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Assessing resource use patterns of Mediterranean loggerhead sea turtles *Caretta caretta* (Linnaeus, 1758) through stable isotope analysis

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Abstract

Stable isotope analysis is a useful tool for studying the ecology of marine consumers, as carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios may reflect individuals' patterns of diet and habitat use. Knowledge of foraging strategies has significant implications for the conservation of endangered loggerhead turtles *Caretta caretta* (Linnaeus, 1758). In this study, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope data were used to assess resource use patterns of the Mediterranean loggerhead turtles (Aeolian Archipelago, Southern Italy). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from carapace scutes of 54 loggerheads of different curved carapace length (CCL) and health status were compared with those of eight potential prey items (benthic, pelagic and fishery discards). MixSIAR results suggested that pelagic prey (from goose barnacles to planktivorous fish) comprised most of loggerheads' diet, with small variations (i.e. benthic prey or fishery discards) depending on size ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and health ($\delta^{15}\text{N}$) of individuals. $\delta^{13}\text{C}$ variations with turtles' size might reflect changes in dietary habitats during life stages. However, the loggerhead turtles and their main source of prey (pelagic prey) had a higher variation in values of $\delta^{15}\text{N}$ compared to $\delta^{13}\text{C}$. This suggested that smaller-sized turtles might preferentially feed on pelagic prey in oceanic habitats and then, as they reach a larger size, gradually enter neritic waters, including in their diet prey sources with higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Some turtles foraging on longline baits/debris also displayed a marked increase in $\delta^{15}\text{N}$. These $\delta^{15}\text{N}$ variations might be explained by differences in diet (trophic differences) and somatic growth rates among individuals, or dietary dilution.

Keywords: *Stable isotope, loggerhead turtle, Mediterranean, trophic ecology*

Introduction

The loggerhead sea turtle (*Caretta caretta*) is the most abundant marine turtle species in the Mediterranean Sea, where it occurs in several areas (Luschi & Casale 2014). Despite this, little is known about its trophic ecology, particularly that of immatures and adults in their feeding and overwintering habitats.

After attainment of a hatchling pelagic phase (Boyle & Limpus 2008; Wallace et al. 2009), immature loggerheads may gradually enter feeding areas on the continental shelf (Laurent et al. 1998) and stay in these habitats over several decades before sexual maturation (Musick & Limpus 1997; Bolten 2003; Casale et al. 2008). Sexually mature loggerheads move to specific

mating and nesting sites during the breeding season (Bolten et al. 1992; Bowen et al. 1995, 2004; Boyle et al. 2009) and return thereafter to the foraging and wintering areas where they spend much of their life (Casale et al. 2012). Upon reaching the adult stage, loggerheads are typically carnivorous, their diet largely comprising neritic molluscs and crustaceans (Dodd 1988; Bjørndal 1997). However, there are exceptions to this general foraging behaviour (Hatase et al. 2002, 2006; Hawkes et al. 2006; McClellan & Read 2007; Revelles et al. 2007b; Casale et al. 2008; Zbinden et al. 2011). For example, the prolonged residence of loggerhead females in oceanic areas (Hatase et al. 2002; Hawkes et al. 2006) suggests pelagic feeding in some

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adult turtles (Reich et al. 2010). Loggerheads also feed opportunistically (Dodd 1988; Burke et al. 1993; Revelles et al. 2007b; Seney & Musick 2007) depending on local prey distribution and abundances (Hawkes et al. 2006; Zbinden et al. 2011), and show significant spatial variation in diet (Hatase et al. 2002; Hawkes et al. 2006; McClellan & Read 2007; Revelles et al. 2007b; Casale et al. 2008; Zbinden et al. 2011).

Habitat degradation and overfishing may in addition influence prey availability and consequently lead to spatial/temporal variations in loggerhead diet (Seney & Musick 2007). In the Mediterranean Sea, fishing activities are responsible for high levels of bycatch and mortality of loggerhead turtles (Casale et al. 2010; Casale 2011). Mediterranean loggerhead turtles, in fact, feed on longline baits and fishery discards or the catch found in trammel nets (Dodd 1988; Tomás et al. 2001; Tomás et al. 2002; Revelles et al. 2007b; Casale et al. 2010; Casale 2011) and fishing aggregating devices (FADs; Blasi et al. 2016; Blasi & Mattei 2017). Furthermore, ingestion of marine debris is a source of concern in the Mediterranean area (Bjorndal 1997; McCauley & Bjorndal 1999; Tomás et al. 2002), leading to lethal and sub-lethal effects in loggerhead turtles (Bjorndal 1997; McCauley & Bjorndal 1999; Nelms et al. 2015).

The Aeolian Archipelago (Southern Tyrrhenian Sea, Sicily, Italy; Figure 1) is a geomorphologically varied area of volcanic origin with adjacent extensive neritic and oceanic habitats (Favalli et al. 2005). Loggerhead turtle nesting sites have never been found in this area; however, immature and adult loggerhead turtles forage and overwinter there (Blasi et al. 2016). In the Aeolian Archipelago the loggerhead turtles may

exploit food resources in fishing zones, apparently attracted by longline bait and fishing discard materials (Blasi & Mattei 2017). Furthermore, in this area, loggerhead turtles may forage on prey settled on anthropogenic debris or found in the proximity of FADs (Blasi et al. 2016), which provide a reliable food source for foraging individuals and offer a concentration of pelagic prey items in open waters (D'Anna et al. 1999; Andaloro et al. 2007).

Stable isotope investigation offers a powerful and practical alternative to stomach content analyses to explore the feeding behaviour of different species (Kelly 2000). In marine environments, a consumer's stable isotopic ratio of $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$) is directly influenced by longitude and latitude, reflecting foraging habitat (from coastal to open waters, from benthonic to pelagic areas and from northern to southern regions; Fry & Sherr 1984; France 1995a,b), while the ratio $^{15}\text{N}/^{14}\text{N}$ ($\delta^{15}\text{N}$) indicates the trophic position (Peterson & Fry 1987; McClellan et al. 2010; Pajuelo et al. 2012a). Additionally, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can provide information on foraging location, as a result of the effects of biogeochemical processes that determine the baseline $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Pajuelo et al. 2010, 2012a,b; Ceriani et al. 2014). Consequently, an assumed constant trophic fractionation of $^{15}\text{N}/^{14}\text{N}$ between consumer and preys allows us to make inferences about feeding interactions and trophic level in food web studies (Fry & Sherr 1984; France 1995a,b; Post 2002).

The analysis of stable carbon and nitrogen isotopes can be performed using tissue samples collected non-lethally from live animals (Michener & Schell 1994; Post 2002). Carapace scutes seem to be a good matrix

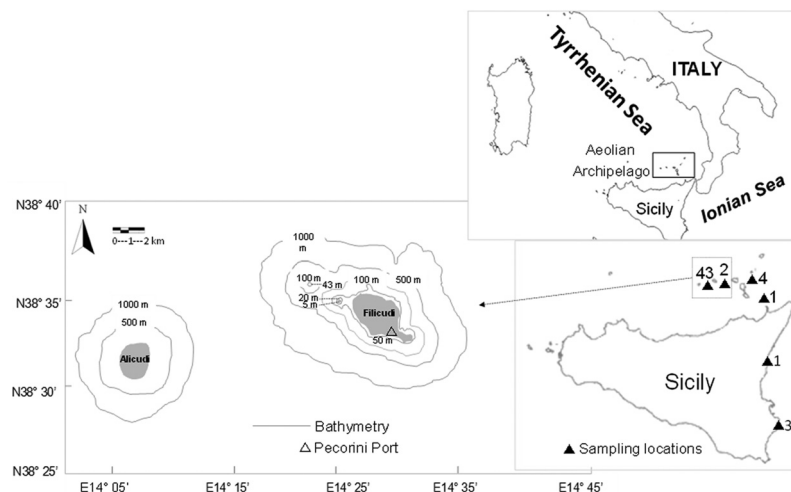


Figure 1. Capture locations of 54 loggerhead turtles *Caretta caretta* from which samples were collected in this study, between 2009 and 2013, in the Aeolian Archipelago located in Southern Tyrrhenian Sea (Sicily, Italy) and along the coast of Sicily (Northern Ionian Sea, Italy). The sampling locations and the number of samples (n) for each location are also indicated.

for examining feeding behaviour in sea turtles (Revelles et al. 2007a; Cardona et al. 2010, 2012; Vander Zanden et al. 2010; Zbinden et al. 2011) as carapaces do not decline with time, in contrast to other tissues and organs (Zanden & Rasmussen 1999), and consequently this trait allows the comparison of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic values between live and dead individuals (Revelles et al. 2007a). In addition, carapace scutes reflect the turtle's dietary history over a long period (several years) (Vander Zanden et al. 2010). Its patterns, however, are less enriched in $\delta^{15}\text{N}$ than those of other tissues (muscle, skin and blood), based on investigations conducted on loggerhead turtles (Revelles et al. 2007a; Reich et al. 2008).

