprus (% of those surveyed).

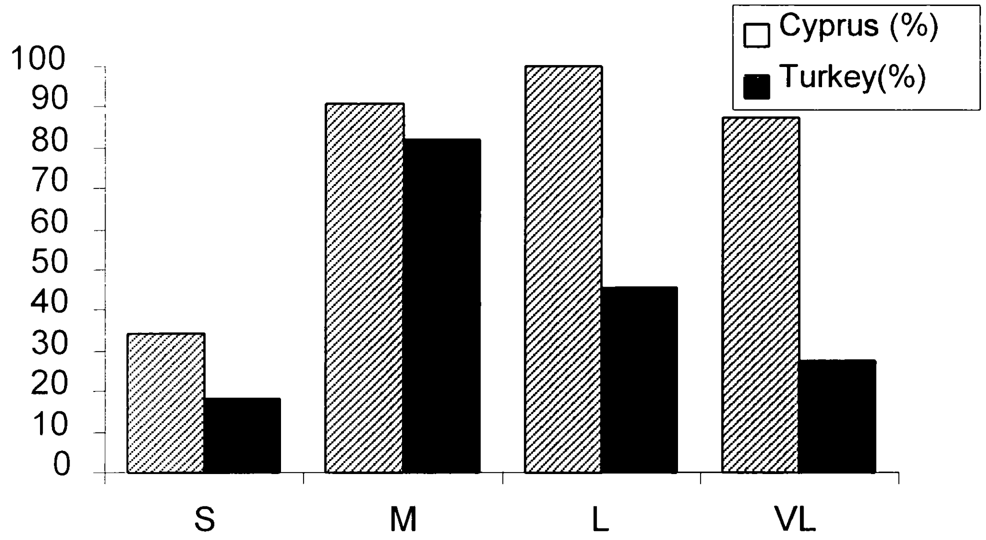

Fig. 3. Size distribution of size classes of sea turtles observed by fishermen (as % of fishermen surveyed) for both turkey and northern Cyprus. (Key: S=small <30cm; M= medium 30-50cm; L= large 50-90cm, VL=very large >90cm)

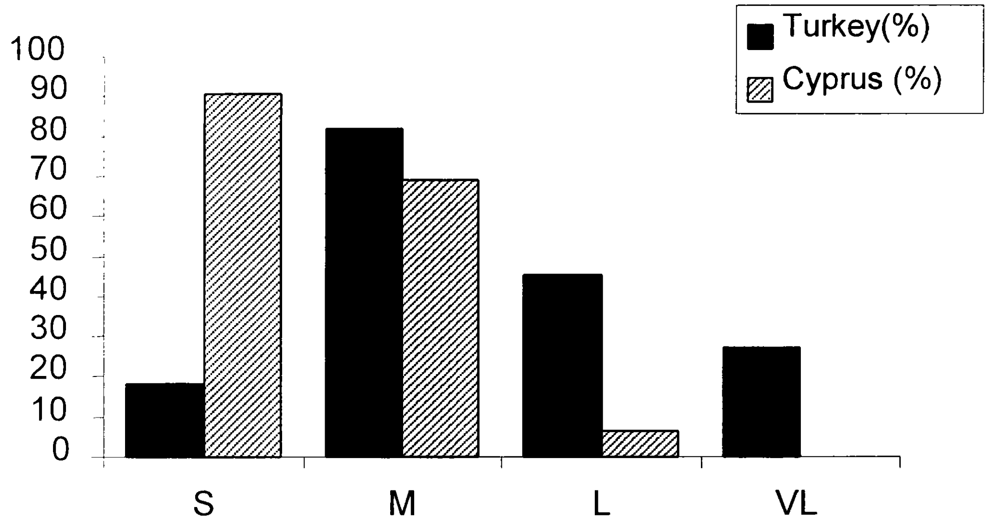

Fig. 4. Size distribution of size classes of sea turtles observed by fishermen (as % of fishermen surveyed) for both turkey and northern Cyprus. (Key: S=small <30cm; M= medium 30-50cm; L= large 50-90cm, VL=very large >90cm).

Chapter 6

Molluscan and Crustacean Items in the Diet of the Loggerhead Turtle.

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Introduction

The loggerhead turtle (*Caretta caretta*) has a circumglobal distribution in temperate and subtropical areas including the whole of the Mediterranean.[1] However, little dietary information is available for those individuals foraging in this region.

After hatching on the natal beach, marine turtles undergo a poorly understood pelagic life history phase, where they are thought to feed upon planktonic items for a number of years[2,3]. Following this, turtles migrate into a juvenile developmental habitat, usually demersal and neritic. It is thought that they remain in these areas until they reach adulthood, at which time they begin migrations to seasonal breeding grounds, which they may undertake every few years.[3]

For both post-pelagic juveniles and adults of this species, study of prey items in other regions of the world have found the diet to be dominated by benthic molluscs, crustaceans and coelenterates.[1] Loggerhead turtles off Australia have recently been observed "mining" such prey items in soft sediments.[4] Individuals sold in markets in Sfax, Tunisia in 1989, were observed to have previously ingested benthic molluscs and crustacea.[5] Identification of gut contents from 31 individuals of varying sizes, caught by trawlers in Tunisian waters, showed the diet to consist largely of these two groups, but also of echinoderms and other items in relatively small proportions.[6] These data suggest that certainly in this region, the loggerhead is a fairly non-selective predator upon sessile and slow moving prey.

Methods

Necropsy examinations of two loggerhead turtles were undertaken as a part of an ongoing study of mortality patterns in Mediterranean marine turtles (B.G.). Lesions present strongly suggested that both individuals had been killed as the result of interactions with local artisanal fisheries in Famagusta Bay, off the coast of northern Cyprus. Both individuals showed signs of cranial trauma, were recently dead, in good general body condition and had digestive tracts relatively full of prey items when they were washed ashore. The first turtle, a subadult female (individual A), with a standard curved carapace length of 49 cm, was discovered on 6th September, 1994. This individual was likely to have been resident in the region. An adult male (individual B), with a standard curved carapace length of 76cm, was discovered on the 7th June, 1995. The breeding season, in this region, is known to occur at the same time of year as the discovery of this individual.[7] Although this male may have been locally resident, it might also have been a breeding migrant from a distant feeding ground.

Guts were incised and all dietary items expected to be identifiable were collected and fixed for later taxonomic identification. Mollusc shell fragments were identified according to an established guide[8] (S.S.) and opercula were identified by comparison with specimens in the Mollusca collections of the Natural History Museum (J.T.). Crustacea were identified according to existing publications for [Anomura:Paguridae] and [Brachyura: Majidae][9-13] and then compared with material in the reference collection of the Natural History Museum (P.C.).

Results and Discussion

The dietary items identified, substrate and depth where the species are normally found, and minimum numbers of each prey species recorded for each individual are shown in Table 1. Five of these genera (*Cerithium*, *Hexaplex, Fasciolaria, Pagurus* and *Paguristes*) have been previously recorded as prey items in this turtle species in the Mediterranean.[6] Outside this region, at least six of these genera (*Astrea*, *Cerithium*, *Conus*, *Strombus*, *Pagurus*, *Paguristes*) have previously been recorded as prey items for the loggerhead turtle.[1]

The occurrence of *Strombus decorus raybaudi* (identified from opercula) in the diet is noteworthy since this species has diversified morphologically since invading the eastern Mediterranean via the Suez canal from the Indian Ocean-Red Sea.[14]

It is difficult to generalise from such a limited sample size, however there was considerable overlap in prey items between both individuals. From general inspection of fragments, several other facts could be ascertained. Both individuals crushed, even the most robust, prey items before swallowing and there was no apparent difference in size of prey selected by different individuals, in that most mollusc fragments originated from shells of range 10-30mm. Distribution data pertaining to the prey species suggest that loggerhead turtles in the eastern Mediterranean do indeed feed upon benthic molluscs and crustacea, at shallow to moderate depths, from both rocky and sedimentary habitats. It is strongly recommended that similar studies are carried out upon incidentally caught or stranded turtles within this region, to allow greater understanding of the synecology of this endangered population.

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Phylum: Mollusca				A	B
Class: Gastropoda	*Astraea rugosa* (Linnaeus, 1767)	rock	8-50m	6 (SH.)	-
	Cerithium vulgatum (Bruguière, 1792)	sand & mud	intertidal-20m	6 (SH.)	-
	Strombus decorus raybaudi (Nikolay & Manoja, 1983)	sand	0-20m	-	100 (O.)
	Naticarius stercusmuscarum (Gmelin, 1791)	sand & mud	>25m	1 (SH.)	-
	Phalium undulatum (Gmelin, 1791)	sand	8-80m	40 (SH.)	39 (SH.)
	Hexaplex trunculus (Linnaeus, 1758)	sand & seagrass	1-100m	30 (SH.&O.)	5 (SH.&O.)
	Buccinulum corneum (Linnaeus, 1758)	rock	intertidal-27m	4 (O.)	-
	Fusinus syracusanus (Linnaeus, 1758)	sand & mud	1-50m	4 (SH.)	6 (SH.)
	Fasciolaria lignaria (Linnaeus, 1758)	rock	0-20m	20 SH.	8 SH.
	Conus ventricosus (Gmelin, 1791)	rock	0-20m	1 (SH.)	20 (SH.)
Class:Bivalva	Anomiidae spp.	rock	0-20m	1 SH.	-
Superclass: Crustacea Class: Malacostraca					
Infraorder: Anomura Family: Paguridae	*Pagurus anachoretus* (Risso, 1827)	rock	6-10m occ. 100m	1	-
	Pagurus cuanensis (Bell, 1845)	rock	15-91m	1	-
	Paguristes eremita (Linnaeus, 1767)	sand	10-80m	1	1
Infraorder: Brachyura Family: Majidae	*Maja goltziana* (d'Oliviera, 1888)	sandy-mud	15-200m	2	-

Table 1. Classification of prey items found, substrate and depths where they are normally found. Minimum numbers in both turtle individuals A and B are given. (Key: SH.= Identified by shell fragments, O.: Identified by opercula).

Chapter 7

The Trophic Status of Marine Turtles as Determined by Stable Isotope Analysis.

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Abstract:

Stable isotope ratios of nitrogen and carbon were determined in bone collagen, egg proteins and keratin from epidermal carapace scutes of loggerhead *Caretta caretta*, green *Chelonia mydas*, and leatherback *Dermochelys coriacea* turtles from the Mediterranean Sea and the European Atlantic Ocean. Isotope ratios in proteins from loggerhead turtles indicate that this species occupies a higher trophic position than green turtles. Leatherback turtles are apparently intermediate in trophic status. Within loggerhead turtles, both isotope ratios correlated positively with body size, indicating a trend of increasing trophic level with age. Within the size range of green turtles sampled, there was apparently no change in trophic level in relation to body size. In absolute terms, isotope signatures of egg proteins were markedly different from those of bone collagen. However, interspecific differences in isotope values were consistent with those in bone protein. The novel application of this technique to marine turtles generally confirmed dietary information collected conventionally in these species, and has also afforded additional insights into shifts in diet and trophic status as turtles mature.

Introduction

In the Mediterranean, there are breeding populations of both green *Chelonia mydas* and loggerhead *Caretta caretta* turtles. It has been estimated that these populations number as few as 300-400 female green turtles and 2000 female loggerhead turtles nesting annually, and both species are considered regionally "endangered" (Groombridge 1990). Green turtles nest in Turkey and Cyprus, with occasional small numbers nesting in Israel. Loggerhead turtles nest more widely; large breeding populations have been recorded in Greece, Turkey, Cyprus and Libya, with low levels of nesting found elsewhere (Broderick & Godley 1996). In addition, although not known to reproduce in the region, leatherback turtles *Dermochelys coriacea* are regularly recorded in the European Atlantic Ocean (Brongersma 1972; Godley et al. in press) and in the Mediterranean Sea (Margaritoulis 1986; Taskavak et al.1998).

After hatching, marine turtles undergo a little known pelagic phase, during which all species are thought to be omnivorous, feeding upon planktonic items for a number of years. Following this, it is hypothesised that individuals migrate to a juvenile developmental habitat, usually neritic (see Musick & Limpus 1997 for review). Studies suggest that, in general, juvenile turtles remain there until adulthood, at which time they begin seasonal migrations between foraging areas and seasonal breeding grounds, which may be undertaken every few years.

In a recent review of marine turtle feeding ecology, Bjorndal (1997) suggested that although green turtles are largely herbivorous during most life history stages, in the pelagic juvenile phase this species is likely to be omnivorous, with a strong tendency towards carnivory. There are, however, no published records of the diet of green turtles in the Mediterranean and there is a paucity of dietary information regarding pelagic juvenile stages of all turtle species world-wide. For post-pelagic juvenile and adult loggerhead turtles, a review of prey items taken in other regions found the diet to be dominated by benthic molluscs, crustaceans and coelenterates (Dodd 1988). The small number of conventional dietary studies undertaken in the Mediterranean have reported similar findings (Laurent & Lescure 1992, 1994, Godley et al. 1997), indicating that individuals stranded dead or incidentally caught in fishing gear had been feeding largely on benthic molluscs and crustacea (Tunisia and Cyprus), but also upon echinoderms and other items in relatively small proportions

(Tunisia). The leatherback turtle is thought to be the most pelagic of all sea turtle species, feeding upon jellyfish, salps and other gelatinous organisms (Bjorndal 1997).

Without direct observation, it is virtually impossible to obtain meaningful dietary information from live, free-living turtles. Animals found dead provide the only realistic opportunity to gather dietary data. However, data obtained from analysis of gut contents from dead turtles have several associated limitations (Duffy & Jackson 1986): ingested food items need to be present and identifiable at the time of examination, there is a likelihood of overestimating the proportion of relatively non-digestible, hard-bodied items and such data only yield a relatively proximate indication of dietary choice.

Stable isotope analysis of assimilated proteins overcomes some of these problems. The technique utilises the fact that the ratios of stable isotopes in proteins of consumers tend to reflect those in their diets in a predictable way (DeNiro & Epstein 1978, 1981, Peterson & Fry 1987). For nitrogen, the ratio of ^{15}N to ^{14}N (conventionally expressed as $\delta^{15}N$) exhibits a stepwise enrichment at each trophic level (Minagawa & Wada 1984, Schoeniger & DeNiro 1984, Hobson et al. 1994), which is thought to be due to differential excretion of the lighter isotope (Peterson & Fry 1987). Hence, biota feeding at relatively high trophic positions will exhibit correspondingly high $\delta^{15}N$ values.

In the case of carbon, the ratio of ^{13}C to ^{12}C (conventionally expressed as $\delta^{13}C$) also shows a tendency to increase with trophic level, but to a lesser degree than $\delta^{15}N$ (Chisholm et al. 1982, McConnaughy & McRoy 1979, Rau et al. 1983, Fry & Sherr 1984, Hobson & Welch 1992). However, the $\delta^{13}C$ value can provide information about the source of carbon entering the food chain, for example distinguishing between marine and freshwater systems (Hobson 1987, Mizutani et al. 1990, Hobson & Sealy 1991). Furthermore, the timescale over which dietary information is represented by a given isotope signature varies with tissue type and depends largely upon metabolic turnover (Tieszen et al. 1983, Hobson & Sealy 1991, Hobson & Clark 1992a, , Hobson 1993, Hobson & Clark 1993). For example, isotope signatures of bone collagen, a protein with a relatively slow turnover, are thought to represent an integration of dietary information over an extended period, possibly approaching the lifetime of the individual (see Stenhouse & Baxter 1979, Tieszen et al. 1983, Hobson 1987).

The analysis of stable isotopes has been used in a wide range of dietary studies in other marine vertebrates, such as fish (e.g. Harrigan et al. 1989, Sholto-Douglas et al. 1991), seabirds (e.g. Hobson 1993, Hobson et al. 1994, Minami et al. 1995, Thompson & Furness 1995, Thompson et al. 1995, Sydeman et al. 1997) and marine mammals, (e.g. Hobson et al. 1995, Ames et al. 1996, Best & Schell 1996, Hobson et al. 1996, Smith et al. 1996). Indeed attempts have been made to describe inter-species relationships in whole food webs using stable isotope markers (e.g. Fry 1988, Rau et al. 1992, Hobson et al. 1995). This study employed stable nitrogen and carbon isotope analysis of collagen extracted from bones, egg derived proteins and of carapace scutes to investigate trophic patterns in four species of marine turtles. This is the first time that stable isotope analysis has been applied to investigate trophic relationships in this group.

Materials and Methods

Sampling.

Humeri and epidermal carapace scutes were collected from turtles found dead on the coast of northern Cyprus and southern Turkey (loggerhead turtles n=11 bone samples; green turtles n=38 bone samples, n=17 scutes). At time of collection, medial curved carapace length (CCL) was measured as an index of size (to the nearest cm) using a flexible tape-measure. A small sample of humeri from adult leatherback turtles stranded on the coasts of Scotland (n=1) and Wales (n=2) were obtained for comparative purposes. Nest contents i.e. whole dead hatchlings, whole embryos in late stages of development (including yolk) and whole eggs which failed to develop were obtained from nests of both loggerhead turtles (n=19) and green turtles (n=20) subsequent to hatching at Alagadi, northern Cyprus. This was carried out according to an established protocol for nest excavation (Broderick & Godley 1996). Only a single sample was taken from any one nest. For comparative purposes, carbon and nitrogen isotope data were made available from a preliminary study (Moncada et al. 1997) investigating the isotopic signatures of "tortoise-shell" from hawksbill turtles *Eretmochelys imbricata* collected from a wide range of sites including Cuba, Mexico, Solomon Islands, Fiji, Indonesia, Philippines and Africa (n=89).

Sample preparation.

Bones were cleaned of muscle and associated connective tissue and then oven-dried at 50°C to constant mass. They were then coarsely divided using a band saw before being ground to a fine powder using an impactor mill operating at liquid nitrogen temperatures. For large bones, the sample consisted of approximately equal portions of epiphysis and diaphysis, whereas, for turtles less than 35cm CCL, both humeri were used. Collagen extraction of bone samples was carried out using a protocol based on that of Chisholm et al. (1983) and used previously in the same laboratory (Thompson & Furness 1995). Lipids were removed from the collagen extract using a Soxhlet apparatus with refluxing chloroform. Following this, samples were re-dried to remove solvent. Nest contents (minus egg shells) were ground and subjected to lipid extraction as for bone samples. It was assumed that carapace keratin samples would contain negligible amounts of lipid and these were rinsed with distilled water to remove any surface contaminants and then ground.

Stable isotope analysis.

All isotopic measurements were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) using a Finnigan Tracer Matt. Isotope ratios are expressed as δ values in parts per thousand (‰) according to the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where X is ^{15}N or ^{13}C and R_{sample} is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. R_{standard} for ^{13}C is PeeDee Belemnite (PDB) and R_{standard} for ^{15}N is atmospheric nitrogen (AIR). All groups of analyses included standard reference materials for routine quality control. All samples were determined in triplicate, with the outlying datum being discarded and mean value being calculated from the remaining data.

In addition, as a quantitative test of analytical consistency, a random selection of samples (n= 23) were determined in triplicate on two separate occasions. On each occasion, the outlying datum was discarded and the mean calculated of the remaining two values. These data were then subjected to repeatability analysis (Harper 1994). This involved calculating the intra-class correlation coefficient which was calculated from the variance components derived from a one-way analysis of variance (ANOVA) as:

$$r = \frac{S_a^2}{(S^2 + S_a^2)}$$

where S_a^2 is the among-groups variance component and S^2 is the within group variance component. Results of both isotope signatures were found to be very highly repeatable (r=0.94 for $\delta^{13}C$; r=0.96 for $\delta^{15}N$) showing the analytical protocol to produce highly consistent values for individual samples. All statistical tests were two tailed.

Results

Size Class Distribution of individuals sampled

The size class distributions of the three species were markedly different (loggerhead turtles: mean CCL = 62.1cm, sd = 15.48, range = 23-79cm, n = 11; green turtles: mean CCL = 41.2, sd = 18.5. range = 21-98cm , n = 38; leatherback turtles: mean CCL = 182cm, sd = 3.5, range = 180-186cm, n = 3). Small sample size precluded leatherback turtles from statistical analyses. However, the difference between mean CCL of green turtles and loggerhead turtles was statistically significant (t_{47}=3.71, p= 0.0016).

