

The diet of pelagic loggerhead sea turtles (*Caretta caretta*) off the Balearic archipelago (western Mediterranean): relevance of long-line baits

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Gut content and stable isotope analyses were used to investigate the feeding habits of loggerhead sea turtles (*Caretta caretta*) inhabiting the Balearic archipelago. Turtles caught in the pelagic realm ($\delta^{13}\text{C}$: -16.0 ± 0.6 ‰ and $\delta^{15}\text{N}$: 8.5 ± 0.9 ‰) and turtles caught on the continental shelf ($\delta^{13}\text{C}$: -16.7 ± 0.6 ‰ and $\delta^{15}\text{N}$: 8.4 ± 1.3 ‰) did not differ in their isotopic signatures, suggesting that their diets include the same prey types. A mixing model revealed that for the turtles here analysed, squid and the jellyfish *Cotylorhiza tuberculata* were the most relevant prey, although stomach contents analysis revealed that carnivorous jellyplankton, neuston, fish and other cephalopods were also consumed. Gut content analysis also indicated that most of the prey identified, including all fish and most cephalopods, were of pelagic origin. Thus, loggerhead sea turtles inhabiting central regions of the western Mediterranean appear to seldom exploit benthic prey, even while on the continental shelf.

INTRODUCTION

The loggerhead sea turtle (*Caretta caretta* (Linnaeus, 1758)) is the most common of the sea turtles that frequent the Mediterranean. This species has a complex life cycle, early juveniles being oceanic and later juveniles and adults being typically neritic (Bolten, 2003 but see Hatase et al., 2002). The western Mediterranean is a feeding ground for juvenile loggerhead sea turtles from rookeries located in the eastern Mediterranean and the north-western Atlantic (Carreras et al., 2006), but there they experience high rates of long-line by-catch (Carreras et al., 2004). Dietary information will be useful to explain those high rates of by-catch, but the available information about the feeding habits of loggerhead sea turtles in the western Mediterranean is scarce and restricted to specimens caught while on the continental shelf (Tomás et al., 2001; Bentivegna et al., 2003).

Although Houghton et al. (2000) succeeded in studying the diet of adult loggerhead sea turtles by means of direct observation, this technique is impractical for diving oceanic animals such as young sea turtles. Thus, for a long time the standard techniques applied to this type of research have been the analysis of gut contents of stranded individuals and the stomach lavage of those alive. Unfortunately, these techniques provide a skewed vision of diet, as the relative importance of rapidly-digested soft prey is underestimated. Furthermore, stranded turtles and their stomachs are typically found in poor condition, whilst stomach lavage relies on a highly stressful procedure (for the turtles involved) and a time-consuming collection of live specimens.

Stable isotope analysis is an alternative technique for studying feeding habits that solves some of these

shortcomings. However, it has seldom been applied to marine turtles (Godley et al., 1998; Hatase et al., 2002). This method is based on the fact that the isotopic composition of an animal depends upon that of its food sources (DeNiro & Epstein, 1978, 1981), though the heavier isotope is steadily enriched going up the trophic web, from prey to predator. Nitrogen and carbon are the elements most often used as tracers; the ratio $^{15}\text{N}/^{14}\text{N}$ (expressed as $\delta^{15}\text{N}$) increases at about 3.4 ‰ per trophic level due to the preferential excretion of the lighter isotope (Minagawa & Wada, 1984), and the ratio $^{13}\text{C}/^{12}\text{C}$ (expressed as $\delta^{13}\text{C}$) increases at about 1.1 ‰ per trophic level in oceanic ecosystems (France & Peters, 1997). However, fractionation factors are also affected by diet composition, feeding rate, growth rate and nitrogen excretion metabolism (Hesslein et al., 1993; Fantle et al., 1999) and, thus, the results obtained from mixing models can only be considered as indicative of the relative contribution of each food source (Philips & Gregg, 2003).

On the basis of gut content analysis, Tomás et al. (2001) concluded that immature loggerhead sea turtles inhabiting the north-western Mediterranean behave as opportunistic predators consuming both benthic and pelagic prey, including fish discarded from bottom trawlers. This conclusion is consistent with observations made by Houghton et al. (2000) and the stable isotope results reported by Godley et al. (1998), although these two studies were carried out on specimens from the eastern Mediterranean. Bentivegna et al. (2003) also stated that loggerhead sea turtles inhabiting the Tyrrhenian Sea feed mainly on benthic fauna, including seahorses and other fish, but in this case the author did not invoke discarded fish to explain such a diet composition. Furthermore, Bentivegna et al. (2003) did not provide information

about the size or the life stage of the loggerhead sea turtles they studied.

Apparently contradicting these observations, satellite tracking study has revealed that roughly 80% of young loggerhead sea turtles found in the central and southern regions of the western Mediterranean spend most of their time in the open ocean, in areas deeper than 1400 metres (Cardona et al., 2005) and, hence, far away from the sea floor and the fishing grounds exploited by bottom trawlers. One would predict on that basis that they subsist on other food sources. The remaining 20% visit the continental shelf on a regular basis (Cardona et al., 2005) and thus are likely to have access to benthic prey and fish discarded from bottom trawlers.

Loggerhead sea turtles are considered to be endangered world wide and incidental catch by drifting long-lines and the ingestion of plastic debris have been identified as possible major threats to their conservation in the Mediterranean and elsewhere (Aguilar et al., 1995; Tomás et al., 2002). Both threats are related to feeding in the water column and, hence, accurate information on the feeding habits of loggerhead sea turtles during their oceanic life stage are of relevance for the design of management strategies to reduce human-induced mortality.