Few studies have used stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic values from carapace scutes of loggerhead turtles in the Mediterranean Sea (Revelles et al. 2007a,b) (Table I), where breeding populations with Atlantic individuals coexist (Luschi & Casale 2014). Knowledge of the foraging patterns (pelagic versus benthic) of loggerhead turtles from juveniles to mature adults is extremely limited because of the challenge of sampling such populations in open waters. Some studies used samples from nesting sites (Godley et al. 1998) or nesting females (Zbinden et al. 2011); others were based on dead individuals (Godley et al. 1998), or on organisms caught in different habitats (Revelles et al. 2007b), or used different tissues (Revelles

Table I. Results of the Kruskal-Wallis test (Hc and p) on $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) values between different groups of loggerhead turtles according to year/season of sampling, preservation method, life stage, health condition and cause of injury. The level of significance of the statistical test was < 0.05 . The highest contributions to the statistical output are reported in bold. Only 49 turtles from the Aeolian Archipelago were included in the statistical tests between groups of turtles with different size, health condition level and cause of injury (one single scute found at sea is not included in the analysis). The number of samples (N) and range of values are also reported for each group of turtles. Abbreviations: LIVE = live; DEAD = dead; GH = good health; INJ = injured; BC = boat collision; ILH = ingested longline hook; OC = debris ingestion; Small = curved carapace length (CCL) < 40 cm; Medium = CCL = 40–60 cm; Large = CCL > 60 cm.

Samples	N	CCL (cm)			$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
		Range	Hc	p	Range	Hc	p	Range	Hc	p
ALL	54	25.5 to 76.0	-	-	-18.87 to -16.78	-	-	4.47 to 10.02	-	-
AEOLIAN	49	31.0 to 76.0	-	-	-18.86 to -17.30	-	-	4.47 to 9.24	-	-
2010	2	25.5 to 70.0	1.3	> 0.05	-18.87 to -16.78	1.6	> 0.05	5.28 to 8.65	1.1	> 0.5
2011	8									
2012	2									
2013	42	31.0 to 76.0			-18.80 to -16.93			4.47 to 10.02		
AUTUMN	25	31.0 to 75.0	2.3	> 0.05	-18.86 to -17.30	1.6	> 0.05	4.47 to 8.64	3.4	> 0.05
SUMMER	23	35.0 to 76.0			-18.62 to -16.92			5.07 to 10.02		
SPRING	4	36.1 to 51.1			-18.68 to -18.19			5.26 to 6.28		
FROZEN	10	35.5 to 61.5	0.5	> 0.05	-18.87 to -17.78	1.7	> 0.05	5.28 to 8.65	1.9	> 0.05
SUN DRIED	44	25.5 to 76.0			-18.80 to -16.78			4.47 to 10.02		
SMALL	10	31.0 to 38.0	-	-	-18.87 to -17.74	6.8	0.003	4.47 to 8.64	8.9	0.01
MEDIUM	25	40.4 to 61.0	-	-	-18.63 to -17.70			4.71 to 10.02		
LARGE	14	61.0 to 76.0	-	-	-18.80 to -17.30			6.36 to 8.61		
SMALL + MEDIUM	35	31.0 to 59.0	-	-	-18.86 to -17.64	4.1	0.04	4.47 to 9.24	7.9	0.005
LARGE	14	61.0 to 76.0	-	-	18.79 to -17.30			5.60 to 8.61		
LIVE SMALL	6	31.0 to 38.0	-	-	-18.56 to -17.54	9.8	0.007	4.47 to 6.75	9.1	0.01
LIVE MEDIUM	20	40.5 to 59.0	-	-	-18.63 to -17.71			4.70 to 7.26		
LIVE LARGE	11	61.0 to 76.0	-	-	-18.28 to -17.30			5.61 to 7.17		
LIVE SMALL + LIVE MEDIUM	26	31.0 to 59.0	-	-	-18.62 to -17.64	6.4	0.01	4.47 to 7.26	8.2	0.004
LIVE LARGE	11	61.0 to 76.0	-	-	-18.27 to -17.30			5.61 to 7.17		
GH SMALL	9	33.0 to 52.0	-	-	-18.36 to -17.92	8.0	0.004	4.47 to 6.34	5.6	0.01
GH MEDIUM + LARGE	6	53.0 to 70.0	-	-	-18.07 to -17.75			5.73 to 6.35		
LIVE	37	31 to 76.0	0.2	> 0.05	-18.62 to -17.30	10.8	0.001	4.47 to 7.26	4.2	0.04
DEAD	12	35.5 to 71.0			-18.87 to -18.05			5.28 to 9.24		
GH	15	33.0 to 70.0	0.7	> 0.05	-18.36 to -17.76	1.9	> 0.05	4.47 to 6.36	6.7	0.009
INJ + DEAD	34	31.0 to 76.0			-18.86 to -17.30			5.27 to 9.24		
GH	15	33.0 to 70.0	1.2	> 0.05	-18.36 to -17.76	0.9	> 0.05	4.47 to 6.36	3.8	0.05
INJ	22	31.0 to 76.0			-18.62 to -17.30			5.07 to 7.26		
GH	15	33.0 to 70.0	3.9	> 0.05	-18.36 to -17.76	13.4	0.004	4.47 to 6.36	9.7	0.02
BC	6	31.0 to 58.0			-18.86 to -17.30			5.04 to 8.64		
ILH	15	35.0 to 76.0			-18.68 to -17.51			5.27 to 9.25		
OC	10	31.0 to 75.0			-18.57 to -17.30			5.64 to 7.26		

et al. 2007a). Moreover, there are discrepancies in the conclusions reached (Supplementary material Table SI).

In this study, stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic data were used to investigate resource use patterns (i.e. integration of diet and foraging habitats) of Mediterranean loggerhead turtles (Aeolian Archipelago, Sicily, Italy). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values from loggerhead carapace scutes were compared with those of potential prey species (benthic, pelagic or fishery discards). Spatial differences in loggerhead $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope values were also analysed among individuals of different size (curved carapace length, CCL) and health status (good health, injured or dead).

Materials and methods

Loggerhead turtle samples

The stable isotope analyses were conducted on 54 loggerhead samples, which consisted of keratin material extracted from the exteriormost layer of the marginal posterior costal scute of the carapace. This body tissue was chosen to avoid possible injuries to the living turtles. Moreover, the exteriormost layer is expected to be the oldest part of the carapace, and therefore to be a good indicator of long-term diet (Reich et al. 2007). Revelles et al. (2007a) demonstrated that the stable isotope values of dorsal and marginal scutes from the same specimen did not differ, and consequently only one scute sample from each turtle was used for further analyses. The samples were obtained by making a small horizontal incision on the carapace with a scalpel blade, and the resulting flake was subsequently peeled from the carapace using forceps.

Fifty carapace samples were collected from loggerhead turtles captured by hand during dedicated boat surveys in multiple years from 2009 to 2013 between May and November around the Aeolian Archipelago waters (neritic and oceanic) (Figure 1). Moreover, four additional samples derived from loggerhead turtles rescued along the coast of Sicily (Northern Ionian Sea; three samples from Siracusa and one from Catania; Figure 1).

Thirty-nine samples were collected from live turtles (i.e. three in 2011 and 36 in 2013) and 15 from dead turtles (stranded, or floating on the sea surface; i.e. two in 2010, five in 2011, two in 2012 and six in 2013). Fifteen samples from live turtles derived from individuals in good health while 24 samples derived from individuals with detectable diseases or problems (injured). All the samples were collected before recovery at the Regional Rescue Centre of

Comiso (Sicily, Italy). The loggerhead samples were preserved by drying in the sun ($n = 44$) or freezing at -20°C ($n = 10$).