Isotope signatures of bone collagen

Isotope signatures of bone collagen in the three species are presented in Table 1. The mean $\delta^{15}N$ signature for loggerhead turtles (19.9‰, Table 1) was significantly higher than that for green turtles (9.9‰, Table 1.; t_{47}=8.89, p<0.0001). There was, however, no significant difference in the mean $\delta^{13}C$ values of these two species (t_{47}=0.60, p=0.55; loggerhead turtles: -14.6‰, green turtles: -15.4‰, Table 1). Although the range in $\delta^{13}C$ values in loggerhead turtles was typically marine (-17.5 to -11.4‰, Table 1) that of green turtles was much wider (-25.7 to -7.7‰, Table 1).

Leatherback turtles had a mean $\delta^{15}N$ signature (14.1‰, Table 1) intermediate to, and a mean $\delta^{13}C$ signature (-19.0‰, Table 1) lower than the other two species. Figure 1 illustrates the combined carbon and nitrogen signatures in extracted bone collagen from all green and loggerhead turtles, in comparison with those from all other tissues analysed in this study.

For loggerhead turtles there was a significant positive correlation between size (CCL) and both $\delta^{15}N$ (Fig 2; R_{10}=0.74, p=0.008) and $\delta^{13}C$ value (R_{10}=0.59, p=0.03). However, similar correlations were not found for green turtles for either $\delta^{15}N$ (Fig. 2; R_{37}=0.01, p=0.89, N.S.) or $\delta^{13}C$ (R_{37}=0.27, p=0.124, N.S.). In addition, although a significant correlation was found between $\delta^{15}N$ and $\delta^{13}C$ in loggerhead turtle collagen samples (R_{10}=0.80, p=0.003) this was not the case for collagen extracted from green turtle bones (R_{37}=0.20, p=0.214, N.S.).

Trophic relationships using nest contents

Summary statistics of the isotope signatures of all nest contents are presented in Table 1. There were insufficient data regarding undeveloped eggs of loggerhead turtles (n = 3) to statistically test for any differences between the levels in these samples and those of conspecific hatchlings or embryos. There was, however, no significant difference between loggerhead turtle hatchlings (mean $\delta^{15}N$ = 7.8‰; mean $\delta^{13}C$ = -17.2‰, Table 1) and loggerhead turtle embryos (mean $\delta^{15}N$ = 7.9‰; mean $\delta^{13}C$ = -16.4‰, Table 1) in either $\delta^{15}N$ signature (t_{14} = 0.72, p = 0.48, N.S.) or $\delta^{13}C$ signature (t_{14} = 0.18, p = 0.86, N.S.). Similarly, there were no significant differences among the three categories of nest contents from green turtle samples for $\delta^{15}N$ (ANOVA, $F_{2,17}$ = 1.5, p = 0.25, N.S.) or $\delta^{13}C$ (ANOVA, $F_{2,17}$ = 1.6, p = 0.224, N.S.).

Since no within-species differences were found, it was assumed that, from each species, all samples of nest contents could be grouped as a single category, "egg-derived protein" for the purpose of inter-specific comparisons (Table 1). There was a marked difference between the isotope signatures of egg-derived protein and conspecific bone collagen. This was true both for green turtles (see below: *Isotopic composition of epidermal carapace scutes*) and loggerhead turtles (Nitrogen: t_{28} = 10.92, p<0.0001; Carbon: t_{28} = 2.77, p = 0.01).

The mean $\delta^{15}N$ value of loggerhead turtle egg proteins (7.6‰, Table 1) was significantly higher than that of green turtles (5.1‰, Table 1; t_{37} = 5.36. p<0.0001). In addition, unlike results for bone collagen (see above), there was a significant difference in $\delta^{13}C$ between the two species (t_{47} = 7.95, p<0.0001), with green turtle egg-derived proteins (mean $\delta^{13}C$ = -

11.7‰, Table 1) exhibiting higher levels than those in loggerhead turtles (mean $\delta^{13}C$ = -16.6‰, Table 1). Figure 1 illustrates the carbon and nitrogen signatures of nest contents from green and loggerhead turtles, compared with conspecific collagen signatures and the levels in carapace scutes of both green and hawksbill turtles.

Isotopic composition of epidermal carapace scutes

Whereas the mean $\delta^{15}N$ of carapace scutes (green turtles: 5.2‰; hawksbill turtles 7.2‰, Table 1) was significantly different between species (t_{104}= 3.4, p=0.001), the mean $\delta^{13}C$ signatures (green turtles: -14.8‰; hawksbill turtles -14.3‰, Table 1) were not (t_{104}= 0.78, p=0.44). Among green turtle samples, the $\delta^{15}N$ signatures of the three different protein extracts were significantly different (ANOVA, $F_{2, 91}$ = 33.06, p < 0.0001), with the $\delta^{15}N$ of bone collagen higher than that for either egg protein or carapace scutes (modified Tukey test for uneven samples; Zar 1984). The $\delta^{13}C$ signatures of the three green turtle protein extracts were also significantly different (ANOVA, $F_{2, 91}$ = 7.09, p = 0.002), with the $\delta^{13}C$ of egg protein significantly higher than that for either bone collagen or carapace scutes (modified Tukey test for uneven samples; Zar 1984). There were no correlations between size (CCL) and $\delta^{15}N$ (R_{16} = 0.17, p = 0.51, N.S.) or $\delta^{13}C$ (R_{16} = 0.30, p = 0.242, N.S.) in the keratin from carapace scutes of green turtles.

Discussion

This is the first study to utilise the power of stable isotope analysis to elucidate trophic relationships in marine turtles. The isotope data for bone collagen show clear species differences. Based on $\delta^{15}N$ signatures, loggerhead turtles feed at the highest trophic level of the species studied here, which, given an average 3-4‰ enrichment for each trophic step (Minagawa & Wada 1984, Schoeniger & DeNiro 1984, Hobson & Welch 1992, Hobson 1993, Hobson et al. 1994) would suggest that they are feeding on average 2-3 trophic levels above green turtles. Although based on a small sample size, leatherback turtles appear to feed at an intermediate trophic level. These isotopic data generally confirm available dietary information collected conventionally regarding these species. Green turtles are thought to be largely herbivorous at most life history stages, leatherback turtles specialise on gelatinous organisms, whilst loggerhead turtles are thought to be essentially carnivorous, feeding on molluscs and crustacea (Bjorndal 1997).

Stable isotope analysis has been suggested as a potential means of identifying the origin of tortoise-shell (Moncada et al. 1997), so aiding policing of legitimate trade if limited international trade were ever permitted. Given the substantial overlap in both isotope signatures it is unlikely that green turtle carapace scutes could be differentiated from those of hawksbill turtles using a simple two isotope technique. To differentiate among populations of the same turtle species it is likely that it would be necessary to use isotopic analysis of additional elements and/or incorporate the analysis of trace elements into a multivariate approach, as undertaken previously for African elephant *Loxodonta africana* ivory (Vogel et al. 1990).

The $\delta^{15}N$ values for many loggerhead turtles in this study are higher than might be expected, given published values for most molluscs and crustacea which have been found to range from approximately 7-16 ‰ (McConnaughey & McRoy 1979, Fry 1988, Hobson 1993). There are no published values for fractionation factors between nitrogen isotopic signatures of prey and those in tissues of marine turtles. These have, however, been determined in other taxa. In captive ring-billed gulls *Larus delawarensis*, Hobson & Clark (1992b) reported a diet-collagen fractionation factor of +3.1‰, and in a study of captive seals, Hobson et al. (1996) found that no diet-tissue fractionation factor exceeded +3.1‰ for nitrogen. Bearhop et al. (unpublished data) found diet-feather keratin fractionation factors of between +3.6 and +4.9‰ for three species of piscivorous bird.

It is noteworthy that the mean $\delta^{15}N$ for green turtles is higher than would be expected for an obligate herbivore. The range of $\delta^{15}N$ values in a selection of marine plants from the Mediterranean was 1.1-4.1‰ (Jennings et al. 1997). If the primary food source for green turtles was plant matter, and the diet-tissue fractionation factor was between 3 and 4‰, this would lead to a likely range in collagen $\delta^{15}N$ signature in the region of 4-8‰. The mean $\delta^{15}N$ value reported here (9.9‰, Table 1), would imply that green turtles do not feed solely on marine plants, but also consume animal tissue enriched in ^{15}N. This could be as an incidental part of relatively unselective herbivory. It is likely that a substantial number of animal items such as fish eggs, molluscs and crustacea will be consumed with plant matter and although green turtles are thought to be largely herbivorous, numerous anecdotal accounts exist of animal items being found in gut contents in this species (reviewed by Bjorndal 1997). However, green turtles may selectively feed on animal tissues such as fish eggs and invertebrates, as our isotope data suggest that animal matter may provide a substantial part of the protein in the diet of this species.

Carbon isotope signatures were found to be very similar in bone collagen of both Mediterranean breeding species (Table 1). However, the mean $\delta^{13}C$ signatures in green and loggerhead turtles were elevated compared to that of leatherback turtles (Table 1). From inter-species comparisons of isotope signatures in seabirds, pelagically-feeding species tend to have more negative carbon signatures than inshore or benthically-feeding species (Hobson et al. 1994). The depleted $\delta^{13}C$ values reported here for leatherback turtles would be consistent with the highly pelagic nature of that species, whilst the neritic feeding preferences of both loggerhead and green turtles could explain the relatively elevated $\delta^{13}C$ values found in these species (Table 1).

The relatively high variance of $\delta^{13}C$ values of collagen samples from green turtles (range: -25.7 to -7.7‰, Table 1) reported here is of particular interest. It implies that some green turtles may have fed primarily in a pelagic environment (relatively low $\delta^{13}C$ values, see above), whilst others have fed on a relatively ^{13}C-rich food source, sea grass being the obvious candidate (Boon & Bunn 1994, Bunn et al. 1995, Hemminga & Mateo 1996).

Paradoxically, although the mean size of nesting loggerhead turtles is less than that of green turtles in the eastern Mediterranean (Broderick & Godley 1996), the individuals stranded and subsequently sampled for this study showed the converse trend. This is a pattern which has previously been noted in stranded turtles in the region (Baran & Kasparek 1989). It is thought to be due to small juvenile loggerhead turtles not being present in the eastern Mediterranean, but occupying a relatively remote and, as yet, undiscovered developmental habitat. Notwithstanding, due to the comprehensive sampling regime undertaken, samples were obtained from small juvenile through to adult size ranges for both species.

As loggerhead turtles increase in size, they appear to feed at progressively higher trophic levels (Fig. 2). No size-related relationships were found in the isotope signatures of green turtle bone collagen (see Results, Fig.2). This may suggest that bone collagen, at least in this species and possibly in marine turtles generally, may not represent dietary information integrated over extended periods, approaching the lifetime of the individual, as is thought the case in other groups (see Stenhouse & Baxter 1979, Tieszen et al. 1983, Hobson 1987).

If isotopic values in turtle bone collagen approached a lifetime integration of dietary information, small green turtles would be expected to show higher $\delta^{15}N$ signatures and lower $\delta^{13}C$ signatures due to omnivorous feeding whilst in the pelagic juvenile stage. That this may indeed be the case in marine turtles is further supported by: i) marine turtles are slow to mature, with estimates reported as ranging from at least 9-14 years in leatherback turtles, 19-27 years in green turtles, and in excess of 20 years for loggerhead turtles (Zug & Parham 1996), ii) being reptiles, turtles are likely to experience skeletal growth throughout this maturation period and possibly beyond, and that iii) skeletal growth is likely to involve constant remodelling and metabolism of bone elements. It is unlikely, therefore, that bone collagen will represent more than medium range dietary preferences of say, several years. In addition, no size-related relationships with isotope signatures of carapace scute keratin were found. It is likely that reasons i and ii (see above) compounded by physical wear on the external surface of scutes will mean that this protein is also only indicative of medium-term dietary influences.

The use of isotopic analysis of egg components to trace avian diets has been suggested previously (Hobson 1995). It appears that there are no differences between the isotope signatures of undeveloped eggs, dead-in-shell embryos or dead hatchlings (see Results and Table 1) and that all may be considered as samples indicative of maternally derived "egg proteins". This being the case, dead hatchlings and embryos would make ideal units for future comparisons with other species and locations as they can be sampled non-invasively after the completion of the hatching process.

The fractionation of different isotopes between diet and consumer tissues varies between different tissues within a species (Mizutani et al. 1990, Hobson & Clark 1992a, 1992b). It is not known why egg protein $\delta^{15}N$ was markedly lower than that of bone collagen in both Mediterranean species (Table 1). Diet-tissue fractionation factors are not known for marine turtles, and would require controlled feeding trials in order to be determined. The between-species differences in $\delta^{15}N$ were, however, consistent with the general difference in feeding preferences already discussed. A significant difference existed in egg-derived protein $\delta^{13}C$ values between loggerhead and green turtles (Table 1) which was not found in the analysis of collagen. This may be complicated by the fact that collagen samples were collected from a wide range of size classes of both species from small juveniles to full grown adults. Egg proteins are solely of adult origin where the difference in feeding between these species is likely to be most divergent, and any inter-specific dietary-related differences in isotope signatures are more likely to be elucidated. The most enriched $\delta^{13}C$ signature of all tissues analysed was that of the green turtle eggs, this would be consistent of a diet dominated by vegetation such as seagrass (Boon & Bunn 1994, Bunn et al. 1995, Hemminga & Mateo 1996, Jennings et al. 1997). These marked inter-tissue differences from the same species highlight the fact that the most rigorous interspecific comparisons of dietary intake of consumers through stable isotope analysis should, wherever possible, involve the same tissue type.

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Sample Type		n	d15N ppt		d13C ppt	
			Mean (SD)	range	Mean (SD)	range
Bone Collagen						
Loggerhead turtles		11	19.95 (3.53)	13.1 to 26.4	-14.60 (1.84)	-17.5 to -11.4
Green turtles		38	9.92 (1.84)	3.7 to 17.3	-15.40 (4.31)	-25.7 to -7.7
Leatherback turtles		3	14.06 (0.52)	13.6 to 14.6	-19.02 (5.43)	-25.3 to -15.4
Nest contents						
Loggerhead turtles	Overall "egg derived protein"	19	7.62 (1.65)	3.3 to 10.2	-16.56 (1.92)	-20.5 to -13.6
	Hatchlings	8	7.75 (1.76)	5.6 to 10.2	-17.15 (1.85)	-20.5 to -14.6
	Embryos	8	7.88 (1.01)	6.6 to 9.3	-16.42 (2.18)	-19.6 to -13.6
	Eggs	3	6.57 (2.85)	3.3 to 8.4	-15.41 (1.24)	-16.2 to -14.0
Green turtles	Overall "egg derived protein"	20	5.09 (1.28)	3.3 to 7.5	-11.7 (1.91)	-15.3 to -8.5
	Hatchlings	7	4.43 (0.87)	3.4 to 5.3	-10.8 (1.80)	-13.1 to -8.5
	Embryos	7	5.46 (1.44)	3.4 to 7.5	-11.7 (1.54)	-14.8 to -10.3
	Eggs	6	5.42 (1.38)	3.3 to 7.1	-12.7 (2.21)	-15.3 to -10.1
Carapace Scute						
Green turtles		17	5.19 (1.79)	2.2 to 8.8	-14.81 (3.43)	-22.3 to -10.5
Hawksbill turtles		89	7.15 (2.24)	2.7 to 12.3	-14.35 (1.95)	-19.5 to -8.8

Table 1: Descriptive statistics of both $\delta^{15}N$ and $\delta^{13}C$ for all samples in the study.

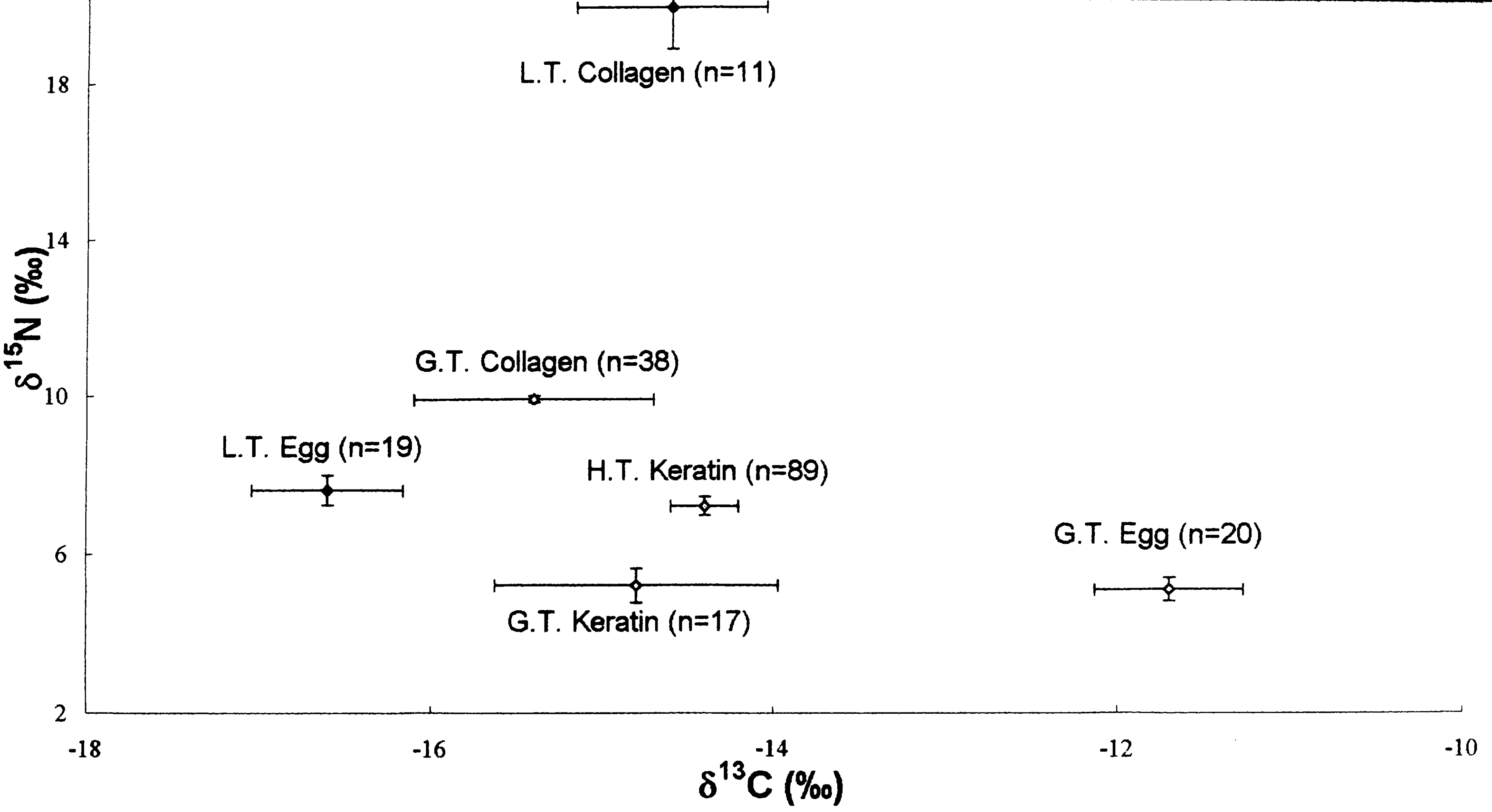

Figure 1: Comparison of isotopic signatures (mean±SE) in the bone collagen (Collagen) and egg protein (Egg) of green (G.T.) and loggerhead turtles (L.T.) with carapace scute keratin (Keratin) from green and hawksbill turtles (H.T.).