The aim of this paper is to identify the prey consumed by immature loggerhead sea turtles off the Balearic archipelago, in the central region of the western Mediterranean, and determine potential differences between specimens caught over the continental shelf and those caught over the slope and the bathyal plains (pelagic).

MATERIALS AND METHODS

Sample collection and classification

Samples were collected on the islands of Majorca and Minorca (Balearic archipelago, western Mediterranean) from 2002 to 2004. Stranded turtles were collected and sampled by the staff of the Conselleria de Medi Ambient and the Fundació AsproNatura. For each specimen, the minimum straight and curved carapace lengths (SCL_{min} and CCL_{min} respectively) were recorded, the carcass was examined for trauma and pathologies, the cause of mortality was established as one of interaction with drifting long-lines, interaction with trammel nets, collision with boats or other causes, and samples from skin and gut contents were collected for analyses. Officers from the Fundació AsproNatura also collected live turtles off both islands while basking. For these animals, SCL_{min} and CCL_{min} were recorded and samples of skin collected. Gut contents and skin samples were stored frozen (−20 °C) until analysis.

Stranded and caught turtles were sampled for analyses. Stranded turtles were further subdivided into two, in an attempt to reflect that different components of the population are likely to be selectively captured by different fishing gears. The three groups of turtles sampled were therefore: (i) captures from trammel nets; since this gear is only used on the continental shelf and lays upon the sea-floor, this group is expected to include turtles with a chance to approach the sea-floor and prey upon benthic species; (ii)

captures from drifting long-lines; this gear is deployed on the slope or in the open ocean and hence it is expected to catch turtles that spend most of their time offshore and have no chance to reach the seabed and consume benthic species; (iii) hand-caught turtles; these were collected on the continental shelf and, therefore, like the first group, may have access to the seabed and to benthic prey; for this reason, trammel net and hand-caught turtles were pooled together and considered a unique group confronted to long-line turtles.

Potential prey were sampled, also off Majorca and Minorca, so as to identify their isotopic signatures. The fishing gear employed to collect the various species were as follows: infralittoral fish (*Bothus podas* (Delaroche, 1809), *Cephalacanthus volitans* (Linnaeus, 1758), *Trachinus draco* (Linnaeus, 1758) and *Xyrichtys novacula* (Linnaeus, 1758)) with trammel nets; circalittoral species (*Homola barbata* (Fabricius, 1793), *Mullus surmuletus* (Linnaeus, 1758), *Octopus vulgaris* Cuvier, 1797, *Palinurus elephas* (Fabricius, 1787), *Scorpaena scrofa* (Linnaeus, 1758), *Scyllorhinus canicula* (Linnaeus, 1758), *Sepia officinalis* Linnaeus, 1758, *Trigloporus lastoviza* (Brünnich, 1768) and *Zeus faber* (Linnaeus, 1758)) with bottom trawling nets; pelagic fish and squids (*Loligo vulgaris* Lamarck, 1798, *Sardina pilchardus* Walbaum, 1792 and *Spicara smaris* (Linnaeus, 1758)) with purse seines; gelatinous zooplankton (*Beroe ovata* Bruguière, 1789, *Cotylorhiza tuberculata* (Macri, 1778), *Pelagia noctiluca* (Forsskål, 1775), *Salpa* sp. and *Veleva veleva* (Linnaeus, 1758)) and neustonic species (*Idotea metallica* Bosc, 1802, *Lepas* sp. and fry of *Trachurus* sp.) with a dip net; and pelagic copepods, with a 250 µm plankton net towed during light hours. Moreover, two pelagic top-predators (*Stenella coeruleoalba* (Meyen, 1833), sampled using live biopsy techniques, and *Thunnus thynnus* (Linnaeus, 1758), collected with hook and line, were also analysed in order to obtain a more detailed picture of isotopic signature changes along the pelagic food web.

Analysis of stable isotopes

The skin of 21 loggerhead sea turtles was sampled for stable isotope analyses: eight turtles caught in the pelagic realm by drifting long-lines and 13 turtles caught on the continental shelf (eight from trammel nets and five hand-caught while basking). The other organisms analyses were performed in the following tissues: white dorsal muscle in fish, mantle in cephalopods and dorsal muscle in dolphins; all other animals were homogenized fully and an aliquot of the homogenate subsampled for the analysis. Samples were stored at −20 °C prior to analysis. Once thawed, tissues were dried at 60 °C, ground to a fine powder, and their lipids extracted with a chloroform/methanol (2:1) solution. Pelagic copepods were treated with 0.05 M HCl to remove carbonates. All the samples were weighed into tin cups, combusted at 1000 °C, and analysed in a Flash 1112 IRMS Delta C Series EA Thermo Finnigan continuous flow isotope ratio mass spectrometer. Atropine was used as a system check for elemental analyses. Stable isotope abundances were expressed in δ notation according to the following expression:

$$\delta X = ((R_{\text{sample}}/R_{\text{standard}}) - 1) 1000$$

Table 1. Habitat, sample size (*N*), mean and standard deviation (*SD*) for the isotopic signatures of the species analysed in this study, other than loggerhead sea turtles. Habitat type: pelagic (*P*), circalittoral (*C*) and infralittoral (*I*).