Life stages and health condition

The notch-to-tip CCL (i.e. from the nuchal scute notch to the tip of the supracaudal scute; Bolten 1999) was measured for each loggerhead individual. In addition, visibly injured or dead loggerhead turtles were subjected to veterinary examination to establish the likely cause of injury/death based on a complete external (for all turtles) and internal examination (for dead turtles). A cause of injury/death was assigned only if clear injuries were evident on the turtle body (e.g. bycatch in longlines or evidence of collision with boats). Bycatch in longline fishery was assigned when the hook and/or the line were found in the tongue, oesophagus, stomach, or intestinal tract upon examination of the individual. A metal detector was used to determine whether the hook was in the oesophagus, stomach, or intestinal tract of living turtles if the fishing line did not come out of the turtle beak or cloaca. Gastrointestinal occlusion due to ingestion of anthropogenic debris was assigned from the massive presence of plastic and debris in the faeces of rescued turtles during turtle recovery at the Regional Rescue Centre of Comiso. The oesophagus, stomach, and intestinal tract of dead individuals were analysed to search for evidence of large amounts of debris and plastic. Finally, collision with boats was assigned if clear injuries were identified in loggerheads' carapace, neck, plastron, flippers, head, or limbs. Multiple injuries were also assigned; in particular the most severe and recent was assigned as the primary cause while less severe injury was assigned as a secondary cause.

To investigate differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope values with life stages, the loggerhead turtles were classified based on CCL values of (a) 20–40 cm (small size, $n = 12$), (b) 40–60 cm (medium size, $n = 27$), and (c) > 60 cm (large size, $n = 15$). We could not determine sexual maturity in loggerhead individuals, but 70 cm is generally considered the minimum CCL for Mediterranean loggerheads to be classified as adult (Laurent et al. 1993; Casale et al. 2009; Piovano et al. 2011). Therefore, we assumed that our samples were composed mainly by early ($n = 12$; CCL < 40 cm) and late ($n = 37$; CCL = 40–60 cm) juveniles and few adults ($n = 5$; CCL > 70 cm), which are the developmental stages of loggerhead turtles typically found in the western Mediterranean (Laurent et al. 1993). For the isotopic comparison, the loggerhead samples were also classified according to turtles being: (a) in good health, (b) injured (live turtles with problems) or (c) dead. Finally, injured/dead turtles were grouped by cause of

rescue/death: (a) ingested longline hook, (b) gastrointestinal occlusion due to debris ingestion, and (c) collision with boats.

Food web marine species

Twenty-five samples from eight potential prey items for loggerhead turtles were analysed in this study. Prey samples were collected by hand during breath-holding dives and boat surveys (Aeolian Archipelago; Figure 1). Fishery discharge and longline baits were also provided by local fishermen in Filicudi island during 2013 and selected for the food items (supplementary material Table SII).

Benthic prey samples included muscle tissue from the abdominal region for the grapsid crab *Percnon gibbesi* ($n = 3$), soft body without external shell for the gastropod *Osilinus turbinatus* ($n = 3$) and gonad material for the purple sea urchin *Paracentrotus lividus* ($n = 3$). Pelagic prey samples included homogenised bodies of goose barnacle *Lepas* spp. ($n = 3$) and white muscle from one fish species, the big-scale sand smelt *Atherina boyeri* ($n = 3$). Finally, samples from prey items acquired from fishery discharge included white muscle samples from three fish species, i.e. salema porgy *Sarpa salpa* ($n = 3$), greater amberjack *Seriola dumerili* ($n = 3$) and tuna fish *Thunnus* spp. ($n = 4$) (Supplementary material Table SII). The muscle tissue of fish species was chosen because it tends to vary less in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than do other tissues (Pinnegar & Polunin 1999).

Furthermore, data available in the literature for different organisms sampled in the Mediterranean Sea were included to make inferences about the isotopic composition of the trophic chain present in the waters around the Aeolian Archipelago. Specifically, we included $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ patterns of particulate organic matter (POM), *Posidonia oceanica*, *Verella verella*, *Pelagia noctiluca*, *Tuberculata* sp. and *Salpa maxima* from the north-western portion of the Mediterranean (Cardona et al. 2007, 2012); sediment organic matter (SOM), *Posidonia oceanica*, amphipods and copepod species from the south-west coast of Sicily (Vizzini et al. 2002); benthic and pelagic fish species, copepods, malacostraca, fish larvae and brachiopods from the southern portion of Sicily (Rumolo et al. 2016, 2017); and *Sarda sarda* larvae, juveniles and adults, *Scomber scomber*, and cephalopods *Loligo vulgaris* and *Todares sagittatus*, from two locations in the Southern Tyrrhenian Sea (Western Mediterranean Sea) (Sarà & Sarà 2007).

Sample preparation

To avoid possible contamination arising from epibionts present on the turtle carapace, all carapace

samples were rubbed with sterile gauze and deionised water prior to being put in vials. All samples were transported to Newcastle University laboratories where they were prepared for the stable isotope analyses. All 2013 loggerhead samples were sun dried, whereas loggerhead sea turtle samples from the previous years were either stored frozen or left to dry in the sun. The remaining samples were stored frozen and then dried in an oven for 24 hours at a constant temperature of 50°C before transport to Newcastle. All of the samples were then frozen at -80°C prior to freeze-drying for 72 hours. Samples were hand ground to a fine powder using mortar and pestle. *Lepas* spp. samples were further treated by adding 1M hydrochloric acid to eliminate the inorganic carbon present in the exoskeleton (DeNiro & Epstein 1978), which does not indicate food sources; these samples were then dried again at 50°C for 24 hours. *Thunnus* spp. samples were the only ones with a C:N ratio above 3.5 (Post et al. 2007), and therefore, due to their high concentration of lipids, were further treated by adding a 2:1 solution of chloroform/methanol to eliminate lipids which tend to be more depleted in $\delta^{13}\text{C}$ than proteins are (Sweeting et al. 2004, 2006). The treated samples were dried again at 50°C for 24 hours after lipid extraction. Carbonate and lipid extractions alter the $\delta^{15}\text{N}$ signatures of the samples; therefore, the treated samples were analysed both before and after the treatments. One milligramme of each sample was weighed and put into a tin capsule for isotopic determination.

Stable isotope analyses

An EA-IRMS Europa Scientific 20-20 mass spectrometer was used for the analysis of both samples and reference materials, to calibrate the system and compensate for drift with time. In detail, the reference material used for analysis was IA-R042 (powdered bovine liver), with a $\delta^{15}\text{N}$ value of +7.65‰ vs. air and a $\delta^{13}\text{C}$ value of -21.60‰ vs. V-PDB. IA-R042 was chosen as a reference material as it most closely matches the organic matrix of the analysed samples. Moreover, other reference Iso-Analytical working standards such as IA-R045 (ammonium sulphate, $\delta^{15}\text{N} = -4.71\text{‰}$ vs. air), IA-R046 (ammonium sulphate, $\delta^{15}\text{N} = 22.04\text{‰}$ vs. air), IA-R005 (beet sugar, $\delta^{13}\text{C} = -26.03\text{‰}$ vs. V-PDB) and IA-R006 (cane sugar, $\delta^{13}\text{C} = -11.64\text{‰}$ vs. V-PDB) were measured as quality control check samples during analysis. Isotope ratios were expressed as parts per thousand (‰) differences from a standard reference material:

$$\delta X = \left/ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right. \times 10^{-3}$$

where X is ^{15}N or ^{13}C , R is the corresponding ratio $^{14}\text{N}:^{15}\text{N}$ or $^{13}\text{C}:^{12}\text{C}$, and δ is the index of heavy to light isotope in the sample. The standard reference materials were Vienna Pee Dee Belemnite (V-PDB) for carbon and atmospheric N_2 for nitrogen (Polunin et al. 2001). Experimental precision based on the standard deviation of replicates of the internal standard was 0.13‰ for $\delta^{13}\text{C}$ and 0.06‰ for $\delta^{15}\text{N}$ ($n = 8$).

Bayesian mixing models and statistical analyses

Bayesian mixing models available as an open source R package (MixSIAR GUI; Moore & Semmens 2008; Semmens et al. 2009; Stock & Semmens 2013) were performed to estimate the proportion of each potential food source to the diet of loggerhead turtles. The isotopic values obtained from the literature were not included in the models, due to the different sampling locations (north-western part of the Mediterranean, Tyrrhenian Sea and other parts of Sicily) and time frames. The literature data were used instead to make some general inferences about the possible structure of the Aeolian Archipelago's trophic chain and the interpretation of the differences between isotopic patterns. MixSIAR estimates the probability distributions (5th, 25th, 50th, 75th and 95th percentiles) of each source (potential prey item) to a consumer's stable isotope values while accounting for variability among consumer and source isotopic values, and uncertainty associated with tissue–diet discrimination factors (Phillips et al. 2014). The model was run once including all turtles to examine the proportions of potential prey items for the population, and once for each specimen to examine this ratio on an individual level. The Markov Chain Monte Carlo (MCMC) (three replicate chains) was run for 300,000 iterations, discarding the first 200,000 samples and then thinning by 100. Model convergence was confirmed using Gelman–Rubin and Geweke diagnostic tests. Before running MixSIAR, the potential preys with known diet/trophic position in the food web were grouped on the basis of ecological similarity, in three categories that represent the different habitats in which turtles might forage: pelagic (i.e. *Lepas* spp. and *Atherina boyeri*), benthic (i.e. *Osilinus turbinatus*, *Percnon gibbesi* and *Paracentrotus lividus*), and prey items from longline baits or fishery discards (i.e. *Tunnus* spp., *Seriola dumerili* and *Sarpa salpa*) (Supplementary material Table SII). Few studies