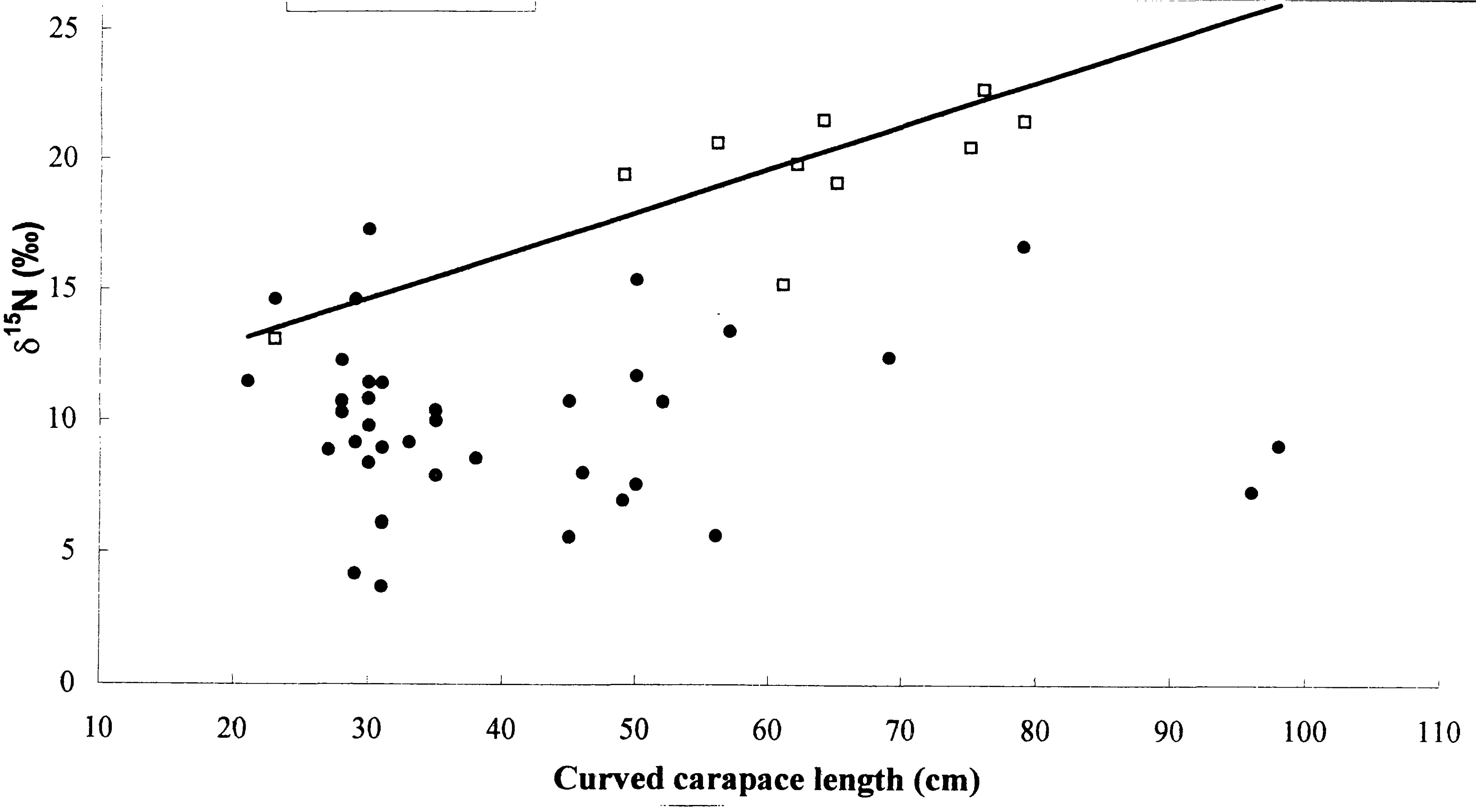

Figure 2: Plot of $\delta^{15}N$ in bone collagen *versus* curved carapace length in both green and loggerhead turtles. Coefficient of correlation (r) is shown for loggerhead turtles.

Chapter 8

Concentrations and Patterns of OrganochlorineContaminants in Marine Turtles from Mediterranean and Atlantic Waters.

In press as:

McKenzie C., B.J. Godley, R.W. Furness and D.E. Wells. Concentrations and Patterns of Organochlorine Contaminants in Marine Turtles from Mediterranean and Atlantic Waters. Marine Environmental Research.

Abstract:

Concentrations of individual chlorobiphenyls (CBs) and organochlorine pesticides (OCPs) in marine turtle tissues collected from Mediterranean (Cyprus and Greece) and European Atlantic waters (Scotland) between 1994 and 1996 are described. CB concentrations were highest in adipose tissue and ranged from 775-893, 39-261 and 47-178 μg/kg wet weight in loggerhead, green and leatherback turtles respectively. Omnivorous loggerhead turtles had the highest organochlorine contaminant (OC) concentrations in all tissue sampled, with dietary preferences likely to be the main differentiating factor among species. Decreasing lipid contaminant burdens with turtle size were observed in green turtles, most likely attributable to a change in diet with age. Principal Components Analysis of data indicated that there were also pattern differences among species, confirming bioaccumulation differences.

Introduction

The three sea turtle species most commonly found in Mediterranean and European Atlantic waters are the green turtle (*Chelonia mydas*), the loggerhead turtle (*Caretta caretta*) and the leatherback turtle (*Dermochelys coriacea*) (Brongersma, 1972; Groombridge, 1990). Only the green turtle and the loggerhead turtle are thought to breed in the Mediterranean region, with nesting mainly taking place on the shores of the eastern Mediterranean (Broderick and Godley, 1996), both populations being considered as regionally endangered. In addition, marine turtles from distant breeding populations, especially leatherback and loggerhead turtles, are often recorded in the European Atlantic and the Mediterranean. These populations are considered as globally endangered and globally threatened respectively.

An extensive review of environmental contaminants in turtles has been conducted by Meyers-Shöne and Walton (1994), however most information relates to freshwater species. Studies have found a mean concentration of OCs in the freshwater species, snapping turtles (*Chelydra serpentina*), from the Hudson river of 2,990 µg/g in adipose tissue. High concentrations of CBs and OCPs in the eggs of this species have been correlated with population effects such as decreased hatching success, increased hatchling deformities and disorientation (Bishop *et al.*,1991; Bishop *et al.*, 1994). There have been few studies on the concentrations and bioaccumulation of organochlorine contaminants in marine turtles. In the majority of cases reported only the total CB concentrations have been provided with no information about the congener profile.

Clark and Krynitsky (1980) measured CBs in loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) eggs. CB concentrations in the eggs of loggerhead turtles ranged from 32-201 µg/kg and were below detectable limits in the green turtles. Loggerhead turtles from the east coast of Florida contained <5.0-133 µg/kg arochlor equivalents in their livers whilst green turtles from the same area had liver CB burdens ranging from <5.0-70 µg/kg Aroclor equivalents.

Levels of contaminants have been the subject of prior concern in other megafaunal taxa in the Mediterranean. Cetaceans, particularly striped (*Stenella coeruleoalba*) and bottlenose dolphins (*Tursiops truncatus*), from the Western Mediterranean have been shown to contain high organochlorine levels ranging from 90-1,000 µg/g for CB (Aguilar and Borrell, 1994;

Corsolini *et al.*, 1995). These levels have been tentatively linked with the 1990/1991 striped dolphin epizootic in the Mediterranean (Aguilar and Borrell, 1994). The only study of top predators in Greek waters found lower concentrations in a striped dolphin and a monk seal (*Monachus monachus*) from the South Aegean sea (Georgakopoulou-Gregariadou *et al.*, 1995).

The diet preferences of each of the sea turtle species sampled are markedly different and have been recently reviewed (Bjorndal, 1997). Loggerhead turtles worldwide are generally carnivorous, their diet thought to be dominated by benthic invertebrates. This has recently been supported by a dietary study carried out on two individuals from the eastern Mediterranean which were found to have been feeding on benthic molluscs and crustaceans (Godley *et al.*, 1997). Leatherback turtles are the most pelagic of the three species studied and their diet consists almost exclusively of jellyfish, salps and other gelatinous organisms. Juvenile green turtles are omnivorous but as they mature their diet becomes herbivorous, consisting mainly of sea grass.

There are similarities in the life history of the species involved (Musick and Limpus, 1997). After hatching on the natal beach, marine turtles undergo a poorly understood pelagic life history phase, where they are thought to feed upon planktonic items for a number of years. Following this, they migrate into a juvenile developmental habitat, usually demersal and neritic, where it is likely that they remain until they reach adulthood. It is during this post-pelagic phase that species appear to develop a greater degree of dietary specialisation.

In this study, 15 marine turtles comprising three species were analysed for 22 CB congeners and 17 organochlorine pesticides. In addition, OC concentrations were determined in a small number of Mediterranean loggerhead and green turtle hatchlings and an egg from each species.

Materials and Methods

Sampling

Samples of liver and adipose tissue from stranded turtles from Cyprus, Greece and Scotland were collected for organochlorine analysis. In addition, a small number of dead hatchlings and undeveloped eggs were collected from nests hatched at Alagadi, northern Cyprus, according to an established protocol (Broderick and Godley, 1996). Individual samples were wrapped in aluminium foil and transported frozen.

Analytical methodology

Tissue samples, whole hatchlings or eggs were macerated and mixed with anhydrous sodium sulphate and extracted using Soxhlet apparatus for eight hours with methyl tertiary butyl ether (300 ml). Following extraction the percentage extractable lipid was determined gravimetrically.

An aliquot containing *ca.* 200 mg lipid was removed from the bulk extract and transferred to hexane. The lipids were removed using a 6 g alumina column (5% H_2O) and the organochlorines eluted with hexane. The eluent was further cleaned up and the CBs and OCPs separated using an alumina column (3 g) deactivated to 5% H_20, followed by a silica column (3 g) deactivated to 3% H_20 (Wells and Johnston, 1977; Wells *et al.*, 1985). The CB fraction was then transferred to iso-octane and the internal standards (D6/D16) were added.

Final determination of CBs and OCPs was carried out using a Varian 3500 gas chromatograph fitted with an electron capture detector (GC-ECD). The analysis used a CPSil 8 and CPSil 19 column (50 m x 0.25 mm) for CBs and OCPs respectively. GC conditions and quality assurance protocols have been published previously (Megginson *et al.*, 1994).

Contaminant concentrations below the limits of quantitation (LOQ) are presented as less than values. The LOQ is the analyte concentration in the sample giving rise to a chromatographic peak of the same height as the lowest standard and is therefore sample specific.

All solvents used for the chemical analyses were of the highest purity and were obtained as glass distilled grade from Rathburn Chemicals (Walkerburn, Scotland). The individual pure solid CB's were obtained from the Community Bureau of Reference (CB's 28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170, 180), Promochem GmBH (CB's 31, 44, 70, 74, 128, 157, 158, 194) and Ultra Scientific (CB's 49, 110, 187).

A number of samples were analysed using a Hewlett Packard 5989A mass spectrometer engine equipped with a HP 5890 gas chromatograph (GC-MS). Samples were manually injected onto a CPSil 8CB column using helium as a carrier gas and the analysis was carried out under electron impact (EI) ionisation.

Results

Sample information is given in Table 1. The concentrations of organic contaminants measured in marine turtle adipose and liver tissues are given in Tables 2 and 3 respectively. Data from hatchlings and eggs are given in Table 4. The CB and p,p'-DDE data in all tissues analysed are shown graphically in Figures 1a and b.

CB concentrations were highest in the loggerhead turtles for all tissues measured. Concentrations in adipose tissue ranged from 775-893 µg/kg wet weight, considerably higher than observed in green turtles from the Mediterranean and leatherback turtles washed ashore on the Scottish coast, which had ranges of 39-261 µg/kg and 47-178 µg/kg respectively (Table 3). The same conclusion is drawn when the data are presented as lipid weight concentrations although the within species variability increases. In all species, adipose tissue contained the highest organochlorine concentrations followed by liver, kidney and pectoral muscle.

As previously observed by Lake *et al.* (1994), the predominant congeners in all species were CBs 138, 153, 180 and 187. These compounds are found in higher proportions in industrial CB formulations and are not susceptible to metabolic degradation as they have no vicinal H atoms, a requirement necessary to allow oxidative metabolism (Boon *et al.*, 1994). There was little difference in the proportional concentrations of CBs in different organs of the same animal.

Analysis of DDT and its isomers in the turtle samples showed that p,p'-DDE was present in the greatest concentrations, making up >95% of total DDT in the majority of samples. The observed DDT component pattern in most samples was p,p'-DDE >> p,p'-DDT > p,p'-DDD. This pattern is similar to the pattern observed in most top predators distant from DDT point source contamination (Aguilar, 1987). The p,p'-DDE component of DDT in turtle tissues was proportionately higher than for other marine species previously analysed, in agreement with data from the east coast of the USA for loggerhead and Kemp's ridley turtles (Lake *et al.*, 1994; Rybitsky *et al.*, 1995). As observed for the chlorobiphenyls, the highest p,p'-DDE concentrations were measured in loggerhead adipose tissue.

The relationship between the curved carapace length (cm) and the total CB and p,p'-DDE concentration measured in green turtle livers are given in Figures 2a and b respectively. The decrease of these contaminants with increasing size was tested using Spearman's rank correlation and found to be statistically significant (R_s=0.44, p<0.05 for CB) and (R_s=0.84, p<0.001 for p,p'-DDE). Since size is likely to be proportional to age, these results also correlated with the available knowledge of the green turtle life history. It is highly likely that the smallest of the green turtles are recent recruits into the juvenile developmental habitat (Musick and Limpus, 1997). Inspection of gut contents (Godley, unpublished data) has found all green turtles stranded to be complete herbivores. Prior to this stage they are likely to have passed through the pelagic omnivorous phase when they would feed at a higher trophic level. The main OC burden would be accumulated by green turtles in the pelagic stage and subsequently diluted as the animal grows during the herbivorous stage. This finding is different from observations made in the omnivorous, fresh water snapping turtle in which muscle contaminant concentrations were found to be correlated with length. The more lipophilic CB congeners were found to be highly correlated with age (Hebert *et al.*, 1993).

There were insufficient data to study any potential bioaccumulation of OCs in loggerhead and leatherback turtles.

The insecticide dieldrin was detected in most of the tissues analysed, and was often present as the second most abundant OCP identified in the chromatogram, although at considerably lower concentrations than p,p'-DDE. The concentration ranges for the loggerhead and leatherback turtles were similar in both adipose and liver samples, while the range observed for the green turtles was lower.

Other pesticides detected were the main constituents of technical Chlordane; oxychlordane, heptachlor epoxide and trans-nonachlor. Heptachlor, α-chlordene and γ-chlordene were not detected in any samples. The concentration of chlordanes in the marine turtles were in the order loggerhead > leatherback > green turtle. Chlordane residues were not detected in the three green turtle adipose samples but were detected in two of the liver samples, G5 and G8.

Lindane, (hexachlorochyclohexane, γ-HCH) was detected in two of the loggerhead turtle samples at concentrations <1 μg/kg. Hexachlorobenzene and α-HCH (an isomer of lindane) were not detected in any of the samples.

The concentrations of organic contaminants in loggerhead and green turtle hatchlings and eggs suggest a similar trend with CB and OCP levels in loggerhead turtles being higher than in green turtles. The loggerhead turtle egg, LE1, had the second highest concentration of ΣDDT in the data set when expressed on a lipid weight basis and concentrations in the loggerhead hatchlings were similar to that of adults when expressed on a lipid weight basis.

Comparative data for ΣCB and p,p'-DDE concentrations in loggerhead, green, and leatherback turtles are given in Table 5. Concentration differences between loggerhead turtles and green turtles have been noted previously, loggerhead turtles having consistently higher concentrations in muscle, liver and eggs than green turtles (Hillestad *et al.*, 1974; Thompson *et al.*, 1974; Clark and Krynitsky, 1980; McKim and Johnson, 1983). The concentrations of OCs measured in loggerhead and green turtles from the Mediterranean lie within the range of those measured by other workers.

The largest peaks observed in the GC-ECD chromatograms of the loggerhead and green turtle CB fractions were not identified as CBs or OCPs. Chromatograms for the livers of a loggerhead turtle, L1 and a green turtle G1 are given in Figures 3a and b with the unknown peaks labelled A-C. The samples were run on GC-MS in EI mode, however, only one of the unknown components, peak C in the GC-ECD chromatogram, was identified using GC-MS. The total ion chromatogram and the mass spectrum for peak C are given in Figures 3c and d. The mass spectrum for peak C gave a good match (>95%) for elemental sulphur, S_8. The sulphur concentrations were not quantified but on the basis of peak size the order of tissue retention of sulphur was liver > kidney > adipose > muscle > hatchlings = eggs.

The observed sulphur is likely to be of dietary origin as both species of turtle, especially green turtles, are known to feed on sea grasses. Additionally, the CB fraction of the leatherback turtle samples, thought to be feeding on jellyfish and other pelagic items, did not contain peaks A-C. Under anaerobic conditions, such as those to be found in the stomach, seagrasses will decompose via sulphate reduction to H_2S. H_2S will then undergo microbial oxidation to S_8 or SO_4^{2-} (Fenchel, 1970).

Chlorinated organics enter marine turtles through their diet and through reproductive transfer from females to eggs. The degree of bioaccumulation of these contaminants depends on the trophic position of a marine turtle species in the marine food web, its habitat and the geographical location of individuals.

For example, in the Mediterranean, loggerhead turtles, which are omnivorous, but mainly carnivorous have higher concentrations of CBs and OCPs than green turtles which are omnivorous after hatching and are subsequently herbivorous as they mature. In the turtles from the Atlantic (1 loggerhead, L6 and 2 leatherbacks LB1 and LB2) it was the loggerhead turtle which had the higher OC concentrations. Leatherback turtles feed almost exclusively on pelagic jellyfish which possibly explains the low exposure to OC contamination measured in this species. Although firm conclusions should not be drawn on the basis of such a small sample size this difference in OC level may reflect a difference in diet between the two species.

The data for hatchlings and eggs (Table 4) further suggest that loggerhead turtles are exposed to relatively higher concentrations (on a lipid weight basis) of OCs, particularly p,p'-DDE, during early developmental stages. There are no published studies correlating OC concentrations with any adverse effects, however, at concentrations considerably higher than those observed in this study an increase in deformities and decreased hatching success rate in the fresh water snapping turtle were observed (Bishop *et al.*, 1994).

OC pattern analysis

The differences in the pattern of OCs in each species can be masked by differences in absolute concentrations both within and among turtle species. Much of this variance may be reduced by studying the ratios of each compound to the recalcitrant congener CB153 (Wells *et al.*, in press). In this study, those congeners which were commonly above the limit of quantitation (LOQ) in most samples and p,p'-DDE were normalised to CB153 to give contaminant patterns. Only samples which had OC concentrations above the LOQs for the selected compounds were normalised.

The biplot of the first two Principal Components Analysis (PCA) was used to study the CB and p.p'-DDE patterns in the turtle samples. When all data was included the loggerhead hatchling, LH1, was clearly separated from the other samples on the basis of the higher chlorinated CBs (CBs 170, 180, 194) and p,p'-DDE. This sample also had the highest absolute concentrations of CB and DDT of the four loggerhead hatchlings analysed. These hatchings would have obtained most of the OC burden directly from the mother, but it is not immediately apparent why the high concentration should also produce a change in OC pattern. To examine the other data more closely LH1 was removed from the data set and the PCA recalculated. The results are given in Figure 4.

Three groups have been identified in the biplot, these being loggerhead turtle adipose tissue, liver, kidney, muscle and egg; loggerhead turtle hatchlings and green turtles. The lines drawn in the PCA biplot to visualise these groups do not have statistical significance. The green turtle samples are relatively scattered in the plot and this may reflect an increase in analytical and sample variance as a result of the low OC concentrations observed, as indicated by the positions of the sample duplicates (GL5A and GL5B). In one case samples of adipose tissue and liver (GA1 and GL1), from the same animal have similar OC patterns indicating little differences between some tissues. However the differences in patterns between the same tissues in a second animal (GA2 and GL2) would appear to contradict this. In the loggerhead turtle samples there is little pattern difference between organs from the same animal (adipose tissue, liver, kidney and muscle from L1 and adipose tissue and liver from L2).

The loggerhead turtle hatchlings have higher concentrations of the lower chlorinated CBs and p,p'-DDE than the other tissues. To evaluate the differences between the hatchlings and the other samples more clearly the green turtle samples were removed and the PCA recalculated with the loggerhead samples alone. The resulting biplot (Fig. 5) shows that, despite the degree of scatter, the three hatchlings have higher scores on PC1 than the other samples. The main conclusions to be drawn from the biplot are that hatchlings have higher relative concentrations of lower chlorinated CBs (52, 70, 101, 105, 110,118) and lower relative concentrations of higher chlorinated CBs (CBs 156, 170, 180, 194) compared with adults of the same species. This indicates that there is a preferential transfer of lower chlorinated, less lipophilic OC components from the mother to hatchling. It is not clear why this same difference is not observed with the egg sample. This preferential reproductive transfer has been previously observed in pinnipeds and cetaceans (Green *et al.*, 1996;

Mckenzie *et al.*, in press) and is based on the lipophilicity (Log K_{ow}) of the compound being transferred. To show this the score of each compound on PC1 was plotted against the Log K_{ow} in Figure 6. There is a significant negative correlation (R^2=0.75, p<0.001) between an individual compounds score on PC1 in Figure 5 and its Log K_{ow}.