Scientific name	Common name	Habitat	N	$\delta^{13}\text{C}$ mean(SD)	$\delta^{15}\text{N}$ mean(SD)
Jellyplankton					
<i>Beroe ovata</i>	Sea mitre	P	9	-19.0 (0.4)	3.6 (0.4)
<i>Pelagia noctiluca</i>	Pink jellyfish	P	12	-20.1 (0.5)	3.8 (0.6)
<i>Salpa</i> sp.	Salps	P	2 ^a	-21.1 (0.3)	1.7 (0.7)
<i>Veella veilla</i>	By-the-wind-sailor	P	10	-19.3 (0.3)	4.4 (0.3)
<i>Cotylorhiza tuberculata</i>		P	9	-17.7 (0.2)	2.2 (0.2)
Crustaceans					
Copepods (several species)		P	5 ^a	-22.4 (0.9)	2.6 (0.3)
<i>Homola barbata</i>		C	1	-18.5	9.4
<i>Idotea metallica</i>		P	10	-18.7 (1.3)	5.9 (0.8)
<i>Lepas</i> sp.	Goose barnacle	P	4	-18.9 (0.9)	6.2 (0.9)
<i>Palinurus elephas</i>	Spiny lobster	C	8	-17.1 (1.4)	12.3 (2.5)
Cephalopods					
<i>Loligo vulgaris</i>	Long-finned squid	P	9	-17.2 (0.4)	8.5 (0.9)
<i>Octopus vulgaris</i>	Common octopus	C	3	-20.0 (0.1)	7.9 (0.4)
<i>Sepia officinalis</i>	Common cuttlefish	C	2	-18.3 (0.2)	8.2 (0.5)
Fish					
<i>Bothus podas</i>	Wide-eyed flounder	I	10	-17.7 (0.3)	7.7 (0.3)
<i>Cephalacanthus volitans</i>	Flying gurnard	I	9	-16.9 (0.7)	9.6 (0.8)
<i>Mullus surmuletus</i>	Striped red mullet	C	10	-17.9 (0.7)	11.0 (1.2)
<i>Sardina pilchardus</i>	European pilchard	P	6	-19.2 (0.2)	7.9 (0.1)
<i>Scorpaena scrofa</i>	Largescaled scorpionfish	C	10	-19.2 (0.5)	9.6 (0.5)
<i>Scyliorhinus canicula</i>	Small-spotted catshark	C	10	-17.1 (0.4)	9.7 (0.3)
<i>Spicara smaris</i>	Picarel	P	5	-18.8 (0.2)	8.2 (0.3)
<i>Trachinus draco</i>	Greater weever	I	10	-18.1 (0.3)	8.4 (0.3)
<i>Trachurus</i> sp.	Horse mackerel	P	8	-18.2 (0.2)	5.4 (0.2)
<i>Trigloporus lastoviza</i>	Streaked gurnard	C	7	-19.2 (1.2)	8.8 (1.0)
<i>Xyrichthys novacula</i>	Pearly razorfish	I	10	-17.1 (0.5)	8.9 (0.3)
<i>Zeus faber</i>	John Dory	C	2	-19.1 (0.2)	10.0 (0.7)
<i>Thunnus thynnus</i>	Northern bluefin tuna	P	2	-18.5 (0.4)	10.2 (1.1)
Mammals					
<i>Stenella coeruleoalba</i>	Striped dolphin	P	5	-17.8 (0.5)	10.1 (0.9)

^a, Collective samples including several individuals.

where *X* is ^{13}C or ^{15}N and R_{sample} and R_{standard} are the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ of the sample and the standard. The standards for ^{13}C and ^{15}N are the Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (air), respectively. International isotope secondary standards for carbon (IAEA CH_6 ($\delta^{13}\text{C} = -10.4\text{‰}$), USGS 24 ($\delta^{13}\text{C} = -16.1\text{‰}$), IAEA CH_7 ($\delta^{13}\text{C} = -31.8\text{‰}$)) were used to a precision of 0.2‰ and for nitrogen (IAEA NO_3 ($\delta^{15}\text{N} = +4.7\text{‰}$), IAEA N_2 ($\delta^{15}\text{N} = +20.3\text{‰}$), IAEA N_1 ($\delta^{15}\text{N} = +0.4\text{‰}$)) were used to a precision of 0.3‰.

Analysis of gut contents

The digestive tract gut contents of 19 turtles were analysed. Mouth, oesophagus and intestine were checked for prey remains, although identifiable remains were found in the intestine only once. Guts were thawed and the contents rinsed on a 1-mm mesh sieve. Prey remains were preserved in 70% ethanol and were identified to the lowest possible taxonomic level. The length and weight of undigested prey individuals were measured before being

preserved in ethanol. As the available sample size was limited, all individuals were pooled in a single group irrespective of the type of fishing gear responsible of their catch. The relative importance of each prey species to the diet of loggerhead sea turtles, as revealed by gut content analysis, was assessed using the frequency of occurrence, numerical importance, and the method of points. When using the method of points, the score for each prey species was based on the weight of the undigested individuals recovered from the gut. These were as follows: 1 point for every barnacle, 2.4 points for every *I. metallica*, 2.9 points for every salp, 4.1 for every *Trachurus* sp., 612.2 points for every clupeid, 4102.0 points for every *Octopus macropus* Risso, 1826 and 1367.3 points for any other cephalopod.