have been conducted to investigate fractionation values in the Cheloniidae family (Seminoff et al. 2006; Revelles et al. 2007a; Reich et al. 2008). The trophic enrichment factor (TEF) of $0.17 \pm 0.03\text{‰}$ for $\delta^{13}\text{C}$ and $2.8 \pm 0.1\text{‰}$ for $\delta^{15}\text{N}$ derived from Seminoff et al. (2006) for skin, that has low isotopic turnover like the carapace, was considered reliable. Despite the fact that it was estimated for juvenile green turtles *Chelona mydas*, the authors actually sampled organisms with a body size comparable to that of our turtles. Using the mentioned TEF, moreover, our results could be more easily compared to previous stable isotope studies of loggerhead prey (Wallace et al. 2009; McClellan et al. 2010; Goodman Hall et al. 2015) that also used the discrimination factor of Seminoff et al. (2006). MixSIAR results were reported, for each source, as a posterior density distribution of proportional contributions to consumer mixture data (mean dietary proportions with associated credibility intervals).

Normal distributions of loggerhead stable isotope and CCL data were checked using Anderson–Darling, and homogeneity of variances using Levene's test. The Kruskal–Wallis test was used to compare stable isotope and CCL data among different groups of turtles. In particular, stable isotope data were compared between turtles grouped by: (a) sampling location (Aeolian islands and Sicily); (b) year (from 2009 to 2013) and season (spring, summer and autumn) of sampling; (c) preservation method (sun drying and freezing); (d) CCL (small, medium and large); and (e) health condition (good health, injured and dead). Stable isotope values were also compared between groups of turtles with different causes of injury/death (ingested longline hook, gastrointestinal occlusion due to debris ingestion and boat collision). Correlations (Spearman rank tests) were used to assess the strength of association between $\delta^{13}\text{C}$ and CCL and between $\delta^{15}\text{N}$ and CCL. These statistical analyses were run in PAST (Hammer et al. 2001).

Results

Results of Bayesian mixing models

Bivariate plots of isotopic signatures of 54 loggerhead turtles and potential prey items within which proximity to the source indicates the greatest contribution to the diet are provided in Figure 2 (Phillips & Gregg 2001).

The MixSIAR Bayesian Mixing model indicated that, for the overall population (Figure 3), the pelagic prey items (*Lepas* spp. and *A. boyeri*) constituted the major part of the loggerhead turtle diet (mean:

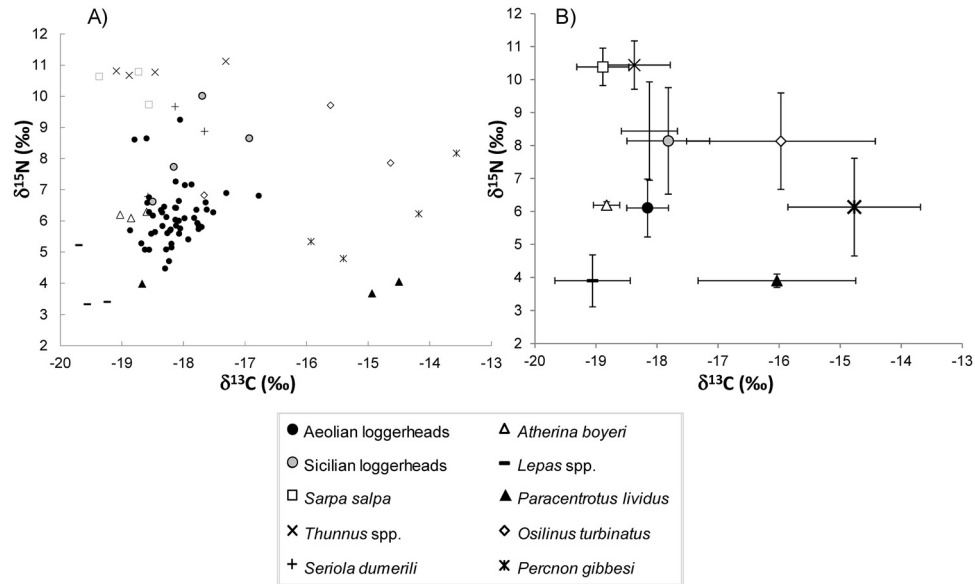


Figure 2. Bivariate plot of stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of 54 loggerhead turtles with those of the other species analysed. Values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are reported in panel A while means and \pm one standard deviation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are reported in panel B.

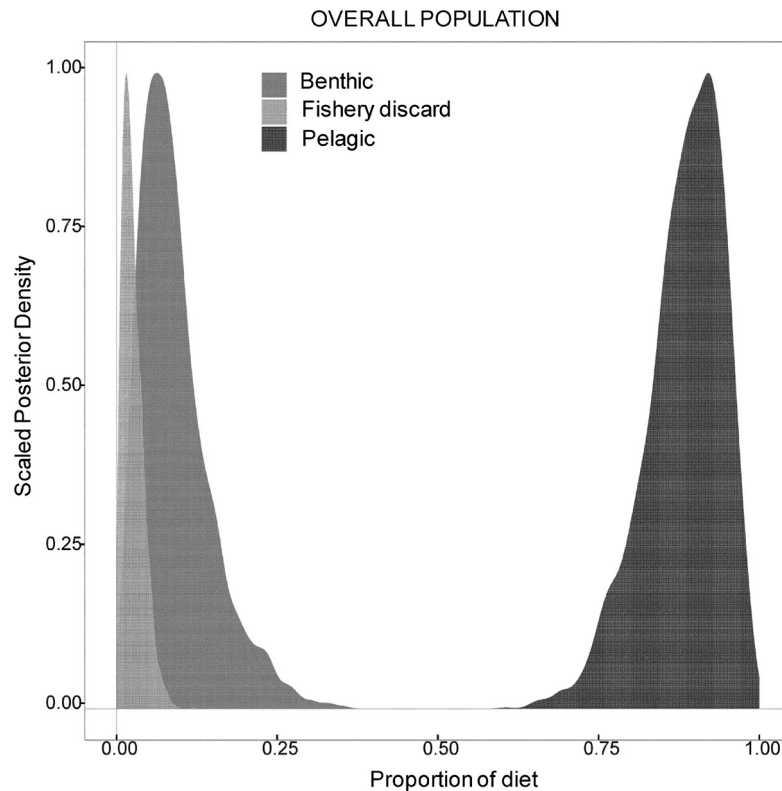


Figure 3. MixSIAR results showing the posterior density distributions of proportional contributions of the potential prey groups (pelagic, benthic and fish discards) to the diet of loggerhead turtle population. Pelagic prey (*Lepas* spp. + *Atherina boyeri*); benthic prey (*Osilinus turbinatus* + *Percnon gibbesi* + *Paracentrotus lividus*); fish discards (*Thunnus* spp. + *Sarpa salpa* + *Seriola dumerili*).

88.3%; 5th and 95th quantiles: 76.7% and 96.6%) followed by benthic prey (mean: 9.2%; 5th and 95th quantile: 2.2% and 20.5%) and, in minor part, by prey from fishery discards (mean: 2.5%; 5th and 95th quantiles: 0% and 5.5%).

Mixing model results for individual populations confirmed that most of the turtles feed mainly on pelagic prey (mean > 80.0%), with the exception of 15 individuals (i.e. two from Siracusa, one from Catania and 12 from the Aeolian Archipelago) that exhibited a pronounced increase in the mean consumption of benthic resources (max. mean 53.0%; 5th and 95th quantiles: 2.8% and 8.4%; Supplementary material, Table SIII).

Seven of these individuals had CCL > 60 cm, and five individuals had CCL between 40 and 60 cm, while only three individuals had CCL < 40 cm (Supplementary material, Table SIII). In addition, only one of these individuals was in good health, while the others were injured or dead (Supplementary material, Table SIII). Finally, prey items from fishery discards were integrated (50% Confidence Interval (CI) up to 5%) in the diet of only six individuals (max. mean 35.0%; 5th and 95th quantiles: 1.0% and 5.7%), i.e. three dead turtles from the Aeolian Archipelago (CCL > 50 cm), one injured turtle from Siracusa (CCL < 40 cm), one dead turtle from Siracusa (CCL = 40–50 cm) and one dead turtle from Catania (CCL = 40–50 cm) (Supplementary material, Table SIII). The known causes of injury/death for these individuals were debris ingestion (n = 4), longlines (n = 3) and boat collision (n = 1). The trophic mixing space shaped by potential prey groups (benthic, pelagic and fishery discards) that contributed to the diet of each loggerhead individual is reported (Figure 4). All 54 turtles were included to allow for a visual comparison of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with those of potential prey items (Figure 4).