Conclusions

This study has shown that organic contaminant concentrations in three marine turtle species decreased in the order loggerhead > green = leatherback. The OC concentrations within different tissues of the loggerhead turtles were the highest in the adipose tissue, followed by liver > eggs > hatchlings > muscle > kidney. although adipose tissue is preferable, liver and eggs are recommended for monitoring studies. this is beacause of the difficulty in sampling adipose correctly and in many individuals, especially juveniles, may lead to sampling inconsistency which may be reflected in a higher variance in the chemical data. The differences in contaminant concentration between species are possibly related to diet, with the varied diet of the loggerhead turtle and its higher position in the marine food web giving rise to greater exposure to contaminants than leatherback and green turtles.

The concentrations of contaminants in marine turtles from Mediterranean and Atlantic waters measured in this study are similar to those determined in the same species elsewhere in the Atlantic. The levels in all these studies are considerably lower than the concentrations shown to cause deleterious effects in freshwater turtles. In green turtles the highest contaminant burdens are found in juvenile animals but as the individual grows and there is a decrease in contaminant intake the initial concentrations are diluted.

Contaminant patterns show differences between green turtles, loggerhead turtles and loggerhead hatchlings indicating different modes of bioaccumulation. With respect to the loggerhead turtles and hatchlings this is due to selective transfer of less lipophillic contaminants from mother to offspring. The loggerhead turtle egg, did not show higher relative concentrations of lower chlorinated CBs but no conclusions have been drawn since only one egg of each species was analysed.

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Sample ID	Species	Area	Sampling date	Sex	CCL (cm)	CCW (cm)
G1	Green	Cyprus	21 06 95	J	33	26
G2*	Green	Cyprus	16 08 95	J	57	55
G3	Green	Cyprus	22 07 95	J	35	35
G4	Green	Cyprus	20 06 96	J	33	31
G5	Green	Cyprus	18 07 96	J	37	32
G6*	Green	Cyprus	05 09 96	J	31	28
G7	Green	Cyprus	01 07 96	J	35	30
G8	Green	Cyprus	15 09 96	J	32	28
G9	Green	Cyprus	11 08 95	J	30	28
LB1	Leatherback	UK	18 10 93	M	151	nm
LB2	Leatherback	UK	24 10 95	M	141	89
L1	Loggerhead	Cyprus	07 06 95	M	67	65
L2	Loggerhead	Cyprus	06 09 94	J	49	47
L3*	Loggerhead	Greece	23 12 95	F	81	74
L4	Loggerhead	Cyprus	02 08 95	J	23	22
L5	Loggerhead	Greece	22 12 95	J	48	43
L6	Loggerhead	UK	07 11 95	J	21	19
GH1	Green	Cyprus	03 08 95	H	nm	nm
GH4	Green	Cyprus	01 09 95	H	nm	nm
GH5	Green	Cyprus	27 09 95	H	nm	nm
LH1	Loggerhead	Cyprus	29 07 95	H	nm	nm
LH2	Loggerhead	Cyprus	31 07 95	H	nm	nm
LH5	Loggerhead	Cyprus	22 08 95	H	nm	nm
LE1	Loggerhead	Cyprus	29 08 95	E	na	na
GE5	Green	Cyprus	14 08 95	E	na	na

Table 1. Turtle samples analysed for CBs and OCPs
M - adult male, F - adult female, J - unsexed juvenile, H - hatchling, EG - egg,
CCL - curved carapace length, CCW - curved carapace width,
***Samples analysed in duplicate, nm - not measured, na - not applicable**

ID	lipid (%)											DDD	DDE	DDT		
L1	51	<5.4	24	28	71	229	132	73	775	<6.0	9.2	<6.0	705	8.0	739	12.0
L2	21	<8.0	37	32	54	232	133	154	893	<2.0	6.2	6.6	376	<2.0	391	14
L3	73*	<4.8	8.6	8	62	261	169	116	853	10	<1.8	<1.8	446	4.6	454	33
LB1	74	<7.2	12	<7.2	<7.2	7.8	<7.2	<7.2	47	<8.1	19.0	<8.1	10	<8.1	14	22
LB2	50	<4.7	12	5.8	8.3	46	25	24	178	<6.2	13.0	<6.2	57	<6.2	58	12.0
G1	33	<3.2	25	12	3.7	14	8.2	7.8	109	<3.2	3.5	<3.2	19	<3.2	23	<3.2
G2	26*	<2.2	13	5.7	<2.2	<2.2	<2.2	<2.2	39	<1.9	<1.9	<1.9	2.4	<1.9	3.3	<1.9
G3	32	<3.7	19	33	27	32	28	13.0	261	<3.0	<3.0	<3.0	6.0	<3.0	11	<3.0

Table 2. CB and OCP concentrations in turtle adipose tissue (μg/kg wet weight)

ΣCB = sum of CBs 31, 28, 52, 49, 44, 74, 70, 101, 110, 149, 118, 153, 105, 138, 158, 187, 128, 156, 157, 180, 170, 189, 194)

ΣDDT = o,p'-DDD + o,p'-DDE + o,p'-DDT + p,p'-DDD + p,p'-DDE + p,p'-DDT

Σchlor = heptachlor + heptachlor epoxide + α-chlordane + γ-chlordane + oxychlordane + trans-nonachlor

Sample ID	Extractable lipid (%)	CB28	CB52	CB101	CB118	CB153	CB138	CB180	ΣCB	Endrin	Dieldrin	p,p'-DDD	p,p'-DDE	p,p'-DDT	ΣDDT	Σchlor
L1	12	<1.6	<1.6	3.1	8.7	29	24	11	102	nm	nm	nm	76	nm	77	nm
L2	10	<0.5	<0.5	3.4	4.9	27	20	18	101	<0.5	2.7	<0.5	49	<0.5	49	2.1
L4	3.9	<0.5	1.3	1.5	3.3	13	12	7.3	50	<0.2	0.3	<0.2	68	0.7	69	1.8
L5	9.5	<0.5	0.8	<0.5	6.4	26	18	11	83	1	2.5	<0.5	49	<0.5	51	5.0
L6	6.8	<0.9	15	16	12	24	20	12	159	nm	nm	nm	149	nm	152	nm
LB1	14	<0.8	<0.8	<0.8	<0.8	1.3	0.8	<0.8	3.7	1.1	2.5	<0.7	1.7	<0.7	9.1	2.3
LB2	11	<0.6	3.9	<0.6	<0.6	1.5	<0.6	<0.6	3.1	1.3	3.1	<0.6	6.5	<0.6	14	2.3
G1	14	<1.5	2.2	4.1	2.8	6.1	6.0	2.5	35	0.6	3.0	<0.7	6.2	0.8	10	<0.7
G2	na	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.0	0.5	<1.0	<1.0	<1.0	1.2	<1.0
G3	16	<1.0	2.2	3.4	3.0	9.2	7.6	5.3	45	nm	nm	nm	2.7	nm	2.7	nm
G4	7.4	<0.8	1.0	1.3	1.3	5.8	6.4	3.5	25	<0.4	2.4	<0.4	1.3	<0.4	2.2	<0.4
G5	15	<0.7	0.7	0.9	<0.7	2.0	2.0	1.1	10	nm	nm	nm	1	nm	1.0	2.7
G6	31	<3.4	4.3	7.4	4.8	13	11	6.7	77	<1.0	<1.0	<1.0	21	<1.0	23	<1.0
G7	15	<1.7	3.5	3.9	1.7	3.4	2.2	1.9	29	<0.8	1.5	<0.8	1.3	<0.8	2.9	<0.8
G8	25	<2.4	3.0	2.8	<2.4	12	10	7.4	47	<1.2	1.9	<1.2	4.2	<1.2	5.1	3.7
G9	7.9	<0.86	2.0	3.2	2.6	6.3	5.2	2.8	34	<0.4	<0.4	<0.4	5.8	<0.4	6.2	<0.4

Table 3. CB and OCP concentrations in turtle liver tissue (μg/kg wet weight)

nm = not measured

ΣCB = sum of CBs 31, 28, 52, 49, 44, 74, 70, 101, 110, 149, 118, 153, 105, 138, 158, 187, 128, 156, 157, 180, 170, 189, 194)

ΣDDT = o,p'-DDD + o,p'-DDE + o,p'-DDT + p,p'-DDD + p,p'-DDE + p,p'-DDT

Σchior = heptachlor + heptachlor epoxide +α-chlordane + γ-chlordane + oxychlordane + trans-nonachlor

ID	lipid (%)											DDD	DDE	DDT		
LH1	8.4	1.0	7.3	4.0	1.5	3.2	1.8	4.1	45	0.4	1.3	1.0	104	1.9	113	3.3
LH2	8.6	0.5	3.8	2.4	2.2	3.3	2.1	0.5	22	1.3	9.2	<0.4	18	0.8	22	7.9
LH3	7.0	0.7	5.6	5.1	6.5	19	9.2	5.3	71	0.4	1.3	<0.4	49	0.4	51	2.6
LH5	4.8	0.6	4.6	3.4	1.1	2.8	1.3	0.4	23	<0.2	<0.2	<0.2	4.5	<0.2	5.3	0.9
GH1	8.1	0.6	4.0	1.9	<0.4	1.1	0.4	<0.4	13	<0.4	0.3	<0.4	0.5	3.4	5.8	0.4
GH4	7.0	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	0.2	<0.4
GH5	7.5	<0.4	0.5	<0.4	<0.4	<0.4	<0.4	<0.4	1.1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
LE1	6.0	0.4	2.7	1.2	7.2	29	14	12	89	<0.3	0.6	0.3	154	0.4	155	1.8
GE5	7.0	0.3	2.2	1.1	<0.3	<0.3	<0.3	<0.3	6.1	<0.3	<0.3	<0.3	2.3	0.5	4.3	<0.3

Table 4. CB and OCP concentrations in turtle hatchlings and eggs (µg/kg wet weight)

ΣCB = sum of CBs 31, 28, 52, 49, 44, 74, 70, 101, 110, 149, 118, 153, 105, 138, 158, 187, 128, 156, 157, 180, 170, 189, 194)

ΣDDT = o,p'-DDD + o,p'-DDE + o,p'-DDT + p,p'-DDD + p,p'-DDE + p,p'-DDT

Σchlor = heptachlor + heptachlor epoxide + -chlordane + -chlordane + oxychlordane + trans-nonachlor

	3	Cyprus Greece	Adipose tissue	775-893	391-739	This study
	17	Virginia / N Carolina, USA	Liver	8.3-514	<2.0-458	Rybitsky *et al.* (1995)
	8	Florida, USA	Liver	<5.0-133	<1.0-51	McKim and Johnson (1983)
	4	Cyprus Greece	Liver	50-102	49-77	This study
	1	Scotland, UK	Liver	159	152	This study
	9	Florida, USA	Muscle	8.0	<1.0-40	McKim and Johnson (1983)
	1	Virginia, USA	Muscle	<2.0	<2.0	Rybitsky *et al.* (1995)
	1	Cyprus	Muscle	49	23	This study
	1	Virginia, USA	Kidney	4.8	<2.0	Rybitsky *et al.* (1995)
	1	Cyprus	Kidney	26	9.5	This study
	9	Florida, USA	Eggs	78*	47**	Clark and Krynitsky (1980)
	56	Florida, USA	Eggs	n.m	99**	Clark and Krynitsky (1985)
	na	Georgia / S Carolina, USA	Eggs	n.m	58-305#	Hillestad *et al.* (1974)
	1	Cyprus	Egg	89	155	This study
Green	3	Cyprus	Adipose tissue	39-261	2.4-19	This study
	4	Florida, USA	Liver	<5.0-70	<1.0	McKim and Johnson (1983)
	9	Cyprus	Liver	<1.1-47	<1.0-21	This study
	2	Florida, USA	Eggs	< 25*	<5.0-42	Clark and Krynitsky (1980)
	10	Ascension Island	Eggs	8***	n.d-9.0	Thompson *et al.* (1974)
	1	Cyprus	Egg	6.1	4.3	This study
Leatherback	2	Scotland, UK	Liver	47-178	10-57#	This study
	1	England, UK	Liver	230	68#	Godley *et al.* (in press)

Table 5. Comparative ΣCB and p,p'-DDE studies in loggerhead, green and leatherback turtles
***Aroclor 1260 equivalents; ** p,p'-DDE+p,p'-DDT; *** Aroclor 1254 equivalents;**
 # p,p'-DDE+p,p'-DDT+p, p'-DDD na - not available

a) Range and median CB (μg/kg wet weight) in marine turtle tissues and eggs
b) Range and median p,p-DDE (μg/kg wet weight) in marine turtle tissues and eggs

Fig. 1a

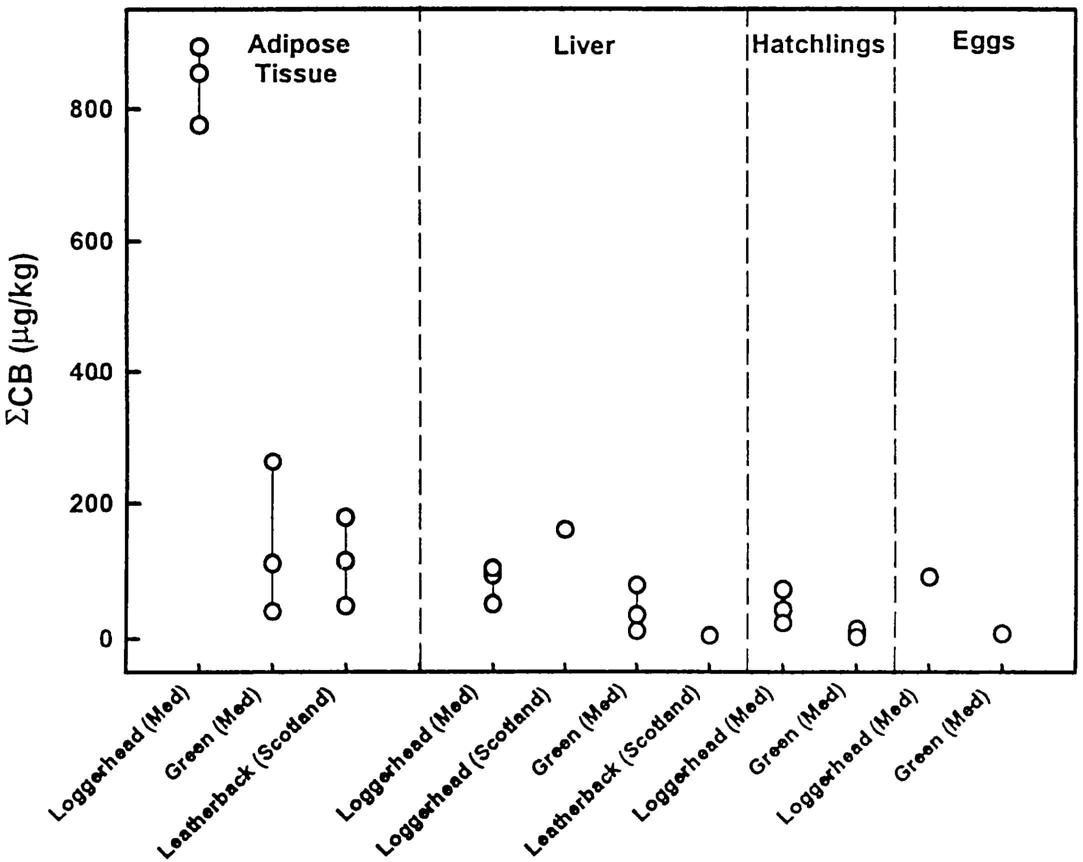
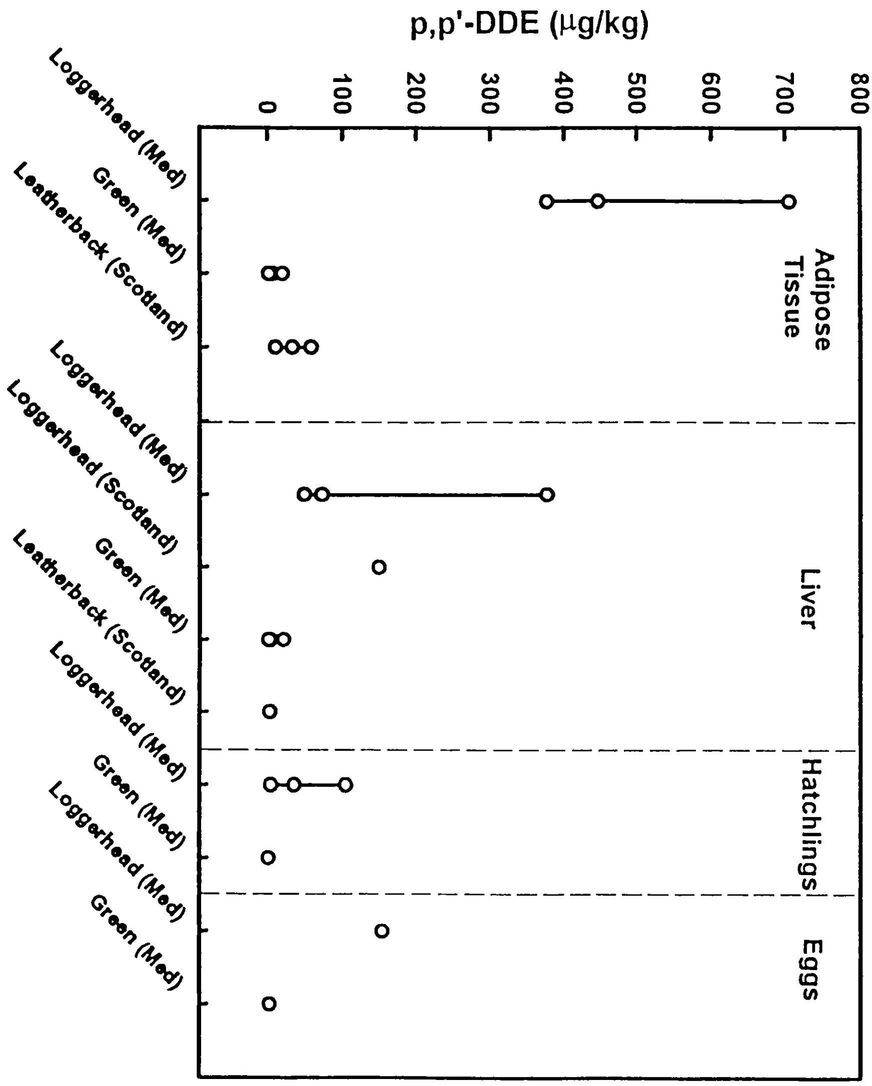

ΣCB (µg/kg)
0
200
400
600
800
Adipose Tissue
Liver
Hatchlings
Eggs
Loggerhead (Med)
Green (Med)
Leatherback (Scotland)
Loggerhead (Med)
Loggerhead (Scotland)
Green (Med)
Leatherback (Scotland)
Loggerhead (Med)
Green (Med)
Loggerhead (Med)
Green (Med)

Fig1b


p,p'-DDE (μg/kg)
0
100
200
300
400
500
600
700
800
Adipose Tissue
Liver
Hatchlings
Eggs
Loggerhead (Med)
Green (Med)
Leatherback (Scotland)
Loggerhead (Med)
Loggerhead (Scotland)
Green (Med)
Leatherback (Scotland)
Loggerhead (Med)
Green (Med)
Loggerhead (Med)
Green (Med)

a) Curved carapace length (cm) vs CB (μg/kg wet weight) in green turtles
b) Curved carapace length (cm) vs p,p'-DDE (μg/kg wet weight) in green turtles