Data analysis

All statistical analyses were conducted using SPSS 11.5 software, aside from the cluster analysis, which was performed with Statgraphics Plus 3.0. Normality and

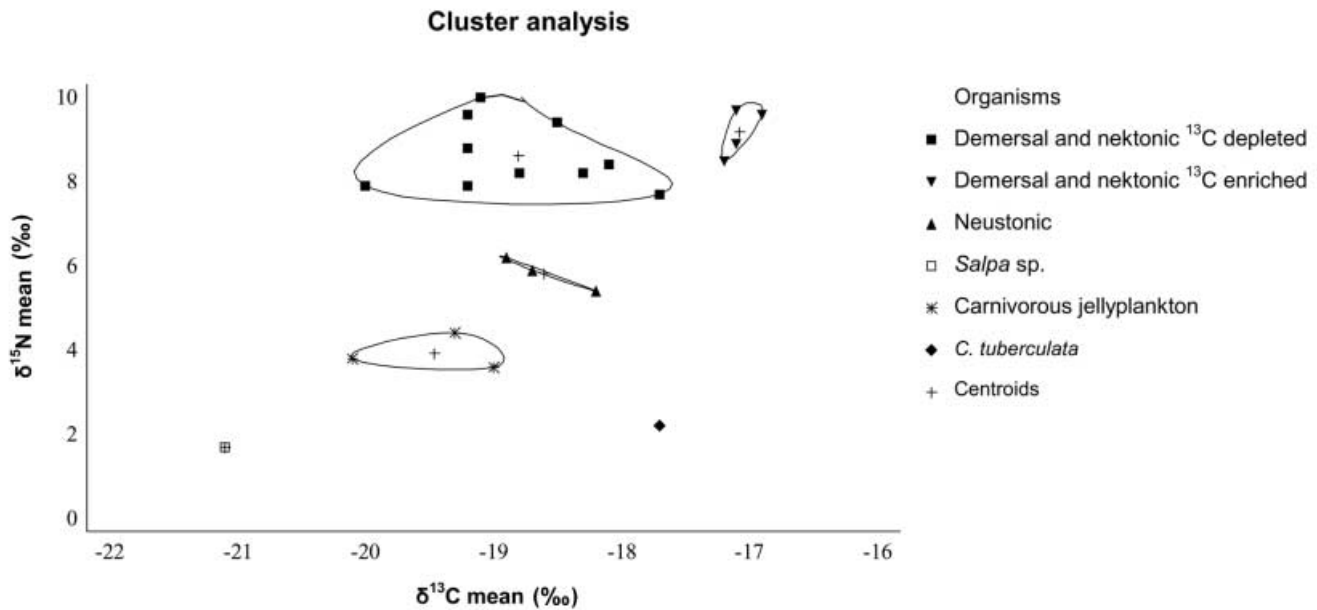


Figure 1. Cluster analysis of the bivariate isotopic signatures of the considered potential prey. The centroids are denoted by +.

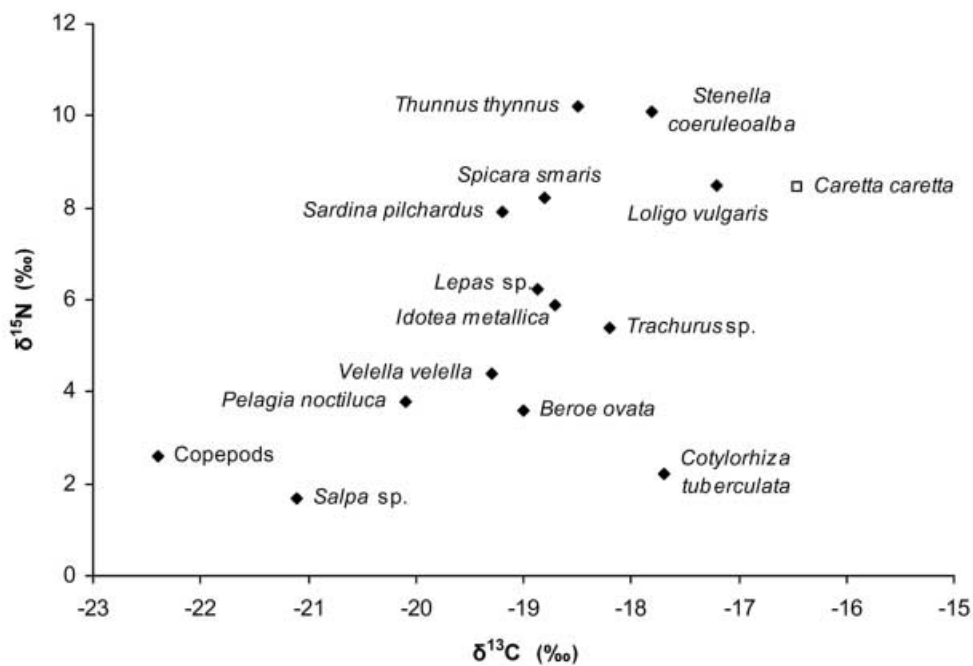


Figure 2. Bivariate isotopic signatures of the considered pelagic species and the analysed loggerhead sea turtles.

homogeneity of variance were tested with Lilliefors' and Levene's tests, respectively, prior to any other analyses. Unless stated, data are shown as mean standard deviation.

The existence of a correlation between SCLmin and $\delta^{15}\text{N}$, and between SCLmin and $\delta^{13}\text{C}$ was investigated for each group of turtles to test whether the trophic level, as revealed by $\delta^{15}\text{N}$, increases with length. In some cases, carapace length was measured using only one of the two systems established in the sampling protocol (CCLmin and SCLmin). As the straight measure is preferred, CCLmin data were transformed to SCLmin using a linear equation calculated from a database including information from about 47 specimens stranded or caught off the

Balearic archipelago (SCLmin=0.924 CCLmin-0.323, $r^2=0.970$, $P<0.001$).

A Student's *t*-test was used to determine whether the two considered groups of turtles (pelagic and continental shelf) differed in their average SCLmin. A Hotelling's *t*-test was also used to test whether the two considered groups of turtles differed in their bivariate isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$).