Stable isotope variations with life stage and health status

The loggerhead CCL data were normally distributed (Anderson-Darling, $p > 0.05$) while the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data were not (Anderson-Darling, $p < 0.05$). There was homogeneity of variances in loggerhead $\delta^{13}\text{C}$ (Levene's test, $L = 2.21$, $p > 0.05$) and $\delta^{15}\text{N}$ (Levene's test, $L = 0.20$, $p > 0.5$) among the three CCL classes (small, medium and large), while loggerhead $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values did not significantly differ with preservation method or among years or season of sampling (Table I).

Values of $\delta^{13}\text{C}$ from Aeolian loggerheads ranged from -18.87‰ to -17.30‰ (mean \pm standard deviation (SD) = $6.2 \pm 1.1\text{‰}$) while $\delta^{15}\text{N}$ values

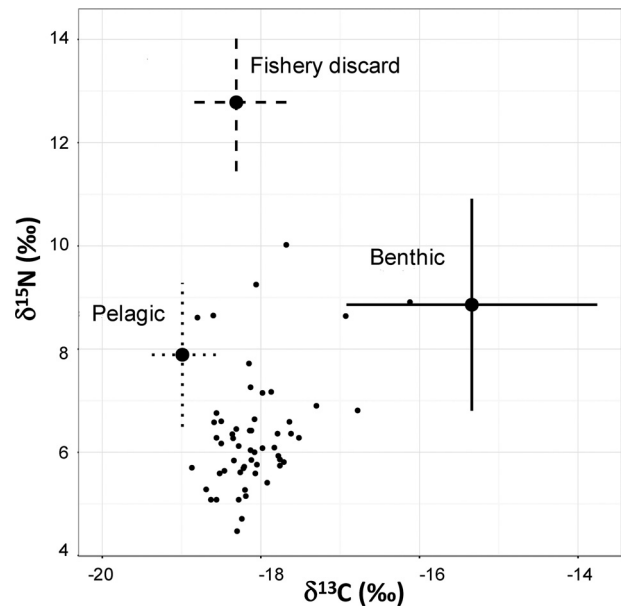


Figure 4. Results of the Bayesian mixing model showing the trophic mixing space shaped by potential pelagic prey (*Lepas* spp. + *A. boyeri*), benthic prey (*O. turbinatus* + *P. gibbesi* + *P. lividus*) and prey items from fish discards (*T.* spp. + *S. salpa* + *S. dumerili*) that have contributed to the diet of loggerhead turtles. Each black circle represents a single $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ sample value associated with an individual turtle. Prey item values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were adjusted for isotopic discrimination between diet and tissue. All 54 turtles were included in this figure to allow for a visual comparison of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with those of potential prey items.

ranged from 4.47‰ to 9.24‰ (mean \pm SD = $-18.1 \pm 0.4\text{‰}$) (Table I). However, the Sicilian turtles had higher $\delta^{15}\text{N}$ values (from 6.17‰ to 10.02‰) and $\delta^{13}\text{C}$ (from -18.49 to -16.92) than the Aeolian ones (Kruskal-Wallis test, $H_c = 9.7$, $p = 0.02$; Table I).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the Aeolian turtles (live and dead) were plotted by size (CCL) and health condition (Figure 5), showing higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ with increasing CCL in Aeolian turtles (live and dead), live turtles (excluding dead turtles) and those in good health (excluding injured and dead turtles) (Table I).

In addition, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were higher in dead (mean CCL = 50.4 ± 10.5 cm) than live (mean CCL = 52.4 ± 12.4 cm) turtles; however, only $\delta^{15}\text{N}$ was higher in injured/dead turtles (mean CCL = 52.8 ± 10.9 cm) compared to those in good health (mean CCL = 49.7 ± 11.0 cm; Table I). Separate analyses for each class showed that turtles injured/killed by boat collision had lower $\delta^{13}\text{C}$ than turtles in good health (Kruskal-Wallis, $H_c = 12.3$, $p = 0.0005$) or those injured/killed by longlines (mean CCL = 54.0 ± 12.4 cm; Kruskal-Wallis, $H_c = 8.2$, $p = 0.004$) or showing debris ingestion

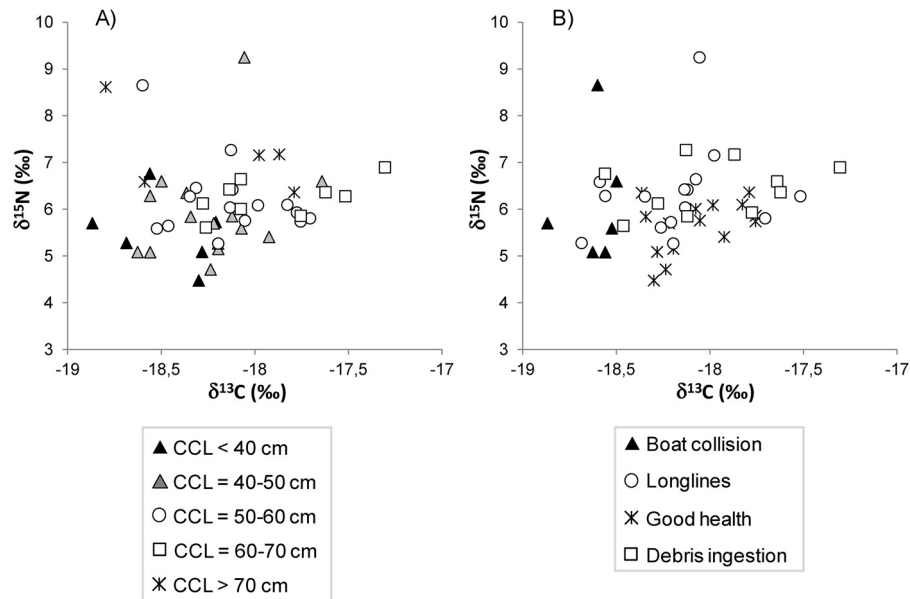


Figure 5. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios in loggerhead turtles from the Aeolian Archipelago plotted by size (curved carapace length (CCL); panel A), health condition and cause of injury (panel B), showing different patterns of isotopic signature among groups of individuals. Only loggerhead turtles with known size and cause of injury/dead were included.

(mean CCL = 53.1 ± 13.1 cm; Kruskal-Wallis, $H_c = 7.8$, $p = 0.005$; Table I). In addition, turtles in good health exhibited lower $\delta^{15}\text{N}$ than those injured by longlines (Kruskal-Wallis, $H_c = 7.4$, $p = 0.006$) or showing debris ingestion (Kruskal-Wallis, $H_c = 9.9$, $p = 0.001$). The mean CCL did not differ among turtles with different problems (Kruskal-Wallis; $H_c = 2.8$, $p = 0.4$); however, turtles injured or killed by boat collision had smaller sizes (mean CCL = 45.2 ± 8.7) than those with other problems (mean CCL = 53.6 ± 7.2 cm; Kruskal-Wallis; $H_c = 10.5$, $p < 0.05$) or in good health (mean CCL = 49.7 ± 11.1 cm; Table I). Finally, only $\delta^{15}\text{N}$ varied among different groups of turtles with similar size (CCL between 40–60 cm, $n = 25$). In particular, $\delta^{15}\text{N}$ was significantly higher in turtles injured/killed by longlines (Kruskal-Wallis, $H_c = 4.9$, $p = 0.02$) or showing debris ingestion (Kruskal-Wallis, $H_c = 3.6$, $p = 0.05$) than in turtles in good health.

Discussion

The feeding ecology of the loggerhead turtle was inferred through the analysis of the stable carbon and nitrogen isotopes that accumulated in its carapace and by the comparison of these values to those for possible preys or food production sources. To our knowledge, this is the first study that uses this methodology to investigate the

influence of size and health condition in different specimens of the Mediterranean loggerhead turtle.