Fig 2a

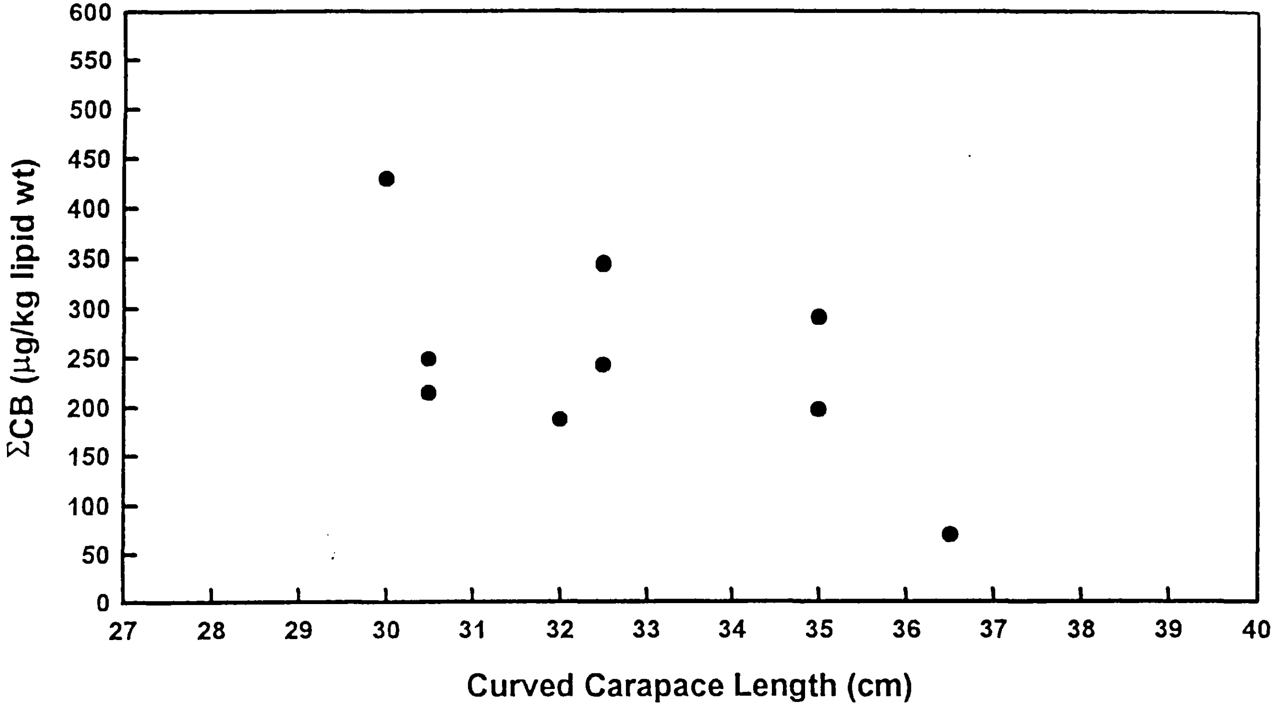
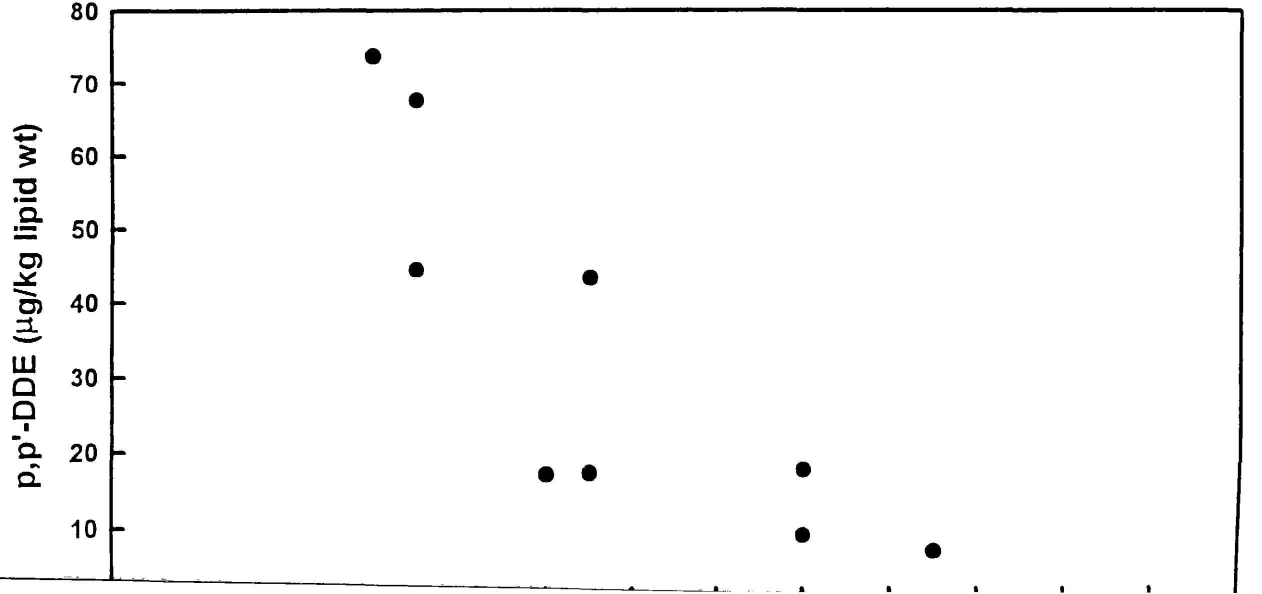

ΣCB (μg/kg lipid wt)
0
50
100
150
200
250
300
350
400
450
500
550
600
27
28
29
30
31
32
33
34
35
36
37
38
39
40
Curved Carapace Length (cm)

Fig 2b


p,p'-DDE (μg/kg lipid wt)
10
20
30
40
50
60
70
80

a) GC-ECD chromatogram of a loggerhead turtle liver, CB fraction.
b) GC-ECD chromatogram of a green turtle liver, CB fraction.
c) GC-MS total ion chromatogram of a loggerhead turtle liver, CB fraction
d) GC-MS mass spectra of peak C detected in a loggerhead turtle liver, CB fraction.
Peak A - unknown; Peak B - unknown; Peak C - elemental sulphur, S_8

Fig 3a

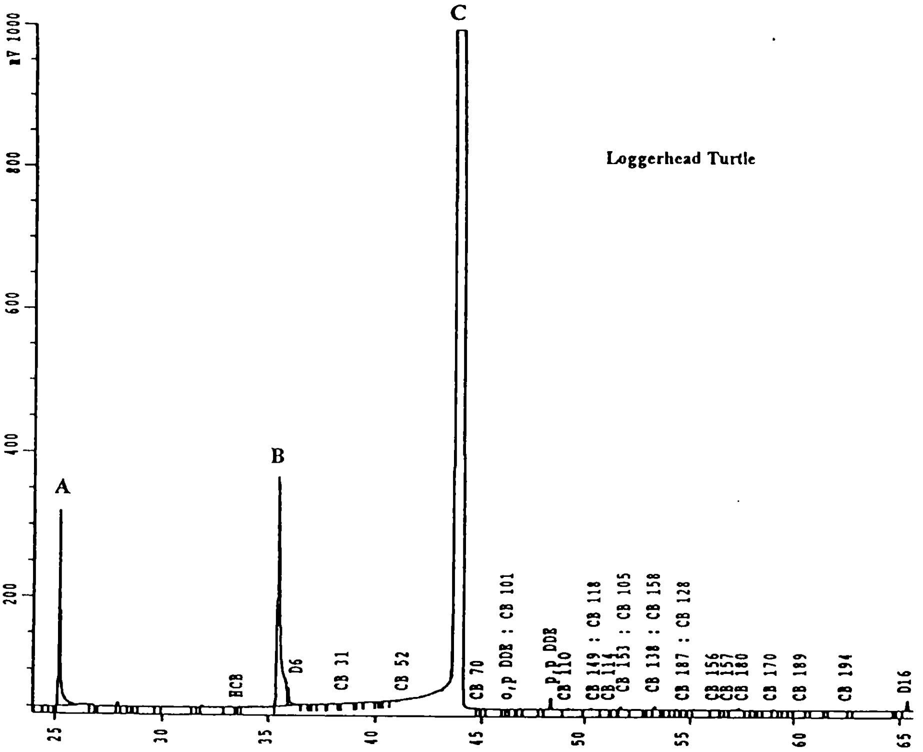


Fig 3b

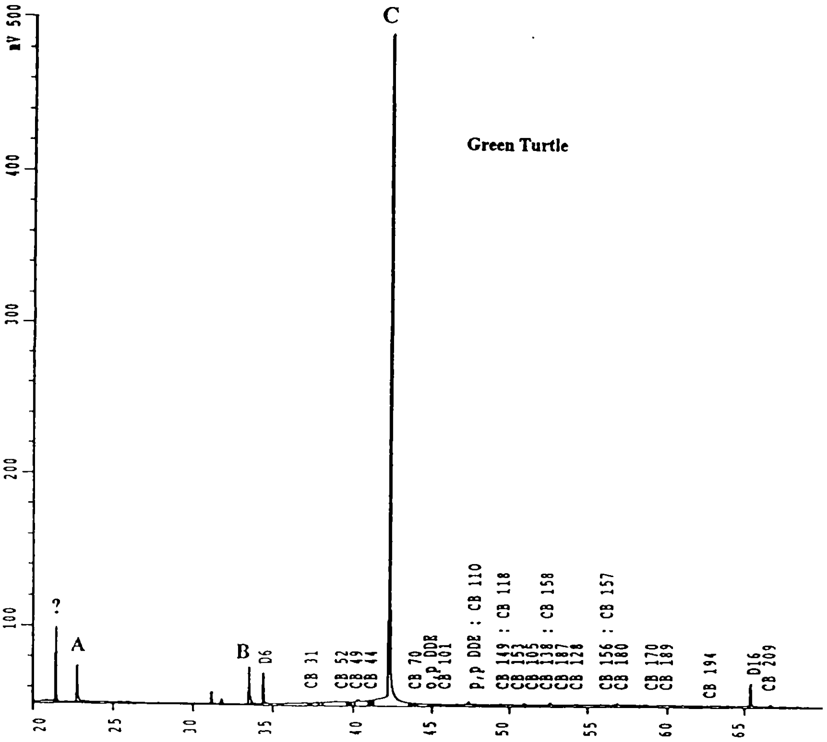


Fig 3c

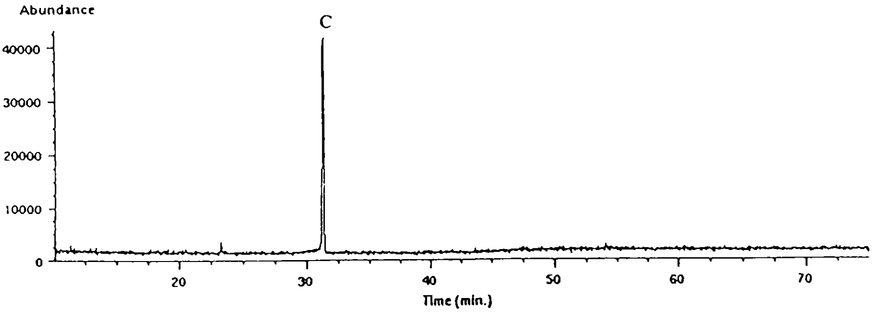
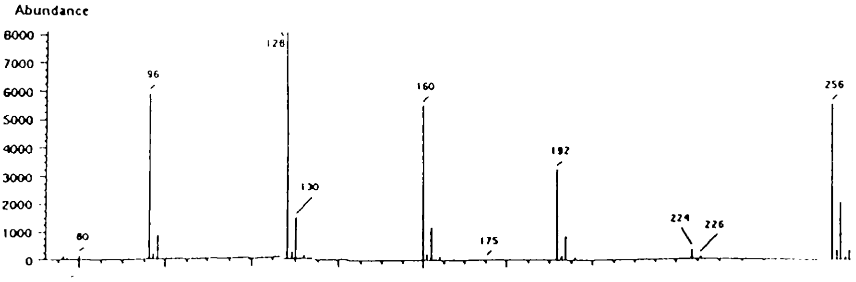


Fig 3d

Scan 1117 (31.274 min) of 22010101001.d



ig. 4. PCA biplot of green and loggerhead turtle data normalised to CB153, excluding loggerhead turtle hatchling, LH1.

Fig 4

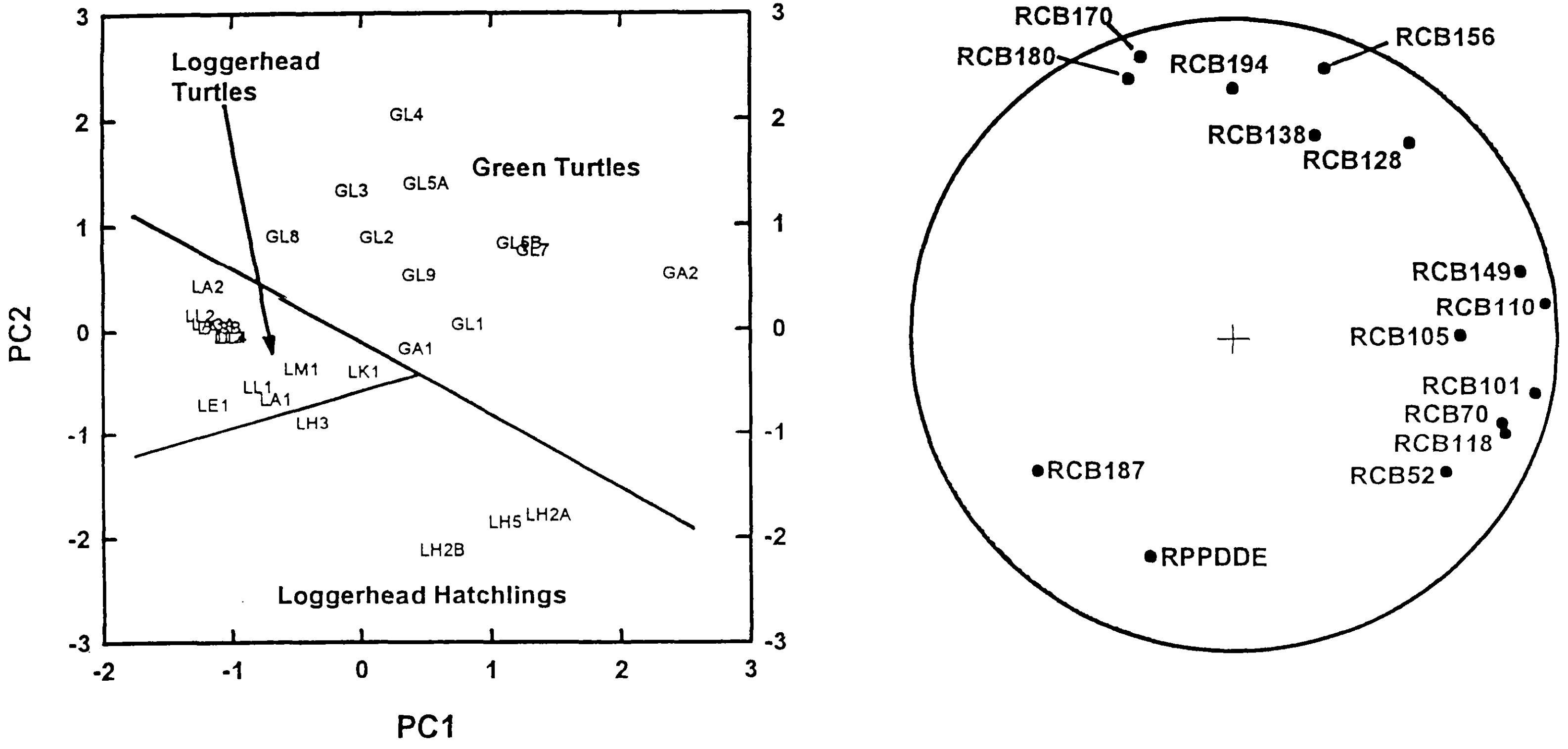
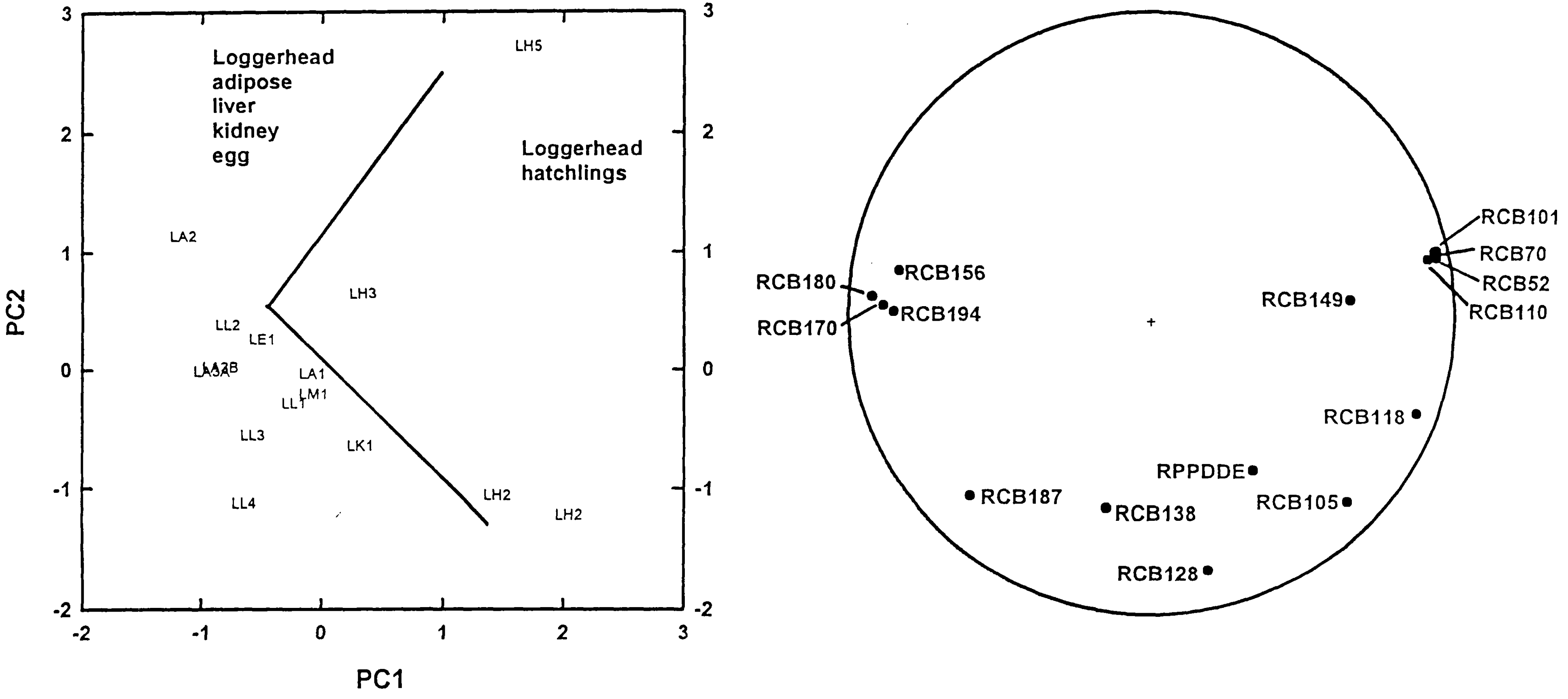
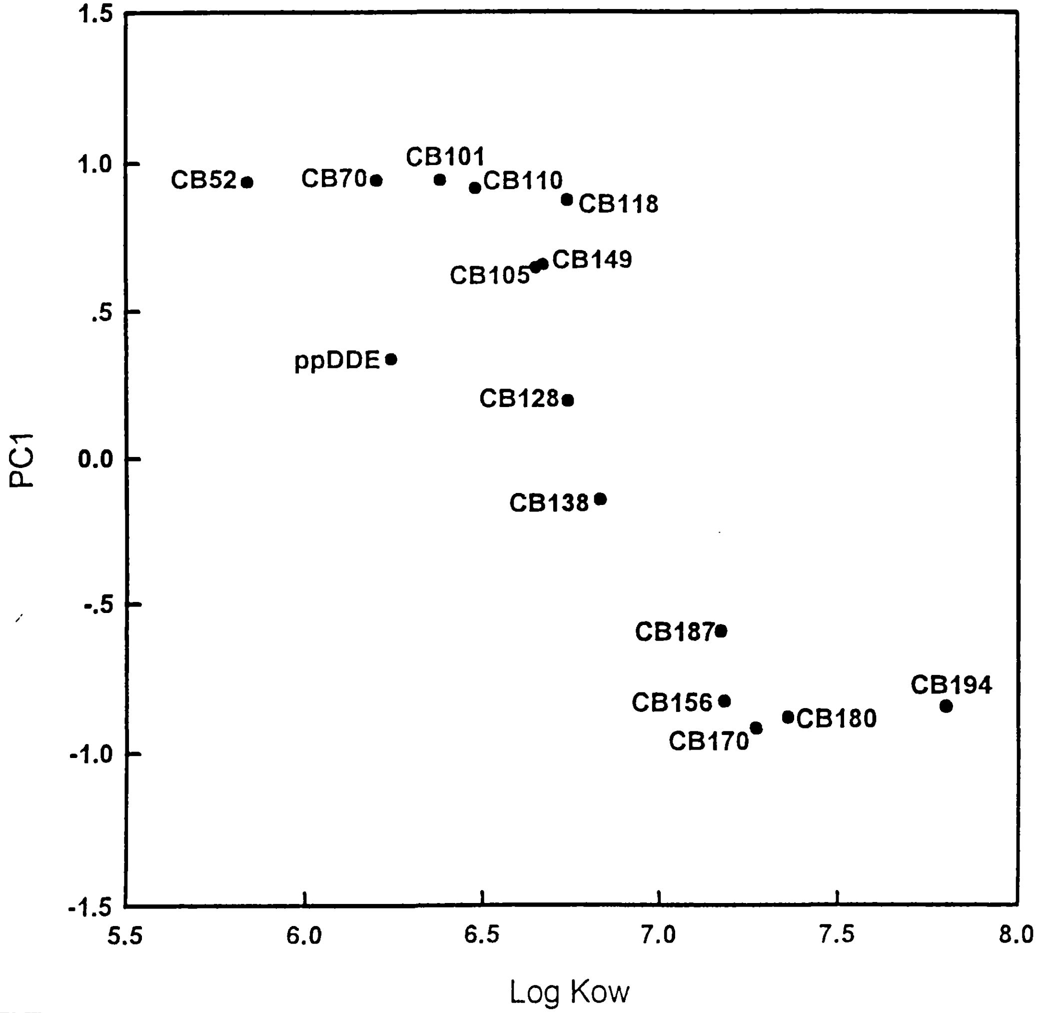

PC2
PC1
3
2
1
0
-1
-2
-3
-2
-1
0
1
2
3
Loggerhead Turtles
Green Turtles
Loggerhead Hatchlings
GL4
GL3
GL5A
GL8
GL2
GL5B
GL7
GA2
GL9
LA2
LL2
GL1
GA1
LM1
LK1
LL1
LA1
LE1
LH3
LH5
LH2A
LH2B
RCB170
RCB180
RCB194
RCB156
RCB138
RCB128
RCB149
RCB110
RCB105
RCB101
RCB70
RCB118
RCB52
RCB187
RPPDDE

Fig 5


PC2
PC1
3
2
1
0
-1
-2
-2
-1
0
1
2
3
Loggerhead
adipose
liver
kidney
egg
Loggerhead
hatchlings
LH5
LA2
LH3
LL2
LE1
LA3A
LA3B
LA1
LM1
LL1
LL3
LK1
LL4
LH2
LH2
RCB180
RCB156
RCB194
RCB170
RCB149
RCB101
RCB70
RCB52
RCB110
RCB118
RPPDDE
RCB187
RCB138
RCB105
RCB128

ig. 6. Contaminant loading scores for PC1 for loggerhead turtle data normalised to CB153, excluding loggerhead turtle hatchling, LH1 vs Log K_{ow}.