The IsoSource mixing model of Phillips & Gregg (2003) was used to analyse the contribution of several potential preys to the loggerhead sea turtles considered in this study. This method examined all possible combinations of each source contribution in small increments (1% in this

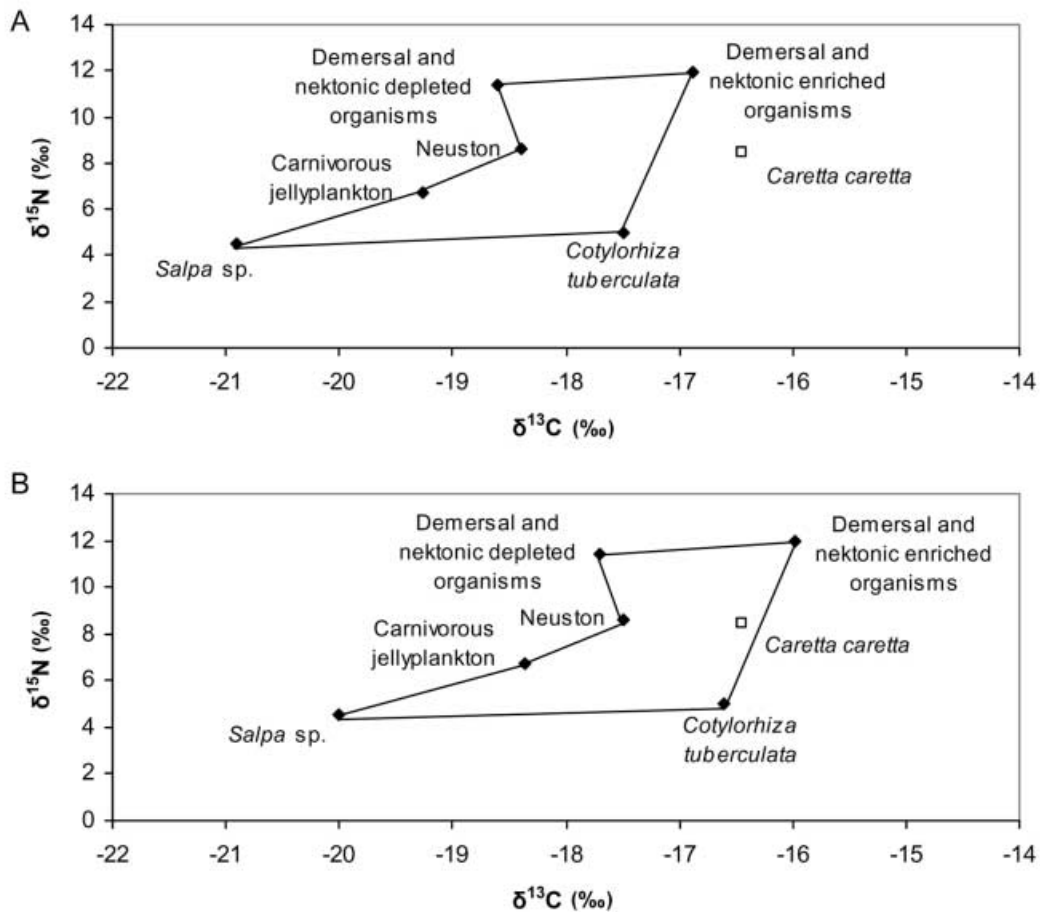


Figure 3. Mixing polygons for the analysed loggerhead sea turtles under two different isotope fractionation scenarios: (A) 0.2‰ for $\delta^{13}\text{C}$ and 2.8‰ for $\delta^{15}\text{N}$; and (B) 1.1‰ for $\delta^{13}\text{C}$ and 2.8‰ for $\delta^{15}\text{N}$.

study). Combinations that sum to the observed mixture isotopic signatures within a small tolerance (± 0.1 ‰ in this study) are considered to be feasible solutions, from which the frequency and range of potential source contributions can be determined (Philips & Gregg, 2003). As the two groups of turtles considered did not differ in their bivariate isotopic signatures, the mean isotopic signature of the 21 analysed turtles was calculated and entered in the IsoSource mixing model. As the IsoSource mixing model (Philips & Gregg, 2003) does not admit a large number of food sources, potential prey with similar isotopic signatures needed to be grouped. Analysis of covariance (ANCOVA), taking $\delta^{15}\text{N}$ as a covariate, was performed to determine whether pelagic, circalittoral and infralittoral species differed in their $\delta^{13}\text{C}$. $\delta^{15}\text{N}$ was used as a covariate because $\delta^{13}\text{C}$ covaries with ecosystem baseline and also species trophic level. Following, a cluster analysis was performed to group potential preys with similar isotopic signatures. Before entering the data in the IsoSource mixing model, the average bivariate isotopic signature of each group of prey was corrected with the fractionation factors reported by Seminoff et al. (2006) for the epidermis of the green turtle (*Chelonia mydas* (Linnaeus, 1758)), i.e. 0.2‰ for the $\delta^{13}\text{C}$ and 2.8‰ for the $\delta^{15}\text{N}$. However, as the mean isotopic signature of the turtles analysed was outside the mixing polygon, no solution was possible and a second analysis using the fractionation factor for the $\delta^{13}\text{C}$ (1.1‰ per trophic level) proposed by

France & Peters (1997) for open-ocean food webs was used instead of that reported by Seminoff et al. (2006) (see Discussion). Other values often used (Roth & Hobson, 2000; Hobson & Bairlein, 2003) were rejected as loggerhead sea turtles are low growing ectotherms and it has been shown that turnover rate is influenced by growth rate (Hesslein et al., 1993).