Integration of diet

Several studies demonstrated that the diet of the Mediterranean loggerhead turtle is highly varied in terms of prey taxa (Bjorndal 1997; Godley et al. 1998; Tomás et al. 2001; Revelles et al. 2007a; Cardona et al. 2010, 2012; Zbinden et al. 2011). If on one hand our MixSIAR results confirmed the availability of a wide range of prey items in the study area, on the other hand they suggested that pelagic prey (from goose barnacles to planktivorous fish) comprised most of the diet of the loggerhead study population. The $\delta^{13}\text{C}$ values of all the turtle specimens, in fact, overlapped with the isotopic pattern of the pelagic organisms sampled around the Aeolian Archipelago, suggesting a similar use of the habitat. Based on the $\delta^{15}\text{N}$ obtained from both direct analysis and the literature, the possible trophic chain used by the Mediterranean loggerhead turtles was constructed (Figure 6; Vizzini et al. 2002; Cardona et al. 2007, 2012; Sarà & Sarà 2007; Rumolo et al. 2016, 2017). Although we suggest caution in using data obtained from areas that are influenced differently in terms of oceanography and isotopic contribution (e.g. northern Mediterranean vs. southern Mediterranean), we felt confident in inferring little

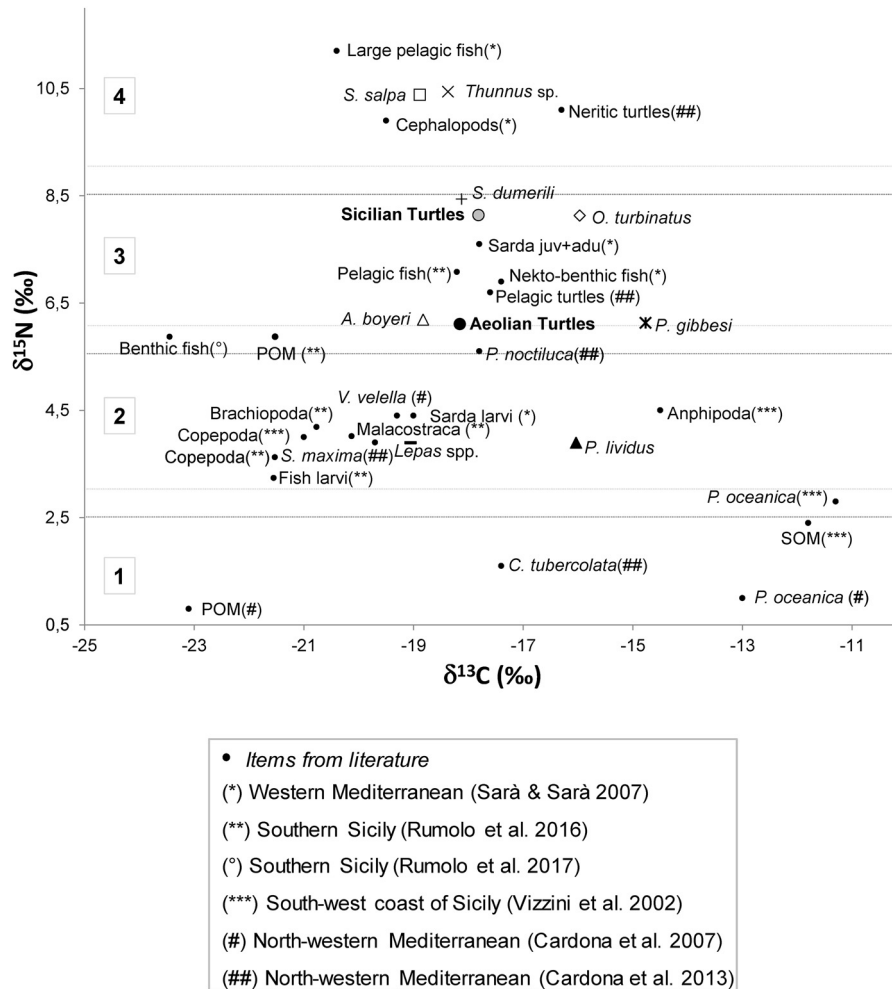


Figure 6. The isotopic composition of loggerhead carapace scutes and potential prey items as found in the present study (species name reported close to symbols) compared with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of loggerhead turtles and potential diet items from other Mediterranean areas extrapolated from the literature (Vizzini et al. 2002; Cardona et al. 2007, 2012; Sarà & Sarà 2007; Rumolo et al. 2016, 2017). Main trophic levels from 1 to 4 for pooled consumer categories are included. Items are from this study.

variability in the trophic role of similar organisms. The $\delta^{15}\text{N}$ of POM, *Tuberculata* sp. and *Posidonia oceanica* samples characterised them as primary producers and constituents of the basis of the food web (Figure 6). Assuming an enrichment of ^{15}N comprised between 2 and 3‰ for consecutive trophic positions (DeNiro & Epstein 1978; Minagawa & Wada 1984; Post 2002), all the sampled loggerhead turtles were considered secondary consumers (third level; Figure 6).

This classification was coherent with the isotopic values of zooplanktivorous, barnacles (*Lepas* spp.), copepods, fish larvae, amphipods and sea urchins, which are normally classified as primary consumers and could be part of the diet of the loggerhead turtles, like fish larvae and barnacles (Figure 6). Documented observations of feeding events

directed to the consumption of gelatinous and planktonic organisms, like jellyfish (e.g. *Pelagia noctiluca*), planktonic tunicates (e.g. *Salpa maxima*) and hydrozoans (e.g. *Velella velella*) (Blasi et al. 2016; Blasi & Mattei 2017), were partially confirmed by the $\delta^{15}\text{N}$ analysis. *Salpa maxima* and *Velella velella* were classified as primary consumers, and the ^{15}N isotopic enrichment between them and the loggerhead turtle was compatible with the expected value (Figure 6). *Pelagia noctiluca*, on the other hand, was found at the same trophic level as the turtles, suggesting its contribution to the isotopic composition of the sampled loggerheads is not major (Figure 6). Based on the work of Cardona et al. (2012) conducted in the northern portion of the Mediterranean Sea, however, jellyfish should be consumed by loggerhead turtles in

their oceanic stage, while they are consumed only in minor part during the neritic stage. The $\delta^{15}\text{N}$ values of the specimens analysed in this study were also consistent with a pelagic diet, but relatively lower than those registered in the northern part of the basin (Figure 6). This evidence suggests either that the southern population feeds on preys that are depleted in ^{15}N if compared to the northern ones, or that the tissue sampled from our organisms did not yet fully reflect the contribution of the ingested preys. The ^{15}N , however, accumulates rapidly in turtle scutes, especially if compared to the enrichment in ^{13}C (Reich et al. 2007). Further investigation will need to take into consideration the sampling of both turtles and gelatinous organisms in the same study area, in order to directly relate their isotopic composition.

The same discrepancy of *P. noctiluca* in terms of isotopic accumulation was found for *Atherina boyeri*, a zooplanktophagous fish that may be part of the loggerhead's diet, which here appeared to lie in the same trophic level (Figure 6). All turtle specimens were, in fact, isotopically grouped for $\delta^{15}\text{N}$ in the third trophic level, like the pelagic fish species, samples of crabs and molluscs. Finally, all the samples collected as fish discards or longline baits appeared to be representative of the higher trophic level, that of the tertiary consumers (Figure 6), like other typical ichthyophagous species and cephalopods (Jennings et al. 1997; Lepoint et al. 2000; Pinnegar & Polunin 2000; Polunin et al. 2001; Sarà & Sarà 2007).

On an individual basis, the accumulation of ^{15}N did not seem to follow a defined pattern as did ^{13}C . The model confirmed a general preference for pelagic prey items, but some turtles were also found to feed primarily on benthic species or fish discards. These results suggest a higher variability in diet composition between the specimens than that expected only on the basis of $\delta^{13}\text{C}$ values.

In the following sections, all of the possible factors that might influence the different isotopic discrimination in the target loggerhead population are analysed.

Geographic location

As previously mentioned, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ records at the base of the food web are conserved and amplified by $\sim 1\text{‰}$ and $\sim 3\text{‰}$, respectively, through higher trophic levels (DeNiro & Epstein 1978; Pajuelo et al. 2010, 2012a,b). Those values, however, are not fixed but are subject to variation due to variables such as habitat characteristics and local environmental conditions of different geographic areas. Consequently, this

diversity influences the isotopic accumulation in the tissues of consumers such as loggerhead turtles (Seney & Musick 2007; Pajuelo et al. 2010).