Fig 6


PC1
1.5
1.0
.5
0.0
-.5
-1.0
-1.5
5.5
6.0
6.5
7.0
7.5
8.0
Log Kow
CB52
CB70
CB101
CB110
CB118
CB105
CB149
ppDDE
CB128
CB138
CB187
CB194
CB156
CB180
CB170

Chapter 9

Heavy Metal Concentrations in Tissues and Eggs of Marine Turtles from the Mediterranean Sea.

Submitted as:

Godley, B.J., D.R. Thompson and R.W. Furness. Heavy metal concentrations in tissues and eggs of marine turtles from the Mediterranean sea. Marine Pollution Bulletin.

Abstract

Concentrations of heavy metals (Hg, Cd and Pb) were determined in internal organs and nest contents of green *Chelonia mydas* and loggerhead *Caretta caretta* turtles from the eastern Mediterranean Sea. Highest concentrations of mercury were found in liver tissue of loggerhead turtles (median 2.41 $\mu g\ g^{-1}$ dry weight). The corresponding value for green turtles was an order of magnitude less (0.55 $\mu g\ g^{-1}$ dry weight). Cadmium concentrations were highest in kidney tissue of loggerhead turtles (median 30.50 $\mu g\ g^{-1}$ dry weight), whereas the highest value in green turtles tissues was in the liver (median 5.89 $\mu g\ g^{-1}$ dry weight). Concentrations of lead in internal tissues were often below analytical detection limits in both species, but when measurable, tended to be higher in loggerhead turtles. Heavy metal concentrations in egg contents from both these species were uniformly low, often below analytical detection limits. When measurable, concentrations of all three metals tended to be higher in loggerhead turtle nest contents than in green turtle nest contents. We compare these results to the findings of similar studies of marine turtles from other locations, and we discuss the suitability of marine turtle nest contents as a tool in the monitoring of heavy metal contamination in these species.

Introduction

Only three species of marine turtle occur regularly in the Mediterranean Sea. These are loggerhead *Caretta caretta*, green *Chelonia mydas* and leatherback *Dermochelys coriacea* turtles (Groombridge, 1990). Of these, loggerhead and green turtles breed within the region, whilst leatherback turtles are thought to be non-breeding visitors. On a world-wide scale, marine turtle populations are generally in decline (Eckert, 1995; Limpus, 1995).Within the Mediterranean region, it is estimated that the annual breeding populations are as few as 2,000 female loggerhead turtles and 300-400 female green turtles, and it is recommended that both species should be considered regionally endangered (Groombridge, 1990).

One of the cited threats to the survival of marine turtles is pollution (Hutchinson and Simmonds, 1992; Lutcavage *et al.*, 1997). Of the major categories of potential pollutants, the impacts upon marine turtles of: solid debris (Gramentz, 1988; Hobson *et al.*, in press); oil and tar (Gramentz, 1988); and organochlorine residues (McKenzie *et al.*, in press) have been investigated within the Mediterranean region. As far as we are aware, the only previous study which has investigated heavy metal contamination in marine turtles in the Mediterranean region was that of Kaska (1998), in which metal concentrations were determined in eggshells, yolk and embryonic livers of loggerhead turtles from Turkey.

Because of the semi-closed nature of the Mediterranean Sea and the relatively large centres of human population that impinge upon its shores, the levels of marine contaminants to which biota from this ecosystem are exposed are thought to be relatively high (Bacci, 1989; Meadows, 1989; Kuetting, 1994; Borrell *et al.*, 1997). There is a clear need, therefore, to augment the relatively small amount of data regarding heavy metal burdens in marine turtles from the Mediterranean Sea.

In this paper, we present heavy metal (mercury, cadmium and lead) concentrations in the organs and nest contents of loggerhead and green turtles from northern Cyprus, eastern Mediterranean, and evaluate the usefulness with which turtle egg contents may be used to monitor heavy metal contamination in this group.

Materials and Methods

Sample collection and preparation

As part of an investigation of turtles washed ashore dead in northern Cyprus, 1994-1996, curved carapace length (CCL) was measured (using a flexible tape-measure, to the nearest 0.5cm), and organs (liver, kidney and muscle tissues where possible) were dissected from loggerhead (n = 7, mean CCL = 63.5cm, s.d. = 14.2, range = 56.0-79.0cm) and green (n = 6, mean CCL = 49.5, s.d. = 16.6, range = 27.5-56.0cm) turtles. As beaches were patrolled regularly as part of other turtle studies, it was possible to obtain samples from turtles that were located within 24 hours of stranding. Samples of nest contents (dead hatchlings, dead embryos and undeveloped eggs) were opportunistically sampled from remains of previously hatched nests of both species (green turtles: n = 69; loggerhead turtles: n = 48) at Alagadi beach, northern Cyprus, according to an established protocol (Broderick and Godley, 1996). Only one sample per nest was included in the study. All samples were stored frozen (at *ca.* -20^{0}C) until further treatment. Prior to metal analyses, tissues were thawed at ambient room temperature (*ca.* 20^{o}C), then dried to constant mass in an oven at 50^{o}C. Approximate water content, expressed as a percentage of fresh weight, was calculated. However, since samples had been frozen, fresh weights are not presented.

Metal analyses

Total (organic and inorganic combined) mercury, cadmium and lead concentrations were measured. Total mercury concentrations were determined using a cold vapour atomic absorption spectrophotometry technique following an established methodology (Furness *et al.*, 1986; Thompson and Furness, 1989). Cadmium and lead concentrations were measured using flame atomic absorption spectrophotometry according to the methodology described in Stewart *et al.* (1994). All metal concentrations are presented as μg g^{-1} (ppm) of tissue on a dry weight basis. Analytical limits of detection were determined as 0.01μg g^{-1} dry weight.

Results

Concentrations of mercury, cadmium and lead in internal tissues of loggerhead and green turtles are presented in Table 1. Loggerhead turtles tended to exhibit higher metal concentrations than found in green turtles (Table 1). Maximum concentrations in liver tissue of 7.50, 12.97 and 4.90 μg g^{-1} for mercury, cadmium and lead, respectively, were recorded in loggerhead turtles with corresponding maximum concentrations of 1.37, 10.73 and 1.84 μg g^{-1} in green turtles (Table 1). Mercury concentrations were highest in liver tissue > kidney > muscle for both species, cadmium concentrations in kidney tissue were as high or higher than those in liver tissue, but lowest in muscle tissue, whilst lead concentrations appeared to be fairly similar in all three tissues (Table 1). Mean water contents of liver, kidney and muscle tissues were 78%, 72% and 79%, respectively.

Metal concentrations in nest contents from green and loggerhead turtles are presented in Table 2. Concentrations of mercury were generally low, with maximum values of 0.24 μg g^{-1} in green turtle hatchlings and 0.75 μg g^{-1} in loggerhead turtle hatchlings (Table 2). Maximum cadmium concentrations were recorded in yolk and albumen of green turtles (1.22 μg g^{-1}; Table 2), and in hatchling loggerhead turtles (1.45 μg g^{-1}; Table 2). Lead concentrations were higher, with maximum concentrations in hatchlings of 3.86 and 10.56 μg g^{-1} in green and loggerhead turtles, respectively. There was relatively little variation in metal concentrations among the three nest content categories (Table 2). Water content in eggs, embryos and hatchlings ranged from 70-75%.

Discussion

Data regarding stranded turtles presented in this study (Table 1), although based on relatively small sample sizes, offer convincing evidence that for these two Mediterranean marine turtle species, the concentrations of heavy metals in tissues are likely to reflect marked differences in their diet. Green turtles are thought to be generally herbivorous, whilst loggerhead turtles are carnivorous (Bjorndal, 1997). Although there is a paucity of data regarding diet of these species in the region, these broad dietary differences, leading to corresponding differences in trophic status, were recently confirmed using stable isotope techniques (Godley *et al.*, in press a). The higher concentrations of metals in loggerhead turtles compared to green turtles reported here (Table 1) are entirely in keeping with these pronounced trophic differences. A similar pattern was also found in a recent study of organochlorine contaminants in tissues from turtles from the same populations (McKenzie *et al.*, in press). Within both species, patterns of metal accumulation followed those described for other marine vertebrates (see Thompson, 1990 for a review), in that mercury concentrations tended to be highest in liver tissue, cadmium concentrations tended to be highest in kidney tissue and lead concentrations tended to be higher in liver and kidney, than in muscle (Table 1).

For comparative purposes, a summary of heavy metal concentrations in internal tissues of marine turtles determined by other studies is presented in Table 3. To enable comparison with data presented in this study, data from Aguirre *et al.* (1994) and from Sakai *et al.* (1995), which were originally presented on a wet weight basis, have been converted to approximate dry weight basis using mean water content values determined in this study of liver 78%, kidney 72% muscle 78% (see Results). Mercury concentrations in loggerhead turtles from Japan (Sakai *et al.*, 1995) were similar to those reported here (Tables 1 & 3). In liver tissue, for example, the median mercury concentration in loggerhead turtles reported by Sakai *et al.* (1995) was ca. 1.73 $\mu g\ g^{-1}$ (converted dry weight; Table 3), compared to 2.41 $\mu g\ g^{-1}$ in this study (Table 1). In contrast, cadmium concentrations in both green turtles from Hawaii (median kidney concentration ca. 56.79 $\mu g\ g^{-1}$ converted dry weight; Table 3. Aguirre *et al.*, 1994) and from loggerhead turtles from Japan (median kidney concentration ca. 162.50 $\mu g\ g^{-1}$ converted dry weight; Table 3. Sakai *et al.*, 1995) were considerably higher than those reported here (median kidney concentration: green turtle 3.46 $\mu g\ g^{-1}$, loggerhead turtle 30.5 $\mu g\ g^{-1}$; Table 1).

Differences in heavy metal concentrations between populations, may be explained by differences in diet, prevailing environmental contamination in their foraging ranges, by age of individuals sampled, or by a combination of these. Both the study in Japan (Sakai *et al.*, 1995) and in Hawaii (Aguirre *et al.*, 1994) incorporated larger individual turtles than those of the same species in the Mediterranean, which were likely, therefore, to have been older. Since cadmium, for example, is known to accumulate in marine vertebrates with age(Stewart *et al.*, 1994; Dietz *et al.*, 1996), this may explain why cadmium concentrations in both conspecific sample sets (Table 3) were higher than those from the Mediterranean reported here (Table 1). Mercury levels were not determined in green turtles from Hawaii (Aguirre *et al.*, 1994), and mercury concentrations in turtles from the Mediterranean (Table 1) were comparable with those from Japan (Sakai *et al.*, 1995. Table 3). The fact that mercury concentrations did not show the same spatial differences as for cadmium (see above) may be explained by the fact that, in general, organisms from the Mediterranean would be expected to have relatively high mercury levels due to the presence of a natural mercury bed in the region (Bacci, 1989).

Concentrations of metals in leatherback turtles (Davenport and Wrench, 1990; Godley *et al.*, in press b. Table 3) are generally similar to those reported in green turtles in this study

(Table 1), but lower than those reported for loggerhead turtles from Japan (Sakai *et al.*, 1995. Table 3) and in this study (Table 1). Godley *et al.* (in press b) hypothesised that the highly pelagic nature of this species was likely to contribute to low contaminant burdens, both due to avoidance of the contaminated neritic, and through feeding within a food web which is likely to contain few trophic (and therefore bioaccumulative) steps.

Heavy metal concentrations in nest contents of both species in the present study were generally low, often below quantifiable limits (Table 2). However, metal levels appeared lower in green turtle eggs compared to burdens measured in loggerhead turtle nest contents. This was also the case in a preliminary analysis of levels of organochlorines in eggs and hatchlings from the same site (McKenzie *et al.*, in press). Whilst most egg content samples exhibited low metal concentrations (Table 2), a small number of individual samples exhibited relatively high lead concentrations. Hatchling green turtles exhibited a maximum lead concentration of 3.86 $\mu g\ g^{-1}$ and the corresponding value for loggerhead turtle hatchlings was 10.56 $\mu g\ g^{-1}$ (Table 2). Whilst not directly comparable, in a recent review of the toxicity of lead in birds, Franson (1996) suggested that liver concentrations of lead of as low as ca. 2 $\mu g\ g^{-1}$ wet weight (approximately 10 $\mu g\ g^{-1}$ dry weight) could cause subclinical toxic effects in some species. Lead concentrations in loggerhead turtle hatchlings, in particular, would appear to be approaching those concentrations, and would warrant further study.

In a previous study of metal burdens in turtles from the eastern Mediterranean, uniformly low levels of all three contaminants determined here were also reported by Kaska (1998). Although mean concentrations of all three metals in both shells and in yolks were similar to the median values of those in the loggerhead turtle nest contents from northern Cyprus reported here (Table 2), mean levels of all three metals in embryonic loggerhead turtle livers (mercury 0.51; cadmium 1.36; lead 2.48 $\mu g\ g^{-1}$ dry weight. Kaska, 1998) were higher. This might suggest the development of a degree of differential organ partitioning of metals prior to hatching. Similarly, Sakai *et al.* (1995) reported relatively low egg concentrations of mercury (0.01 $\mu g\ g^{-1}$ wet weight) and cadmium (0.03 $\mu g\ g^{-1}$ wet weight) in loggerhead turtles from Japan.

Early work by Stoneburner *et al.* (1980) determined a range of metals (including mercury, cadmium and lead) in loggerhead turtle egg yolk from four sites in the USA. The concentrations of metals presented by Stoneburner *et al.* (1980) were up to two orders of magnitude higher than concentrations in nest contents reported more recently (Aguirre *et al.*, 1994; Sakai *et al.*, 1995; Kaska, 1998; this study). This may be due to relatively high levels of contamination in the USA neritic. Alternatively, it is possible that early analytical methods may not have been as reliable as those currently used.

In conclusion, the preliminary data presented here suggest that metal levels in both green and loggerhead turtles are not likely to be high enough to affect the health of these endangered species. The only exception to this might be relatively high lead concentrations in loggerhead turtle hatchlings (Table 2). It is suggested that for non-invasive monitoring of the potential impact of metal pollution on these species that undeveloped egg, dead embryo or dead hatchling are equally useful monitoring units. That nest contents would constitute a non-invasive and meaningful monitor of heavy metal burdens in marine turtles is further supported by the findings of Sakai *et al.* (1995), who demonstrated that egg concentrations correlated with the those in the female from which they were sampled. In addition, although metal concentrations tend to be generally low in eggs, given the large number laid by reproductively active females (Broderick and Godley, 1996), excretion of metal burdens through eggs may be a substantial elimination route in this group. Although Sakai *et al.* (1995) suggested that this might not be important (<0.5% cadmium and <5% mercury burden per clutch), these authors ignored the fact that turtles lay multiple clutches, over many years. At the very least, further monitoring of metal burdens in marine turtles in the Mediterranean Sea region would seem prudent, especially from those ranging into more intensively industrialised regions such as Spain, Italy and Greece.

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TABLE 1. Mercury, cadmium and lead concentrations ($\mu g\ g^{-1}$ dry weight) in internal organs from loggerhead and green turtles found stranded in northern Cyprus. Values are medians, sample size in parentheses and ranges are given. For analytical limits of detection, see Methods.

Species	Tissue	Mercury Median (n) Range	Cadmium Median (n) Range	Lead Median (n) Range
Loggerhead	Liver	2.41 (5)	8.64 (4)	BDL (4)
		0.82-7.50	5.14-12.97	BDL-4.90
	Kidney	0.47 (2)	30.50 (2)	2.45 (2)
		0.13-0.80	18.8-42.2	BDL-4.90
	Muscle	0.24 (7)	0.57 (4)	2.20 (4)
		BDL-1.78	0.30-1.43	BDL-5.53
Green	Liver	0.55 (6)	5.89 (6)	BDL (6)
		0.27-1.37	2.53-10.73	BDL-1.84
	Kidney	BDL (1)	3.46 (1)	1.81 (1)
		na	na	na
	Muscle	0.09 (5)	0.37 (6)	BDL (6)
		BDL-0.37	0.12-0.78	BDL-2.45

BDL: Below detection limit
na: not applicable

TABLE 2. Mercury, cadmium and lead concentrations (µg g^{-1} dry weight) in nest contents of green and loggerhead turtles from Alagadi beach, northern Cyprus. Values are medians, sample sizes in parentheses and ranges. For analytical limits of detection, see Methods.

Species	Sample type	Mercury Median (n) Range	Cadmium Median (n) Range	Lead Median (n) Range
Loggerhead	Hatchling	0.02 (16)	0.34 (16)	0.13 (16)
		BDL-0.75	BDL-1.45	BDL-10.56
	Embryo	0.01 (27)	0.21 (29)	BDL
		BDL-0.22	BDL-1.09	BDL-6.48
	Yolk & Albumen	0.19 (3)	0.23 (3)	0.19 (3)
		0.16-0.57	0.23-0.56	BDL-3.93
Green	Hatchling	BDL (24)	0.23 (29)	BDL (29)
		BDL-0.24	BDL-0.94	BDL-3.86
	Embryo	BDL (18)	0.33 (16)	0.66 (16)
		BDL-0.12	BDL-0.93	BDL-3.41
	Yolk & Albumen	BDL (17)	0.27 (24)	BDL (24)
		BDL-0.19	0.05-1.22	BDL-1.61

BDL: Below detection limit

TABLE 3. Heavy metal concentrations ($\mu g\ g^{-1}$ dry weight) in internal tissues in marine turtles from other locations. Values are medians, samples sizes in parentheses and ranges.

Species	Location	Tissue	Mercury Median (n) Range	Cadmium Median (n) Range	Lead Median (n) Range	Reference
Loggerhead	Japan	Liver	1.73 (7) 1.13-37.05	47.73 (7) 25.73-66.36	ND	Sakai *et al.* (1995)
	Japan	Kidney	1.00 (7) 0.43-1.57	162.50 (7) 64.64-201.79	ND	Sakai *et al.* (1995)
	Japan	Muscle	0.43 (7) 0.24-0.90	0.29(7) 0.19-0.56	ND	Sakai *et al.* (1995)
Green	Hawaii	Liver	ND	23.82 (13) 1.77-118.18	ND	Aguirre *et al.* (1994)
	Hawaii	Kidney	ND	56.79 (12) 16.86-250.71	ND	Aguirre *et al.* (1994)
Leatherback	UK	Liver	0.61 (4) 0.29-1.20	8.50(4) 0.22-88.00	0.08 (4) 0.02-14.00	Godley *et al.*(in press b)*
	UK	Muscle	0.12 (4) 0.04-0.29	2.10 (4) 0.06-7.50	0.08 (4) BDL-0.31	Godley *et al.*(in press b)*

BDL: Below detection limit, ND: Not determined *also incorporated data from Davenport and Wrench (1990)

Chapter 10

Molecular Profile of Loggerhead Turtles Nesting in Northern Cyprus.