RESULTS

No correlation was found between SCLmin and the isotopic signature ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) in any of the two considered groups: continental shelf ($\delta^{13}\text{C}$: Pearson $Rho = -0.017$; $P = 0.957$; $N = 13$; and $\delta^{15}\text{N}$: Pearson $Rho = 0.063$; $P = 0.838$; $N = 13$) and pelagic ($\delta^{13}\text{C}$: Pearson $Rho = 0.015$; $P = 0.972$; $N = 8$; and $\delta^{15}\text{N}$: Pearson $Rho = 0.277$; $P = 0.506$; $N = 8$). Differences in the mean SCLmin of the two considered groups (continental shelf: 46.5 ± 10.8 cm; pelagic: 50.6 ± 5.2 cm) were not statistically significant (Student's t -test; $t = -1.002$; $df = 19$; $P = 0.329$). Also, their bivariate isotopic signatures (pelagic: $\delta^{13}\text{C}$: -16.0 ± 0.6 ‰ and $\delta^{15}\text{N}$: 8.5 ± 0.9 ‰; continental shelf: $\delta^{13}\text{C}$: -16.7 ± 0.6 ‰ and $\delta^{15}\text{N}$: 8.4 ± 1.3 ‰) did not differ (Hotelling's t -test; $F = 3.380$; $df = 2$; $P = 0.057$). As a consequence, loggerhead sea turtles found over the continental shelf (hand-caught and trammel net entangled specimens) and pelagic (long-line caught) were grouped and their average bivariate signal

Table 2. Mean, maximum and minimum contributions (%) of each source group to the diet of the analysed turtles taking into account a fractionation of 1.1‰ for carbon and 2.8‰ for nitrogen.

Organisms	Mean (SD)	Minimum	Maximum
<i>Salpa</i> sp.	0.9 (1.1)	0.0	5.0
Carnivorous jellyplankton	1.9 (1.9)	0.0	10.0
<i>Cotylorhiza tuberculata</i>	47.1 (1.9)	43.0	52.0
Neuston	3.3 (3.1)	0.0	16.0
Demersal and nektonic ¹³ C enriched	44.4 (2.3)	38.0	50.0
Demersal and nektonic ¹³ C depleted	2.3 (2.3)	0.0	12.0

Table 3. Results from the gut content analysis of the examined loggerhead sea turtles (*N*=19). For the various prey types, we detail frequency of occurrence, numerical importance and total point score. For non-prey items, we only calculate frequency of occurrence.

Prey category	Frequency of occurrence	Numerical importance	Points
Tunicates			
Salps	37.5	52.5	273.4
Crustaceans			
Isopods			
<i>Idotea metallica</i>	12.5	29.8	128.3
Barnacles	18.8	5.0	9.0
Molluscs			
Cephalopods			
<i>Octopus macropus</i>	6.3	0.6	4,102.0
<i>Ancistrotheutis lichtensteini</i>	12.5	1.7	4,102.0
Unidentified	25.0	3.3	8,204.1
Fish			
Clupeids	43.8	6.6	7,346.9
Carangids			
<i>Trachurus</i> sp.	6.3	0.6	4.1
Other ingested items			
<i>Cymodocea nodosa</i>	6.3	—	—
Seaweed	18.8	—	—
<i>Posidonia oceanica</i>	6.3	—	—
Terrestrial plants	6.3	—	—
Plastics	37.5	—	—
Lines and hooks	25.0	—	—
Wood ^a	31.3	—	—
Tar	6.3	—	—
Bird feathers	6.3	—	—

^a, Includes charcoal and cork.

($\delta^{13}\text{C} = -16.4 \pm 0.7\text{‰}$ and $\delta^{15}\text{N} = 8.5 \pm 1.1\text{‰}$) was used for later analysis.

The isotopic signatures of the other 27 species analysed (potential prey and baseline species) are shown in Table 1. The ANCOVA ($F=6.835$; $df=3$; $P=0.002$) revealed that $\delta^{15}\text{N}$ ($F=11.959$; $df=1$; $P=0.002$), but not habitat type ($F=2.475$; $df=2$; $P=0.106$), contributed to the observed variability in $\delta^{13}\text{C}$. The resulting model explained 40.2% of the observed variance. As a consequence, the grouping of species yielded by the cluster analysis (Figure 1) was determined by trophic level (as revealed by the $\delta^{15}\text{N}$) but not by habitat type (as revealed by the $\delta^{13}\text{C}$). The groups found by the cluster analysis were: herbivorous jellyplankton (*Salpa* sp.), carnivorous jellyplankton (*Beroe ovata*, *Pelagia noctiluca* and *Velella velella*), *Cotylorhiza tuberculata*, neuston associated with flotsam (fry of *Trachurus* sp., *Idotea metallica* and *Lepas* sp.), and two other heterogeneous groups, two including a mixture of demersal and nektonic

organisms: one with higher values of $\delta^{13}\text{C}$ (*Cephalacanthus volitans*, *Loligo vulgaris*, *Scyliorhinus canicula* and *Xyrichtys novacula*) and another with values more negative of $\delta^{13}\text{C}$ (*Bothus podas*, *Homola barbata*, *Octopus vulgaris*, *Sepia officinalis*, *Sardina pilchardus*, *Scorpaena scrofa*, *Spicara smaris*, *Trachinus draco*, *Trigloporus lastoviza* and *Zeus faber*). *Mullus surmuletus* and *Palinurus elephas* were excluded from the cluster analysis because their isotopic signatures are so different from that of the turtles and hence they cannot be relevant preys for them. Furthermore, the performance of the IsoSource mixing model improves when only a few prey groups are considered.