The loggerhead turtles collected around the Aeolian Archipelago were considered to feed on pelagic prey items rather than on benthic species or fishery discards. Their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values had comparable ranges of variance, providing proof of a similar temporal integration of diet and habitat use. On the other hand, the four loggerhead carapaces collected along the Sicilian coast (Ionian Sea) reflected a different feeding pattern. Particularly, the mixing model analysis underlined a significative increase in benthic (mean shift from pelagic to benthic resources = 10.7‰) and fish discard resources (mean shift from pelagic to fish resources for longlines: 8.8‰) when compared to turtles from the Aeolian Archipelago. The complex geomorphology of the Aeolian Archipelago may have a significant influence on the diversity of loggerhead prey, reflecting specific foraging strategies adopted in this area. Due to local tectonic structures involving volcanic islands, which comprise extensive neritic and oceanic habitats within short distances of each other (Blasi & Boitani 2012), pelagic prey might be more common (Mills 2001) and easier to access. Moreover, turtles feeding around the Aeolian Archipelago may have access to pelagic prey that are transported by currents from either the continental shelf (coast of Sicily) or the Tyrrhenian Sea (oceanic habitats). An important contribution to diet could also be provided by gelatinous organisms, such as cnidarians and urochordates, as largely documented for immature and neritic stages of loggerheads worldwide (Dodd 1988; Jennings et al. 1997; Lepoint et al. 2000; Pinnegar & Polunin 2000; Polunin et al. 2001; Revelles et al. 2007a,b).

On the other hand, marine debris and lines used to anchor pelagic FADs (largely used in the study area) to the sea bottom encourage the settlement of algae, crustaceans and barnacles, which in turn attract small fish that may be easily accessible prey items and potential food for loggerhead turtles (Blasi et al. 2017). Lastly, the decline in the contribution of neritic/benthic prey items also indicated their rare contribution to the loggerheads' diet. Fishing activities and lack of protection of coastal habitat may have changed prey distribution, leading to a spatial variation of foraging strategies and reflecting dietary supplementation due to the general decline in loggerheads' typical prey items (Seney & Musick 2007).

Loggerhead turtles are long-living vertebrates and they may bio-accumulate heavy metals and organic contaminants from food, sediment, and water (Hutchinson & Simmonds 1991; D'Ilio et al. 2011), which are known

to increase $\delta^{15}\text{N}$ in coastal areas compared to open-water ecosystems (McKinney et al. 2010). It cannot be excluded that agricultural runoff and anthropogenic waste along the coasts of Sicily (Copat et al. 2012) might be also responsible for the higher $\delta^{15}\text{N}$ found in not Aeolian turtles. Further investigations are needed to confirm these hypotheses.

Seasonal habitats

Differences among seasonal habitats utilised by organisms are known to influence the patterns of isotopic signatures (McClellan et al. 2010; Ceriani et al. 2014). In this study, however, the loggerhead carapaces displayed similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values during different seasons. Although different food sources are available to turtles in different seasons, it is possible that the loggerheads preferentially feed on prey that accessible all through the year. $\delta^{13}\text{C}$ variations among seasonal habitats can be detected in samples from the carapace, due to its keratinous composition. In this tissue, in fact, isotopic signatures accumulate over a long period of time (years), and variation between oceanic and neritic specimens would have been detected, as was previously demonstrated in sections of humeri (comparable to the carapace; Seminoff et al. 2006; Reich et al. 2008; Snover et al. 2010; Avens et al. 2013; Thomas & Crowther 2015).

However, seasonal shifts in loggerheads' foraging habitats (neritic to oceanic) might be not consistent with variations in $\delta^{13}\text{C}$. If prey sources vary seasonally or are unequally distributed along the coastal area, the location of productive foraging patches might potentially influence loggerhead turtles' movements during pelagic feeding (Cardona et al. 2014). Turtles feeding in neritic waters might potentially feed on planktonic prey items that are brought close to the coastal area by the local currents (Blasi et al. 2016; Blasi & Mattei 2017). On the other hand, turtles feeding in oceanic habitats might have access to opportunistic prey, such as longlines baits/fishery discards or prey settled on debris and FADs (Blasi et al. 2016). Accordingly, McClellan and Read (2007) demonstrated that seasonal shifts in loggerheads' foraging behaviour (benthic to pelagic) are not always consistent with movement documented by satellite tracking.

Finally, in this study, we could not demonstrate from loggerheads' location that turtles captured in oceanic habitats had significantly lower $\delta^{13}\text{C}$ than those remaining in neritic waters, since all loggerheads were captured while resting at the water

surface, in locations that might be at a distance from the foraging sites (Goodman Hall et al. 2015).

Life stage

The loggerhead turtles from the Aeolian Archipelago showed increasing ^{13}C and ^{15}N values with increasing CCL. Accordingly, lower $\delta^{13}\text{C}$ values were found in turtles injured by boat collision, which were clearly smaller than the other turtles. The influence of the size in $\delta^{13}\text{C}$ variations might reflect changes in dietary habitats during life stages (Godley et al. 1998; Hatase et al. 2002; Reich et al. 2007; Casale et al. 2009; Snover et al. 2010; Casale 2011; Piovano et al. 2011; Avens et al. 2013; Goodman Hall et al. 2015). Accordingly, Mattei et al. (2015) found a different distribution of heavy metals along scutes of Aeolian loggerheads, reflecting accumulation of these contaminants during life stages characterised by different dietary habitats (D'Ilio et al. 2011). In particular, the loggerheads exhibited a small increase in the use of benthic and fish discard resources (mean shift from pelagic to benthic resources = 9.4‰; mean shift from pelagic prey to fish discards: 3.3‰) between small juveniles (CCL < 40 cm) and large adults (CCL > 70 cm). It is possible that the shift to the benthic habitat might occur over an extended period. Consequently, the loggerhead turtles might progressively enter neritic waters as they attain a larger size (Goodman Hall et al. 2015). As they adapt to the new environment, it is possible that they begin to include sources of prey with higher $\delta^{13}\text{C}$ values (Goodman Hall et al. 2015). However, given the overall low variability of $\delta^{13}\text{C}$ values, these differences are not consistent with a clear habitat transition from oceanic to neritic foraging grounds in immature turtles (Godley et al. 1998; Casale et al. 2009; Casale 2011; Piovano et al. 2011). It is also possible that the shift to the benthic habitat might never occur in some individuals (Hatase et al. 2002; Hawkes et al. 2006; McClellan & Read 2007; Revelles et al. 2007b; Casale et al. 2008; Zbinden et al. 2011). Indeed, most turtles in our study were captured in open water (depth > 200 m) and consisted mostly of large juveniles and some adults, which are not exclusively oceanic. On the other hand, variations in $\delta^{13}\text{C}$ may be partially covered by the low variability of CCL measurements in our samples due to differences in somatic growth rates (Casale et al. 2009; Casale 2011; Piovano et al. 2011).

The significant influence of CCL on loggerhead $\delta^{15}\text{N}$ values might indicate that as turtles increase in

size, they may feed on prey at a higher trophic level. For example, larger size might allow turtles to eat larger prey that also have higher $\delta^{15}\text{N}$ values (Pajuelo et al. 2010; Goodman Hall et al. 2015). Finally, it is also possible that small and large turtles forage on similar prey items, but larger turtles with slower growth rates have higher nitrogen discrimination values resulting in higher $\delta^{15}\text{N}$ values (Reich et al. 2008; Pajuelo et al. 2010).

Health condition

It is well known that the method of storage of samples can influence $\delta^{15}\text{N}$ over different periods of time (Ponsard & Amlou 1999; Sweeting et al. 2004; Barrow et al. 2008; Payo-Payo et al. 2013). Here, however, frozen and dried carapace samples collected over different years did not show significant isotopic variation. Consequently, it was assumed that the $\delta^{15}\text{N}$ variations were not related to microbial degradation (Revelles et al. 2007a). In addition, pre- and post-mortem stress could influence the isotopic accumulation in unpredictable ways, especially for $\delta^{15}\text{N}$ (Seminoff et al. 2006; Revelles et al. 2007a; Payo-Payo et al. 2013). On the other hand, Revelles et al. (2007a) found no significant differences in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ between live and dead loggerhead turtles from the same habitat in the western Mediterranean Sea. These considerations suggest caution in the interpretation of the results when animals with different health condition are compared. Despite foraging consistently in the same habitats, in fact, the $\delta^{15}\text{N}$ values found in this study appear to be significantly influenced by health status. The highest values were found in dead/injured individuals that were known to feed or to have been feeding on longline bait or on marine debris. These specimens were clearly bigger than the other sampled turtles. From this, it was hypothesised that larger turtles might feed on prey at a higher trophic level (Godley et al. 1998; Casale et al. 2009; Casale 2011; Piovano et al. 2011). However, turtles feeding on longlines or debris also had higher $\delta^{15}\text{N}$ than did healthy turtles of the same size (large juveniles). Specifically, these turtles displayed a small increase in the consumption of benthic (mean shift from pelagic to benthic resources for longlines = 1.4‰; mean shift from pelagic to benthic resources for debris ingestion = 5.3‰) and fish discard resources (mean shift from pelagic to fish resources for longlines: 2.9‰; mean shift from pelagic to fish resources for debris ingestion = 3.9‰) compared to turtles in good health. For all these reasons, the observed