The data in this chapter are included in a wider ranging study in press as:

Laurent, L., P. Casale, M.N. Bradai, B.J. Godley, G. Gerosa, 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 C. Gautier. Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. Molecular Ecology

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 those exploited or incidentally caught by fisheries. Tagging programs cannot be carried out at all nesting locations nor is it possible to mark all individuals within any given population. In addition, chronic tag loss further confounds such studies (Limpus 1992). This prevents an understanding of the impacts that fishery related mortality have 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 (Grenon & Batisse 1988), pressure on all marine turtle life history stages is likely to increase in this region. In addition to coastal urbanisation, tourism and pollution, human induced threats include incidental capture in marine fisheries. Fishery activity has become apparent as a major threat which is the most important artificial mortality factor for turtles known in this region. Bottom trawl, longline, driftnet, and small coastal fisheries have a large bycatch of marine turtles causing substantial mortality (Laurent *et al.* (1996)). For example, in the western Mediterranean, Spanish longline fisheries interact with loggerhead turtles, *Caretta caretta*, (Caminas 1988; Aguilar *et al.* 1995), with an estimated annual capture rate of greater than 20,000 in 1991 and 1992, and an estimated mortality rate of 20% of those captured (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 Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (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 large 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 and widespread samples 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 basin indicated that numerous individuals caught in this fishery area originated from the United States of America and elsewhere in the Atlantic (Laurent *et al.* 1993), demonstrating the transatlantic developmental migration of loggerhead turtles suggested by Carr (1987). These molecular findings also confirmed earlier speculation that an entry of Atlantic loggerheads into the Mediterranean may be common (Argano & Baldari 1983; Carr 1987; Laurent 1990a; Bolten *et al.* 1992). However, until now, no large nesting areas have 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.

This chapter summarises the findings of molecular profiling of the loggerhead turtle population nesting at Alagadi, northern Cyprus which was undertaken as part of a Mediterranean-wide study to determine haplotype frequencies at the different nesting colonies and compare them to those in bycatch (Laurent *et al* in press.). The data generated are also used to test a hypothesis suggested in a previous study (Broderick and Godley 1996) that among colony differences in curved carapace lengths represented the existence of sub-populations within the region.

Materials and methods

Sample collection

Samples of dead hatchlings or dead embryos were collected at the excavation of nests subsequent to hatching at Alagadi, northern Cyprus, according to the protocol of Broderick and Godley (1996). To avoid replication of genetic information, samples were only collected from the nests of known, tagged females.

DNA extraction, PCR amplification and sequencing

Analysis of DNA was carried out by Dr. Luc Laurent and colleagues and the full methodology is given in Laurent *et al.* (in press). Total DNA was extracted from muscle, hatchlings and eggs by standard phenol/chloroform protocol. PCR amplifications of the 5' part (L strand) of the control region were obtained. Primers L71 and H599 were subsequently designed to amplify variable 520/526 bp fragments which provided genetic markers for this study. Double-stranded amplifications were performed. Sequencing of control region portions were obtained. Double-stranded PCR products were purified by and cycle sequenced with each of the PCR primers using an Applied Biosystems automated DNA sequencers. Each sample was thus double-stranded sequenced enabling elimination of ambiguous sequence sites and validation of variable ones, leading to final sequence genetic markers of 452 or 458 bp in length. To assure accuracy, new haplotypes were amplified and sequenced twice.

Results

Population structure

Table 1 shows the frequency of occurrence of haplotypes in each of the three nesting areas in the eastern Mediterranean. It can be seen that the haplotype A1 is ubiquitous and is found in a high proportion in all three samples. Although this haplotype makes up over half (19/32) of the samples from Turkey, with the remainder consisting of A3, it is fixed (100%) in Cyprus. In Greece although 90% of samples constituted A1, one individual had the haplotype A2. Because haplotype A1 is ubiquitous and not all individuals can be classified as unique using these methods, it is only by testing relative frequencies of haplotypes that statistical levels of confidence may be generated.

	Haplotype			Mean CCL (cm)	Literature source
Sampling area	**A1**	**A2**	**A3**		
Turkey (n=32)	19	0	13	75.6	Baran & Kasparek 1989
Greece (n=10)	9	1	0	83.1	Margaritoulis 1989
Cyprus (n=35)	35	0	0	73.4	Broderick & Godley 1996

Table 1. The distribution of haplotypes from each of Turkey, Greece and Cyprus and literature values for mean curved carapace lengths for each nesting population.

Taking Turkey as a baseline to compare with Greece and Cyprus, the probability of an individual from this arbitrary population being of the haplotype A1 can be estimated as:

$p\,(A1)_{TURKEY} = 19/(19+13) = 0.59$

Given this probability, the likelihood of all 35 samples from Cyprus being A1, if A1 is present with the same frequency as in the Turkish population is:

$(0.59)^{35} = 1 \times 10^{-8}$ (i.e. highly unlikely)

In addition, the likelihood of 9 out of 10 samples from Greece being A1 is calculated using the binomial theorem (Zar 1984) as:

$10\,(p^{9}(1-p)) = 0.037$ (i.e. unlikely)

Thus using this simple test of molecular data, there is strongly suggestive evidence that the nesting populations of Cyprus and Greece are distinct from that of Turkey.

The frequency of A1 in Greece is 0.9 the probability of all 35 samples from Cyprus being A1 can be defined as:

$0.9^{35} = 0.02$ (i.e. unlikely)

This is suggestive of a difference between Cyprus and Greece. However, caution should be exerted as a larger sample from Greece would be necessary to confirm this situation did not arise as result of small sample size.

Notwithstanding, these results are in broad agreement with a suggestion made by Broderick and Godley (1996) that marked differences in curved carapace length among nesting sites might be an indication of the presence of sub-populations in the Mediterranean due to reproductive isolation. A statistical comparison of loggerhead female size showed that females nesting in Cyprus have significantly smaller curved carapace lengths than those nesting in Greece, and those nesting in Turkey according to data from Margaritoulis (1989) and Baran and Kasparek (1989), respectively.

Discussion

Population structure

The DNA data presented here demonstrate that Turkey holds a nesting population that is genetically distinct from the proximate Cyprus and Greek nesting areas. Within nesting areas, it is likely that population differentiation among adjacent nesting sites occurs. This was recently shown for different nesting sites in Turkey by means of analysis of both mitochondrial and nuclear markers (Schroth *et al.* 1996).

Population management

Each loggerhead nesting area in the Mediterranean should be considered as an independent demographic entity, *i.e.* Management Unit (Moritz 1994). It would, therefore, be unwise to base policy on the monitoring of a few important nesting sites only, *e.g.* Zakynthos (Greece). Each Mediterranean population should be managed separately at all life history stages by considering that immature stages of these populations share pelagic habitats throughout the Mediterranean (and possibly the eastern Atlantic), while large immatures and adults have distinct and more localised benthic habitats in the eastern basin. These habitats need to be identified in further studies using a combination of traditional tagging, the utilisation of molecular techniques, telemetry and studies at sea.

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Chapter 11

Patterns of Marine Turtle Mortality in British Waters (1992–1996) with Reference to Tissue Contaminant Levels.

In press as:

Godley, B.J., M.J. Gaywood, R.J. Law, C.J. M^{c}Carthy, C. M^{c}Kenzie, I.A.P. Patterson, R.S. Penrose, R.J. Reid, and H.M. Ross. Patterns of marine turtle mortality in British waters (1992–1996) with reference to tissue contaminant levels Journal of the Marine Biology Association (UK).

Abstract

Mortality patterns of marine turtles entangled in fishing gear, found dead at sea or stranded dead on and around the coast of Britain in the period 1992–1996 are described. Of a total of 38 dead turtles identified, 35 were leatherback turtles (*Dermochelys coriacea*) and three were loggerhead turtles (*Caretta caretta*). All *D. coriacea* were considered adults or subadults nearing sexual maturity. Six individuals were assessed as females, ten were classified as males and 19 were not sexed. *Dermochelys coriacea* (N=20 measured) ranged from 120 to 210 cm in curved carapace length (mean, 152 cm). The three *C. caretta* were juveniles, and ranged from 15 to 30 cm curved carapace length. Possible origins, causes of mortality and interactions with fisheries are discussed. In addition, contaminant levels were determined in the tissues of three *D. coriacea*. Concentrations of all contaminants determined were found to be low.

Introduction

It has been well documented that marine turtles, especially the leatherback (*Dermochelys coriacea* (Vandelli)) and the loggerhead (*Caretta caretta* (L.)) range widely in european Atlantic waters (Brongersma, 1972; Gaywood 1997; Penhallurick, 1990, 1991, 1993). Whereas the presence of *C. caretta* and any other cheloniid turtles in British waters is thought to be the result of animals being carried by currents from their normal habitat, it is widely accepted that *D. coriacea* should be considered a normal and regular member of the British marine fauna. Indeed, this species has been shown to possess numerous adaptations that allow it to function in temperate waters (see for example Frair et al., 1972; Davenport et al., 1990a).

In the Atlantic, *D. coriacea* nest on beaches of tropical regions which include sites in the Caribbean islands, Florida, Central and South America and the west coast of Africa (Boulon et al., 1996; Campbell *et al.*, 1996; Fretey & Girardin, 1989; Girondot & Fretey, 1996; Spotila et al., 1996). It is thought that outwith reproductive periods, this species ranges widely in search of its medusoid prey, which in British waters, mainly consist of *Rhizostoma* and *Cyanea* (Den Hartog & Van Neirop, 1984; Holland et al., 1990). Anecdotal accounts from around Britain and quantitative evidence from the Atlantic coast of North America suggest that abundance of both *D. coriacea* and their jellyfish prey may be correlated (Grant et al., 1996), however, there appear to be seasons where jellyfish are abundant and *D. coriacea* absent (personal communication, S. Murphy).

There is great concern over the future of the world leatherback population, which appears to be in decline. Some authors have suggested that, from current evidence, it is plausible that this species may be on the verge of extinction (Spotila et al., 1996). Although this view has been questioned (Pritchard, 1996), it is apparent that the status of, and threats to, this species in all parts of its range should be quantified and, where possible, addressed.

Data regarding recent patterns and causes of mortality and baseline tissue contaminant levels in other marine megafauna in British waters are quite well established (see for example Bucke, 1990; Law, 1994; North Sea Task Force, 1993). Information regarding similar parameters in marine turtles in British and other northern European waters is, at best, scant. Only levels of metals and organic pollutants in tissues of a single *D. coriacea*, stranded dead in Wales in 1988, have been described (Davenport & Wrench, 1990; Davenport et al., 1990b). This study attempts to qualify the levels of marine turtle mortality in British waters in recent years and investigates the species, sex, size-class composition of the individuals involved and possible reasons for mortality. In addition, baseline contaminant levels for *D. coriacea* are given as are recommendations as to priorities for further research.

Materials and Methods

Marine turtle strandings

Data regarding marine turtle strandings from around Britain were tabulated for the period 1992–1996. These included cases of turtles stranded dead, turtles entangled in fishing gear which subsequently died, and turtles recovered dead at sea. Information consisted of data collected by both governmental and non-governmental organisations and compiled in numerous separate databases. Due to the retrospective nature of this study, not all data were collected in an identical way. However, similar data were collected in a sufficient portion of cases to allow comparisons to be made. These parameters included; date of discovery, species, size (medial curved carapace length or CCL), weight, and the presence of any lesions. In a limited number of cases, full necropsy was undertaken by veterinary pathologists.

Analysis of contaminant levels

Samples were taken for analysis of contaminant levels in three adult male *Dermochelys coriacea*, all of which drowned as the result of entanglement in fishing gear off the west coast of Britain. The locations, dates of these events and CCL for individuals 1–3 respectively, were: Tenby, Wales (12.09.96, 170 cm); Drumbeg, Sutherland, Scotland (18.10.93, 151 cm); Uig, Isle of Skye, Scotland (24. 10. 95, 141 cm).

A range of trace metals plus arsenic and selenium were determined in liver and muscle tissue of individual 1, using acid digestion with microwave heating followed (except for mercury) by analysis using inductively coupled plasma/mass spectrometry. Total mercury was analysed using atomic fluorescence detection, following reduction with tin (II) chloride. All analyses were conducted under an analytical quality protocol requiring the analysis of blanks and reference materials alongside each batch of samples. Further details of method performance are given elsewhere (Law, 1994; Law et al., 1997). The liver sample from this individual was also analysed for organotin compounds (tributyl and dibutyltin) by gas chromatography with flame-photometric detection (Waldock et al., 1989), but these compounds were not found (limits of detection 0.006 and 0.008 mg kg^{-1} respectively). Total mercury, cadmium and lead concentrations were quantified in muscle and liver samples from individuals 2 and 3. Total mercury was analysed using a cold vapour absorption spectrophotometry technique according to an established methodology (Thompson & Furness, 1989). Cadmium and lead levels were ascertained by atomic absorption spectrophotometry according to the methodology of Stewart et al. (1994).

Adipose tissue samples from all three individuals were screened for organochlorine contaminants at two separate laboratories using comparable methodologies. Concentrations of organochlorine pesticides (including DDT and its metabolites) and a range of chlorobiphenyl (CB) congeners were determined by gas chromatography with electron capture detection (Allchin et al., 1989). The original methodologies have been modified in the light of recommendations from the intercomparison programme organised under the auspices of the International Council for the Exploration of the Sea (ICES) (de Boer et al. 1994). Data from both laboratories have been shown to yield comparable data in collaborative studies within ICES (Boon et al., 1997).

Adipose tissue and liver samples from individual 1 were analysed to determine levels of polycyclic aromatic hydrocarbons (PAH). Samples were analysed by coupled gas chromotography-mass spectrometry. Eighteen individual PAH compounds were determined.

Results

Patterns of mortality

A total of 38 dead turtles was recorded during the period 1992–1996. Of these, three were *Caretta caretta* and 35 were *Dermochelys coriacea*. Table 1 lists the status of animals discovered (dead stranded, dead entangled, found dead at sea), by species, year and major geographic area. The strandings were quite widely distributed around north and western Britain as can be seen in Figure 1. However, there is a conspicuous clustering in Carmarthen Bay, Wales (N=13).

The three *C. caretta* were found dead, stranded in the months of March, October and November and it is not possible to draw conclusions regarding temporal distribution, from such a small sample. In contrast, mortality events of *D. coriacea* (Figure 2) show a marked seasonality, with carcasses beginning to be discovered in July, rising to a pronounced peak in October (16/35=46%) , with few carcasses being discovered in November, December and January. The majority of carcasses were discovered between August and October (28/35=80%).

All *C. caretta* were small juveniles, of the size consistent with those expected to be found in the pelagic phase of marine turtle development (Musick & Limpus, 1997). Only one individual (19cm CCL, weight 0.762 kg) was freshly dead when discovered on 7 November 1995, on Benbecula, Western Isles, Scotland and was subjected to full necropsy, showing it to have no detectable gross lesions. It was judged that this individual was likely to have died from starvation after it had strayed/been carried by currents from its normal developmental habitat.

Mean CCL of all *D. coriacea* in this study was 152 cm (N=20 animals measured, SD=25.0, range 120–210 cm). When compared with available literature values for sizes of nesting females at different Atlantic nesting colonies, all individuals of this species were thought to be adults or subadults approaching sexual maturity (except possibly the individual of 120 cm CCL) (Boulon et al., 1996; Campbell et al., 1996; Fretey & Girardin, 1989; Girondot & Fretey, 1996). Due to sexual dimorphism in tail length, the tail of adult females rarely extends more than a few centimetres beyond the caudal tip of the carapace (M. Girondot, personal communication), it was possible to sex 16 of the 35 individuals. Of these, six (37.5%) were sexed as females and ten (62.5%) as males. Although it is advisable to treat data based solely on sexing according to this criterion with caution since large immature males could feasibly be recorded as adult females, it is likely that individuals, mostly adults, of both sexes are found in British waters.

In at least six cases, the cause of mortality of *D. coriacea* is known to have been entanglement (Table 1). However, most of the turtles for which the cause of death was not known, were not subject to detailed necropsy by a veterinary pathologist. In several cases, evidence suggestive of previous entanglement was present, including lacerations and entangling ropes. In others (N=2), there were possibilities of earlier collision with marine vessels. It could not be established, however, whether these events were ante- or post-mortem.

One individual which appeared to have died as the result of causes other than entanglement was an adult female (167cm CCL, weight 420 kg,) found dead on 26 December 1994, at Kircudbright, Scotland. This turtle was thought to have died as a result of starvation, caused by a primary obstruction of the digestive tract by ingested plastic and metal litter. In addition, the animal was likely to have been further compromised by a chronic necrotic lesion in the shoulder, caused by a large fish hook embedded deep within the pectoral muscle.

Contaminant levels

The results of metal contaminant analyses are given in Tables 2 & 3. There was considerable inter-individual variance in the levels of metals investigated. However the general patterns were similar, with liver levels exceeding those of muscle for all metals in all individuals. Cadmium concentrations in the three individuals in this study ranged from 5 to 88 mg kg^{-1} dry weight in livers and from 1.4 to 7.5 mg kg^{-1} dry weight in muscle. This range is almost tenfold greater than that found in the individual from Porthmadog, Wales (Davenport & Wrench, 1990). Lead levels ranged from 0.02 to 14.0 mg kg^{-1} in liver and were < 0.09 mg kg^{-1} in muscle from all individuals.

Mercury levels in the present study varied between 0.29 and 1.2 mg kg^{-1} in liver and 0.04 and 0.12 mg kg^{-1} in muscle. These levels were similar to the Porthmadog individual. It is not known whether turtles are able to detoxify methylmercury ingested from their diet, immobilizing mercury as the selenide as in marine mammals. The concentrations of mercury, are however very low, as are mercury/selenium ratios observed (Hg:Se < 1), suggesting that mercury is unlikely to have adversely affected the health of these animals.

Concentrations of all organochlorine compounds were low (Table 4). This was especially true of pesticide residues. Only *p, p,'*-DDE could be detected consistently in all three individuals with a range of 0.010 – 0.068 mg kg^{-1} wet weight. The fact that *p, p'*-DDT is essentially present only as *p, p'*-DDE indicates uptake remote from the source of the DDT. In all three cases, the major CB compounds present were, in decreasing levels, CB153, CB138 and CB180. Sum of levels of the ICES7 congeners gave a range of 0.035–0.16 mg kg^{-1} wet weight. Although not all congeners were measured in all individuals, it is thought that the sum of all congeners is representative with a range of 0.047–0.23 mg kg^{-1} wet weight. This range is considerably lower than the level of 1.2 µg g $lipid^{-1}$ described by Davenport et al. (1990b).

Results of PAH analyses are given in Table 5. PAH concentrations are very low or undetectable with ΣPAH concentrations of 12 and 5.5 µg kg^{-1} wet weight in adipose tissue and liver respectively. The higher molecular weight (MW) PAH of primarily combustion origin (MW 228, 252, and 276 Da; benz[*a*]anthracene to benzo[*ghi*]perylene) were not detected in either tissue.

Discussion

There is an apparent increasing trend of incidence of discovery of dead turtles over time (Table 1). However, it is not clear if this is due to a real increase in abundance, an increase in the mortality rate, an increased level of reporting, or any combination of these factors. However, it is apparent that a large number of adult *Dermochelys coriacea* use British waters, at least in some years, possibly when prey items are abundant. Both sexes are found and there is a marked clustering of the discovery of most mortality events into the months of August–November. This is consistent with the widely held belief that *D. coriacea* move into British waters when the water temperatures are highest (i.e. late summer and early autumn), given that there will, on average, be a lag between the peak in live sightings (i.e. peak in abundance), and the peak of strandings or discovery of carcasses. This is in broad agreement with data presented by Brongersma (1972) for the northern zone of the area defined as the European Atlantic.

During the study period, of the hard-shelled turtles, only juvenile *Caretta caretta*, were found stranded dead. Juveniles from the western Atlantic nesting population are known to use the North Atlantic gyre as an ontogenic feeding ground (Musick & Limpus 1997). Juveniles stranded in northern Europe are probably from this population, rather than from the Mediterranean population which is geographically closer, but numerically smaller. This observation has also been made regarding occasional stranded females of reproductive size (Hays & Clarke, 1995). Historically, Kemp's ridley turtles (*Lepidochelys kempi*) were also discovered stranded in Britain and Europe (Brongersma, 1972; Gaywood, 1997). It is likely that the paucity of such records in recent years is indicative of the extreme decline experienced by this species in the latter part of this century (USFWS & NMFS, 1992).