As a consequence, in the IsoSource mixing model we entered only six groups of potential prey: herbivorous jellyplankton, carnivorous jellyplankton, *C. tuberculata*, neuston associated to flotsam, demersal and nektonic organisms enriched in ¹³C and demersal and nektonic organisms depleted in ¹³C. *Cotylorhiza tuberculata* differed in

its isotopic signature with respect to herbivorous and carnivorous jellyplankton probably due to the presence of zooxanthellae. Copepods, *Stenella coeruleoalba* and *Thunnus thynnus* were not included in these analyses as they are not potential prey for loggerhead sea turtles. They were included in the study simply to provide a more comprehensive understanding of how the isotopic signature changes along the considered food web.

The isotopic signature of the analysed turtles was close to that of the squid *Loligo vulgaris* and higher than that of some jellyplankton and neuston associated with flotsam (Figure 2), thus suggesting at a first glance a diet based on these organisms. Otherwise, demersal and nektonic organisms were also considered for stable isotope analysis (Figure 3), as their remnants were often found in the stomach content of turtles. In fact, IsoSource revealed that *C. tuberculata* and the group of nektonic and demersal ^{13}C enriched organisms were the major food sources for the analysed turtles. The remaining four groups of prey (*Salpa* sp., demersal and nektonic ^{13}C depleted organisms, carnivorous jellyplankton and neuston associated with flotsam) had a minor contribution, or no contribution at all (Table 2).

The information supplied by the 181 animal specimens found in the 19 analysed guts (vacuity coefficient=15.8%) did not agree with that from stable isotope analyses (Table 3). On a numerical basis, herbivorous jellyplankton (salps=52.5%) and the neustonic isopod *I. metallica* (29.8%) were the dominant prey. However, the frequency of occurrence revealed that clupeid fish (43.8%), cephalopods (37.5%) and herbivorous jellyplankton (salps=37.5%) were the most widespread prey. Finally, when the method of points was used to integrate numerical abundance and relative volume (weight), cephalopods arose as the major prey type (67.9%), followed by clupeids (30.4%). Only one benthic species (*Octopus macropus*) was identified.

Debris was found in 12 of the analysed guts. Plastic and wood were the most frequent items, followed by lines and hooks and plant matter, both terrestrial and marine (Table 3). Plastic fragments were usually small ($398.8 \pm 891.6 \text{ mm}^2$).

DISCUSSION

Isotopic fractionation has been assessed experimentally for fish (Hesslein et al., 1993), birds (Hobson & Bairlein, 2003), mammals (Roth & Hobson, 2000) and, to our knowledge, for only one species of marine turtle, i.e. the green turtle (Seminoff et al., 2006). Some experimental work has been conducted with loggerhead sea turtles, but data are not yet available (K. Reich, personal communication).

Unfortunately, when the isotopic fractionation values reported by Seminoff et al. (2006) were applied to the loggerhead sea turtles here considered, their mean isotopic signature was outside the mixing polygon and no solution was possible. This is because (1) highly ^{13}C enriched prey have not been included in the analysis or (2) green and loggerhead sea turtles differ in skin isotope fractionation. The first possibility seems unlikely, as potential prey more enriched in ^{13}C than those here considered are unknown for the western Mediterranean (Lepoint et al., 2000). Furthermore, green and loggerhead sea turtles differ in

feeding habits and this may affect actual fractionation values (Seminoff et al., 2006). For these reasons, we run IsoSource combining the isotopic fractionation factor reported by Seminoff et al. (2006) for nitrogen with that reported by France & Peters (1997) for carbon in oceanic ecosystems, as satellite tracking has revealed that the loggerhead sea turtles found off the Balearic archipelago spend most of their time off-shore (Cardona et al., 2005).

In our study, both gut contents and stable isotope analyses substantiated consumption of fish and cephalopods, whilst that of *Cotylorhiza tuberculata* was only supported by results from the stable isotopes. The fish species found in stomach contents belonged to the ^{13}C depleted group and hence they are thought to have a minor contribution to the assimilated organic matter. On the other hand, squid were the only members of the ^{13}C enriched group of carnivores found in the stomach contents and hence they are thought to be the most likely contributors to the organic matter assimilated by the analysed loggerhead sea turtles, together with *C. tuberculata*. Conversely, stomach contents analysis suggested that the herbivorous salps were relevant prey and the same is true for the carnivorous jellyfish *Veleva veleva* on the basis of field observations (L. Cardona, personal observation), and in other areas stomach content analysis (Parker et al., 2005). These contrasting results are explained because stomach content analyses are strongly biased towards the detection of prey with hard or indigestible parts (fish, cephalopods, most neuston species and tunicates), whereas soft prey (jellyplankton) are easily digested and their abundance is hence dramatically underestimated. Moreover, water contents in jellyplankton (salps and jellyfish) is extremely high and hence is hardly surprising that most of them had a negligible contribution to the assimilated organic matter.

Overall evidence suggests that the bulk of the diet of the turtles occurring off the Balearic archipelago is represented by squid and *C. tuberculata* and that differences do not exist between those turtles caught on the continental shelf and pelagic realm. This is not surprising, as satellite tracking has revealed that the turtles inhabiting the area spend most of their time in oceanic habitat, seldom approaching the continental shelf (Cardona et al., 2005). Indeed, only one benthic prey species (*Octopus macropus*) has been identified in the stomach contents.