The likely origin of *D. coriacea* in British waters is a matter for speculation. They could be of African or western Atlantic origin. However, although the African nesting populations have been poorly studied, the western Atlantic population is thought to be much larger (Spotila et al., 1996). In addition, evidence for transatlantic migrations has been presented by Girondot and Fretey (1996), who reported that individuals from a long term tagging programme in French Guyana have not only been captured in south eastern USA and north to Newfoundland, but off Morocco, Spain and France. In addition, leatherback turtles have recently been tracked, using satellite telemetry, from Trinidad, via the north Atlantic, to the West African coast (S.Eckert, personal communication). It is likely that the stocks using British waters will be of mixed origin, if not dominated by individuals from western Atlantic breeding populations. The variability of concentrations of trace metals, arsenic and selenium in turtle tissues (Table 2) could further support the suggestion of a mixed origin for the *D. coriacea* stocks found in British waters.

Although marine turtle tagging studies are undertaken in many regions of the world, *D. coriacea* is known to be prone to high levels of loss of conventional flipper tags, making it difficult to determine the origin of turtles by conventional methods. Data concerning the origin of turtles dying in and around British waters may be obtained by genetic investigation (Dutton, 1996), by analysis of photographs of the 'pineal spot' i.e. unpigmented marks on the cranial skin, or by scanning the pectoral region for the presence of internal passive integrated transponders (PIT tags) (McDonald & Dutton, 1996).

From the data so far tabulated from four individuals, it appears unlikely that *D. coriacea* is exposed to high levels of chemical marine contaminants. It is thought that these data, could be considered representative of the adult *D. coriacea* which visit British waters. All individuals analysed for contaminants died traumatically, so it is likely that the confounding effects of concurrent disease and associated wastage on the monitoring of levels of contaminants, such as chlorobiphenyls, will be minimized. *Dermochelys coriacea* is highly pelagic and a medusivore. These two factors probably act together to lower the possible exposure to contaminants in this species. Firstly, the levels of contaminants in the pelagic environment are likely to be much lower than that of the neritic. Secondly, although *D. coriacea* is a top predator, because of the particular dietary specialisation in this species, its food web is likely to be one with few trophic steps, and therefore less potential for biomagnification of environmental contaminants.

Although levels of contaminants in the tissues of *D. coriacea* appear to be low, this species is not isolated from the detrimental effects of pollution. Ingestion of anthropogenic debris is known to be a problem. Previous studies have demonstrated the ingestion of plastic debris by *D. coriacea* (Berrow & Rogan 1995; Fritts 1982). Information presented here, with one individual dying as the result, clearly demonstrates the potential deleterious outcome of this behaviour.

At least six of the 35 leatherback turtles were entangled in lines of pot-fishing gear. In addition, the majority of stranding events (13/15=87%) in Wales have been in Carmarthen Bay, all of which were in 1995 and 1996. Although anecdotal accounts of marine turtles at sea and stranded on the coast of Wales have existed for many decades (J. Davenport, personal communication), the numbers stranded in 1995 and 1996 are unprecedented. Since 1994, an expanding pot-based whelk (*Buccinum undatum*) fishery has been in operation. This has grown annually since 1994 and was projected to have increased to a value of £1.5 million or more in 1996, but the fishery was interrupted by the 'Sea Empress' oil spill off Milford Haven. Along with other activities, the whelk fishery was closed under the Food and Environment Protection Act (FEPA), 1985, from 28 February to 29 August 1996, and a voluntary ban was operated by fishermen in the area from the time of the grounding on 15 February until the FEPA order was in place. However, fishing was underway by the time peak numbers of *D. coriacea* might have been expected in the region. Circumstantial evidence would suggest that this fishery may be of concern with regard to its interaction with an endangered species.

It is not known how individuals become entangled. It may be that the turtles collide with buoy ropes by chance. Alternatively, turtles may actively attack rope/buoy systems as potential prey items such as jellyfish, or they may be attracted by the scent of bait/captured target species and subsequently become entangled. It is possible that some combination of these factors act together. Amelioration of the problem would appear difficult. In an area consistently shown to yield an unacceptable level of turtle/fishery interactions, consideration should be given to a closed season, coincidental with the peak in turtle abundance. Peak abundance would need to be ascertained, by an aerial surveying regime.

It is worthy of note that, although the absolute number of turtles discovered dead in British waters may be low, it is likely that those discovered are only a portion of those killed or dying. In addition, a modelling study regarding *C. caretta* (Crouse et al., 1987) has suggested that population levels may be strongly influenced by the levels of sub-adult and adult mortality. Therefore, the impact of fisheries and any other causes of mortality in British and European waters may be significant or important, especially given the suspected global decline of *D. coriacea* (Spotila et al., 1996). A further impact of northern European fisheries on *D. coriacea* stocks has recently been highlighted . A study of incidental catch by the British tuna fishing fleet demonstrated a catch rate of eight *D. coriacea* per 10, 000 tuna, with a 100% mortality rate of turtles caught (N. Tregenza, personal communication)..

This study has highlighted the need for further detailed investigations of the biology of these endangered (*D. coriacea*) and threatened (*C. caretta*) species to be undertaken. It is recommended that more information be gathered concerning the dispersion of these species in British and other northern European waters through aerial or sea based surveys. Given the lack of detailed empirical knowledge regarding demographic parameters in these species and given their endangered/threatened status, it would appear that strandings in Europe may offer opportunities to bridge some of the gaps in the knowledge base. For example studies of bones might elucidate age of individuals (Zug et al., 1986; Zug & Parham, 1996). An increase in the proportion of animals subject to full necropsy would further elucidate mortality factors acting upon these populations. Since *D. coriacea* is the top predator of a very poorly studied food-chain (Holland et al., 1990), secondary analyses such as the determination of contaminant levels would not only enable monitoring the health of populations, but in conjunction with identification of dietary items, could augment the scant information of the synecology of this enigmatic species.

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		Year															
		1992			*1993*			*1994*			*1995*			*1996*			
		S	W	E	S	W	E	S	W	E	S	W	E	S	W	E	**Total**
D. coriacea	**Dead Stranding**							2		5		5		6	7	1	**28**
	Dead Entangled				1						1	2		1	1		**6**
	Dead at sea												1				**1**
	Total			2	1			2		5	1	7	1	7	8	1	**35**
C. caretta	**Dead Stranding**	1		1							1						**3**
All species		**1**	**0**	**3**	**1**	**0**	**0**	**2**	**0**	**5**	**2**	**7**	**1**	**7**	**8**	**1**	**38**
Annual total			**4**			**1**			**7**			**10**			**16**		

Table 1. Discovery of marine turtles by year and major geographic region.
(S, Scotland; W, Wales; E, England)

Tissue	**TS%**	**Cr**	**Fe**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Ag**	**Cd**	**Hg**	**Pb**	**Hg:Se**
Liver	31.8	< 0.018	5770	< 0.062	9.7	42	2.6	6.5	0.17	28	0.37	4.3	0.022
Muscle	33.3	0.97	38	0.53	0.76	51	4.7	4.3	< 0.003	2.5	0.013	< 0.031	0.0012

Table 2. Concentrations of trace metals, arsenic and selenium in liver and muscle tissue of individual 1 (mg kg^{-1} wet weight).(Hg:Se , molar ratio of the concentrations of mercury and selenium; TS% ., dry tissue percentage and is presented so data can be expressed on a dry weight basis for comparative purposes.)

Tissue	Individual	Ni	Cu	Zn	As	Se	Cd	Hg	Pb	Hg:Se
Liver	1	< 0.19	31	132	8.2	20	88	1.2	14	0.02
	2	n.d.	n.d.	n.d.	n.d.	n.d.	12	0.82	0.02	n.d.
	3	n.d	n.d.	n.d.	n.d.	n.d.	5	0.29	0.04	n.d.
	Porthmadog	2.1	0.15	2.6	0.58	1.4	0.22	0.39	0.12	0.11
Muscle	1	1.6	2.3	153	14	13	7.5	0.04	< 0.09	0.001
	2	n.d.	n.d.	n.d.	n.d.	n.d.	1.4	0.29	< 0.01	n.d.
	3	n.d.	n.d.	n.d.	n.d.	n.d.	2.8	0.12	< 0.01	n.d.
	Porthmadog	1.6	0.26	1.9	0.21	3.6	0.06	0.12	0.31	0.013

Table 3. Concentrations of trace metals, arsenic and selenium in livers of leatherback turtles from individual 1 (this study) and selected metals in individuals 2 and 3 compared with another individual stranded at Porthmadog, Wales in 1988 (mg kg^{-1} dry weight). The values for the latter are mean values derived from four replicate analyses (Davenport & Wrench, 1990).(n.d. = not determined)

Individual		Lipid%	α-HCH	β-HCH	γ-HCH	HCB	Dieldrin	p, p'-DDD	p, p'-DDE	p, p'-DDT
1		41	< 0.001	< 0.001	< 0.001	0.003	0.033	< 0.001	0.068	< 0.001
2		74	< 0.001	n.d	< 0.001	< 0.001	< 0.001	< 0.001	0.010	< 0.001
3		50	< 0.001	n.d	< 0.001	0.002	0.013	< 0.001	0.057	< 0.001
CB18	**CB31**	**CB28**	**CB52**	**CB49**	**CB47**	**CB44**	**CB66**	**CB70**	**CB74**	**CB101**
< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.006	n.d	n.d	< 0.001
n.d	<0.001	< 0.001	0.012	0.002	n.d	< 0.001	n.d	0.002	<0.001	0.004
n.d	<0.001	0.001	0.012	0.002	n.d	0.003	n.d	0.002	0.001	0.006
CB110	**CB151**	**CB149**	**CB118**	**CB153**	**CB105**	**CB141**	**CB138**	**CB158**	**CB187**	**CB183**
< 0.001	< 0.001	< 0.001	0.012	0.073	0.005	< 0.001	0.047	0.001	0.033	0.006
0.0002	n.d	0.001	0.001	0.008	< 0.001	n.d	0.005	<0.001	0.005	n.d
0.0008	n.d	0.004	0.008	0.046	0.003	n.d	0.025	0.001	0.019	n.d
CB128	**CB156**	**CB157**	**CB180**	**CB170**	**CB194**	**ΣICES7**	**ΣCBs**			
0.007	<0.001	n.d	0.032	0.013	0.003	0.160	0.230			
<0.001	<0.001	<0.001	0.005	0.001	<0.001	0.035	0.047			
0.004	0.001	0.001	0.024	0.011	0.004	0.123	0.178			

Table 4. Concentrations of organochlorine contaminants in adipose tissue of three leatherback turtles (mg kg^{-1} wet weight). (n.d. = not determined as part of specific analysis)

	Adipose tissue	**Liver**
Napthalene	1.2	1.5
C_1-Napthalenes	<0.1	2.2
C_2-Napthalenes	<0.1	<0.1
C_3-Napthalenes	<0.1	<0.1
Phenanthrene	1.5	0.6
Anthracene	<0.1	0.2
C_1-Phenanthrenes	6	<0.1
Fluoranthene	1.4	0.4
Pyrene	1.8	0.6
Benz[*a*]anthracene	<0.1	<0.1
Chrysene	<0.1	<0.1
2,3-Benzanthracene	<0.1	<0.1
Benzofluoranthenes	<0.1	<0.1
Benzo[*e*]pyrene	<0.1	<0.1
Benzo[*a*]pyrene	<0.1	<0.1
Perylene	<0.1	<0.1
Indeno[1,2,3-*cd*]pyrene	<0.1	<0.1
Benzo[*ghi*]perylene	<0.1	<0.1
ΣPAH	**12**	**5.5**

Table 5: Polycyclic aromatic hydrocarbons concentrations in adipose tissue and liver from individual 1 (μg kg^{-1} wet weight).

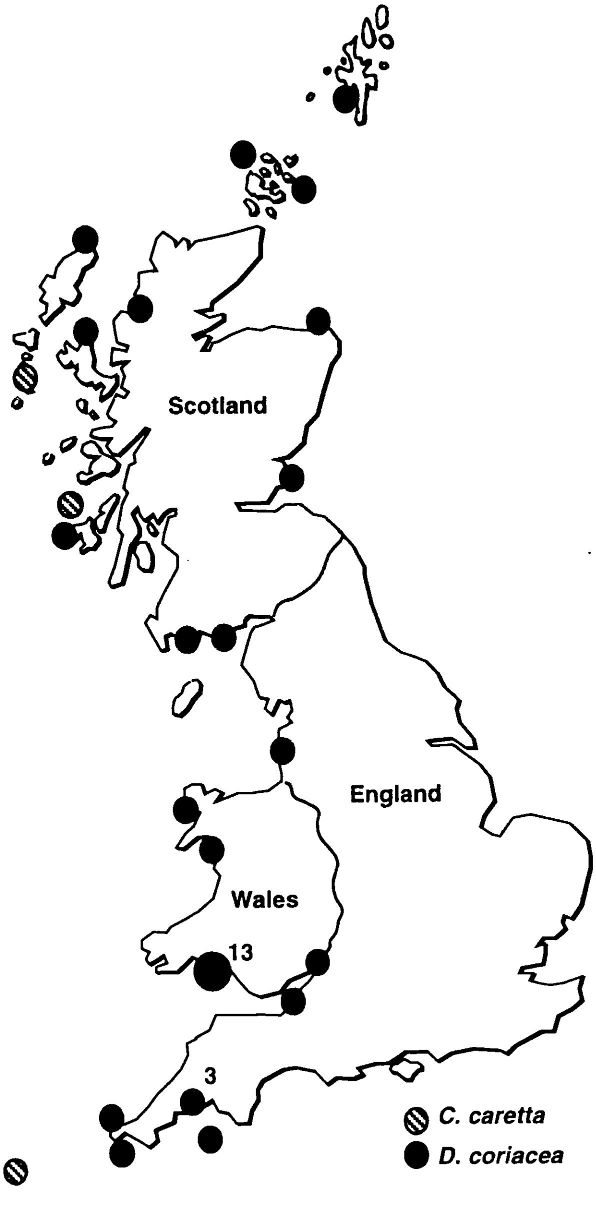

Figure 1. Schematic map of Britain to show locations where dead turtles were discovered (1992–1996).

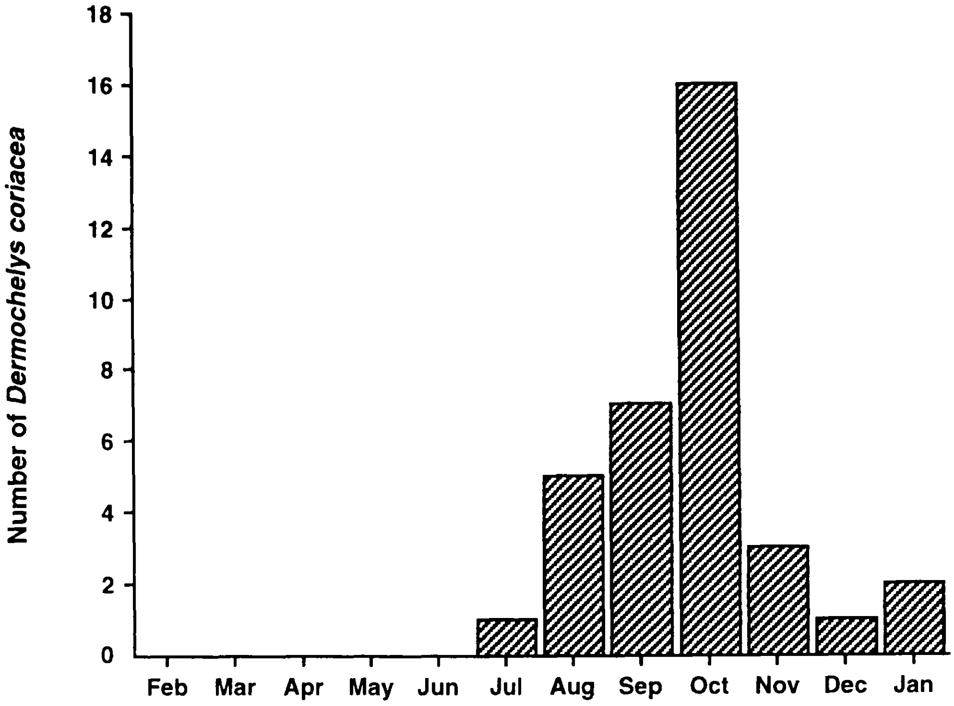

Figure 2. Temporal distribution of discovery of mortality events in *Dermochelys coriacea* (N=35) in British waters (1992–1996).

General Discussion

This section, provides a short synthesis of the conclusions derived from the thesis as a whole, highlighting recommended further research:

As a result of the study of marine turtles initiated in Northern Cyprus (**Chapter 1**) much has been learned of the fundamentals of marine turtle biology in the region. Extension of this longitudinal study will progressively increase the value of the data-set. Part of the power of the data regarding marine turtles recorded in Northern Cyprus has been due to the high degree of observation of individually marked animals. This allows population parameters to be gathered as an average of all individuals rather than of all emergence events or nests. It also allows the calculation of important parameters to be generated such as the internesting interval, the remigration interval and the clutch frequency (i.e. number of clutches laid by a female in one season). These latter two parameters are vital if population size is to be assessed accurately.

Tagging is without doubt a useful technique in the study of marine turtle biology. It is, however, often carried out without ongoing quality assurance as to its effect on subject animals. This study (**Chapter 2**) has demonstrated that although some individuals reacted negatively to the tagging event, there was little quantitative deleterious effect on either the ensuing behaviour or resultant reproductive success. Notwithstanding, an additional query exists regarding the problem of tag loss of traditional tags. To this end it has been successfully demonstrated, at least within one season, that PITs represent a plausible alternative (**Chapter 3**).

Through a study of mortality patterns of Mediterranean turtles (**Chapter 4**) a baseline for future studies in the region has been set. In addition, insights into the differences in relative distribution among species and size classes have also been highlighted. Lesions present are strongly suggestive of the extreme threat of incidental catch in the region. It appears that most fisheries have a level of incidental catch of marine turtles. A survey of fishermen and their attitudes (**Chapter 5**) has highlighted that it is not only industrial fishery methods which are responsible for turtle bycatch. Further detailed studies are needed to investigate mortality patterns and methods of minimisation of incidental catch in marine fisheries.

The work described in the latter part of this thesis was dedicated to deriving ecological information from samples taken from marine turtles stranded both on Mediterranean coasts and around Britain:

By conventional dietary analysis, it was partially confirmed that loggerhead turtles in the Mediterranean feed on molluscs and crustacea (**Chapter 6**). Such conventional studies are difficult to undertake and as such the alternative of using the analysis of stable isotopes in consumer proteins was investigated (**Chapter** 7). This has shown promise allowing clear species differentiation and providing insights which would not have been possible by traditional gut contents analysis.

Among the many factors cited as threats to marine turtles is environmental pollution. Chemical pollution is generally expected to be a problem, especially in the Mediterranean, a nearly closed sea with a large population discharging waste into its waters. Levels of two of the major categories of the potentially harmful environmental contaminants: Organochlorines (**Chapter 8**) and heavy metals (**Chapter 9**), have been determined and have been found to be at generally low concentrations in all marine turtle tissues.

As part of a region-wide study (**Chapter 10**) the molecular profiles of female loggerhead turtles were determined showing the relatively discrete nature of the different nesting populations. This highlights an extremely important conservation message, in that although region-wide management is important, each of the nesting populations should be considered as a discrete management unit. Conservation policy should be based at a local as well as a regional context. Detailed monitoring is important and should be continued.

Finally, a microcosm of many of the aspects of the thesis is carried out on marine turtles stranded in Britain (**Chapter 11**). Although breeding many thousands of miles away, endangered leatherback turtles are being impacted by fisheries in British waters. As well as offering insights into the biology of this most enigmatic of species, it serves to highlight the need for international collaboration in marine turtle conservation across wide geo-political boundaries which may span whole ocean systems.

Marine turtle research in the Mediterranean is beginning to yield a detailed knowledge and understanding of the distribution of marine turtle nesting. Some of the work contained in this thesis has started to yield pieces of information important to understanding at-sea biology of marine turtles in the region. There is still a long way to go. More inwater studies utilising data-logging and transmitting devices are needed. Foraging, wintering and developmental habitats need to be identified, researched and protected. Without a holistic understanding of the range, biology and level of interaction with potential threats, it is unlikely that effective management policies can be formulated.