A relevant role for fish and squid in the diet of pelagic turtles is a result in sharp contrast with previous reports from other regions where jellyplankton and neuston appear to be the major prey of pelagic individuals (Bjørndal, 1997 and references therein; Parker et al., 2005). When fish consumption has been reported, the involved species were slow-swimming (Brongersma, 1972) or small mesopelagic ones (Parker et al., 2005). Likewise, the consumption of cephalopods by oceanic turtles has been reported only by Parker et al. (2005). Conversely, in neritic turtles a number of authors have found slow-swimming fish, fast-swimming fish and cephalopods (Houghton et al., 2000; Limpus et al., 2001; Tomás et al., 2001; Bentivegna et al., 2003), although the latter two were assumed to originate from the catch discarded by fishing vessels (Limpus et al., 2001; Tomás et al., 2001). As the loggerhead sea turtles found off the Balearic archipelago spend most of their time in the off-shore (Cardona et al.,

2005), access to discarded trash-fish must be extremely limited and hence an alternative hypothesis to explain the consumption of fish and squid here reported is required.

Fry of some fish species also aggregate under flotsam (Riera et al., 1999) and thus may be ingested by turtles preying on flotsam-associated neuston such as barnacles and isopods. This is the likely origin of the fry of *Trachurus* sp. found in the gut contents, although incidental ingestion during the consumption of large jellyfish is also a possible origin. However, most of the identified fish remains belong to the clupeid family, whose members do not associate with flotsam off the Balearic archipelago (Riera et al., 1999). Indeed, clupeids inhabit the water column over the continental shelf and the upper slope and hence loggerhead sea turtles may only prey on them when moving to inshore waters. An alternative explanation is that clupeids are often used as bait for drifting long-lines operating in the western Mediterranean (Camiñas et al., 2003), and may thus be scavenged by turtles. Limpus et al. (2001) have indeed reported that Australian loggerhead sea turtles learn quickly to locate floating baits and use them systematically, with a single individual having been caught up to 18 times in such fishing gear. This indicates that scavenging is a likely explanation for the clupeid remnants, although loggerhead sea turtles exploiting that food source are exposed to a high post-release mortality if deep-hooked (Aguilar et al., 1995). Turtles can take the bait and not the hook, but there is no published information about how often they do it. Fish discarded from purse-seiners is an unlikely source of clupeids, as only a few purse-seiners operate off the Balearic archipelago and their fishing grounds are on the continental shelf (Carreras et al., 2004).

Some of the cephalopod remains found in the examined guts may originate from the same source, as squid are also used to bait drifting long-lines (Camiñas et al., 2003). However, *Ancistrotheutis lichtensteini*, one of the prey organisms that gut content analysis would suggest to be important to the turtles' diets (Table 3), is a pelagic species that is rarely caught by fishermen (P. Sánchez, personal communication) and is apparently not used as bait (Camiñas et al., 2003). Bozzano & Sarda (2002) have reported cephalopods scavenging on discarded fish and, therefore, it can be speculated that the turtles may indeed prey on *A. lichtensteini* when they are attracted by long-line bait. Unfortunately, no direct evidence is available to substantiate this hypothesis.

The low amounts of plastic debris and tar found in the gut of the loggerhead sea turtles analysed agree with the results reported by Tomás et al. (2002) for another region of the western Mediterranean, thus revealing that debris ingestion is unlikely to be a major threat for loggerhead sea turtles there. Conversely, high numbers of the isopod *Idotea metallica* were found in some stomachs. This species often lives in the underside of flotsam (Abelló & Frankland, 1997) and hence *I. metallica* might be eaten when loggerhead sea turtles swallow floating debris. An alternative possibility is that *I. metallica* was eaten incidentally when foraging upon jellyplankton if an association like that reported between hyperiid amphipods and jellyplankton (Marilene & Jean, 2001) existed. Unfortunately, such an association has not been reported to our knowledge.

According to Bjørndal et al. (2000), the loggerhead sea turtles found off the Atlantic coast of North America

gradually leave the oceanic realm to become neritic when they reach a CCL of 46–64 cm (42–58 cm SCLmin). This habitat change entails an increase in the consumption of benthic prey as well as an increase in trophic level. Once the turtles have adapted to the neritic environment, they mostly remain on the continental shelf (Bolten, 2003). The situation appears to be different in waters around Japan, where stable isotopes have revealed the existence of both neritic and oceanic adult females (Hatase et al., 2002). Previous information from the eastern Mediterranean and the northern shore of the western Mediterranean suggests a scenario close to that reported for the Atlantic coast of North America, as the trophic level of these loggerhead sea turtles was found to increase with age (Godley et al., 1998) and benthic animals were relevant prey for turtles larger than 31.1 cm SCLmin (Tomás et al., 2001). However, in the loggerhead sea turtles off the Balearic archipelago, pelagic prey represents the bulk of the diet and their trophic level does not seem to increase with age (length) even though the range of lengths of the specimens examined here overlaps with those from the above-reported studies. Satellite tracking has shown that the turtles found off the Balearic archipelago spend most of their time in areas deeper than 1400 m, although a small fraction of the population uses the continental shelf for a short time (Cardona et al., 2005). In this situation, a diet based on pelagic organisms is hardly surprising, although a few individuals may still consume benthic prey while on the continental shelf.

In conclusion, the overall evidence indicates that jellyplankton and squid are the staple food of the immature loggerhead sea turtles off the Balearic archipelago and that long-line bait is the most likely source of some prey species. The attraction of loggerhead sea turtles by long-line bait is hardly surprising considering the low productivity of the waters off the Balearic archipelago.

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