$\delta^{15}\text{N}$ differences could have different origins. First, variations in diet (trophic differences) could occur among individuals (Zbinden et al. 2011). For example, some organisms might preferentially feed on longline baits, discarded fishing material or prey items found settled on FADs and debris (Dodd 1988; Tomás et al. 2001; Bjorndal et al. 2003; Revelles et al. 2007b) and may consequently be more vulnerable to threats. Secondly, it is possible that these turtles may be foraging on similar prey items to the others, but slower growth rates may show higher nitrogen discrimination values, resulting in higher $\delta^{15}\text{N}$ values (Reich et al. 2008; Pajuelo et al. 2010). Third, the ingestion of marine debris was found to be a common secondary consequence for turtles entangled in longlines. The long residence times of these materials in the gastrointestinal system might significantly increase the $\delta^{15}\text{N}$ patterns of these turtles, due to dietary dilution and consequent absorption of toxins (Hutchinson & Simmonds 1991; Godley et al. 1999; Nelms et al. 2015). Accordingly, high levels of contaminants have been found in scutes from Aeolian loggerheads (D'Ilio et al. 2011; Mattei et al. 2015). Lastly, the hypothesis of an alteration of $\delta^{15}\text{N}$ values in animals with different physiological condition could be true as well for the species used as lures. During capture, in fact, fishes suffer intense muscular stress and hypoxia, conditions that alter the metabolic state and could increase the accumulation of nitrogen in their tissues (Lowe et al. 1993). This enrichment would be consequently reflected in the tissues of the turtles that feed on them, and the expected isotopic patterns would be altered.

Based on all these considerations, we underline the need for further investigations to clarify the importance of health conditions in the interpretation of $\delta^{15}\text{N}$ values, especially in animals that are threatened by anthropogenic impacts.

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Supplemental data

Supplemental data for this article can be accessed [here](#).

References

- Andaloro F, Campo D, Castriota L, Sinopoli M. 2007. Annual trend of fish assemblages associated with FADs in the southern Tyrrhenian Sea. *Journal of Applied Ichthyology* 23:258–263. DOI:10.1111/jai.2007.23.issue-3.
- Avens L, Goshe LR, Pajuelo M, Bjorndal KA, MacDonald BD, Lemons GE, Bolten AB, Seminoff JA. 2013. Complementary skeletochronology and stable isotope analyses offer new insight into juvenile loggerhead sea turtle oceanic stage duration and growth dynamics. *Marine Ecology Progress Series* 491:235–251. DOI:10.3354/meps10454.
- Barrow LM, Bjorndal KA, Reich KJ. 2008. Effects of preservation method on stable carbon and nitrogen isotope values. *Physiological and Biochemical Zoology* 81:688–693. DOI:10.1086/588172.
- Bjorndal KA, Bolten AB, Dellinger T, Delgado C, Martins H. 2003. Compensatory growth in oceanic loggerhead sea turtles: Response to a stochastic environment. *Ecology* 84:1237–1249. DOI:10.1890/0012-9658(2003)084[1237:CGIOLS]2.0.CO;2.
- Bjorndal KA. 1997. Foraging ecology and nutrition of sea turtles. Chapter 8. In: Lutz PL, Musick JA, editors. *The biology of sea turtles*. Boca Raton, FL: CRC Press. pp. 199–231.
- Blasi MF, Boitani L. 2012. Modelling fine-scale distribution of the bottlenose dolphin *Tursiops truncatus* using physiographic features on Filicudi (southern Tyrrhenian Sea, Italy). *Endangered Species Research* 17:269–288. DOI:10.3354/esr00422.
- Blasi MF, Mattei D. 2017. Seasonal encounter rate, life stages and main threats to the loggerhead sea turtle (*Caretta caretta*) in the Aeolian Archipelago (southern Tyrrhenian Sea). *Aquatic Conservation: Marine and Freshwater Ecosystems* 27:617–630. DOI:10.1002/aqc.v27.3.
- Blasi MF, Roscioni F, Mattei D. 2016. Interactions of loggerhead turtles (*Caretta caretta*) with traditional fish aggregating devices (FADs) in the Mediterranean Sea. *Herpetological Conservation and Biology* 11:386–401.
- Bolten AB. 1999. Techniques for measuring sea turtles. *Research and Management Techniques for the Conservation of Sea Turtles* 4:1–5.
- Bolten AB. 2003. Variation in sea turtle life history patterns: Neritic vs. oceanic developmental stages. In: Lutz PL, Musick J, Wyneken J, editors. *The biology of sea turtles*. Boca Raton: CRC Press. Vol. 2. pp. 243–257.
- Bolten AB, Martins HR, Bjorndal KA, Cocco M, Gerosa G. 1992. *Caretta caretta* (loggerhead) pelagic movement and growth. *Herpetological Review* 23:116.
- Bowen BW, Abreu-Grobois FA, Balazs GH, Kamezaki N, Limpus CJ, Ferl RJ. 1995. Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. *Proceedings of the National Academy of Sciences* 92:3731–3734. DOI:10.1073/pnas.92.9.3731.
- Bowen BW, Bass AL, Chow S-M, Bostrom M, Bjorndal KA, Bolten AB, Okuyama T, Bolker BM, Epperly S, Lacasella E, Shaver D, Dodd M, Hopkins-Murphy SR, Musick JA, Swingle M, Rankin-Baransky K, Teas W, Witzell WN, Dutton PH. 2004. Natal homing in juvenile loggerhead turtles (*Caretta caretta*). *Molecular Ecology* 13:3797–3808. DOI:10.1111/j.1365-294X.2004.02356.x.
- Boyle MC, FitzSimmons NN, Limpus CJ, Kelez S, Velez-Zuazo X, Waycott M. 2009. Evidence for transoceanic migrations by loggerhead sea turtles in the southern Pacific Ocean. *Proceedings of the Royal Society B: Biological Sciences* 276:1993–1999. DOI:10.1098/rspb.2008.1931.
- Boyle MC, Limpus CJ. 2008. The stomach contents of post-hatchling green and loggerhead sea turtles in the southwest Pacific: An insight into habitat association. *Marine Biology* 155:233–241. DOI:10.1007/s00227-008-1022-z.
- Burke VJ, Standora EA, Morreale SJ. 1993. Diet of juvenile Kemp's ridley and loggerhead sea turtles from Long Island, New York. *Copeia* 1993:1176–1180. DOI:10.2307/1447107.
- Cardona L, Campos P, Levy Y, Demetropoulos A, Margaritoulis D. 2010. Asynchrony between dietary and nutritional shifts during the ontogeny of green turtles (*Chelonia mydas*) in the Mediterranean. *Journal of Experimental Marine Biology and Ecology* 393:83–89. DOI:10.1016/j.jembe.2010.07.004.
- Cardona L, Clusa M, Eder E, Demetropoulos A, Margaritoulis D, Rees AF, Hamza AA, Khalil M, Levy Y, Türkozan O, Marin I, Aguilar A. 2014. Distribution patterns and foraging ground productivity determine clutch size in Mediterranean loggerhead turtles. *Marine Ecology Progress Series* 497:229–241. DOI:10.3354/meps10595.
- Cardona L, De Quevedo IÁ, Borrell A, Aguilar A. 2012. Massive consumption of gelatinous plankton by Mediterranean apex predators. *PLoS ONE* 7:e31329. DOI:10.1371/journal.pone.0031329.
- Cardona L, Revelles M, Sales M, Aguilar A, Borrell A. 2007. Meadows of the seagrass *Posidonia oceanica* are a significant source of organic matter for adjoining ecosystems. *Marine Ecology Progress Series* 335:123–131. DOI:10.3354/meps335123.
- Casale P. 2011. Sea turtle by-catch in the Mediterranean. *Fish Fisheries* 12:299–316. DOI:10.1111/j.1467-2979.2010.00394.x.
- Casale P, Abbate G, Freggi D, Conte N, Oliverio M, Argano R. 2008. Foraging ecology of loggerhead sea turtles *Caretta caretta* in the central Mediterranean Sea: Evidence for a relaxed life history model. *Marine Ecology Progress Series* 372:265–276. DOI:10.3354/meps07702.
- Casale P, Affronte M, Insacco G, Freggi D, Vallini C, Pino d'Astore P, Basso R, Paolillo G, Abbate G, Argano R. 2010. Sea turtle strandings reveal high anthropogenic mortality in Italian waters. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20:611–620. DOI:10.1002/aqc.v20:6.
- Casale P, Affronte M, Scaravelli D, Lazar B, Vallini C, Luschi P. 2012. Foraging grounds, movement patterns and habitat connectivity of juvenile loggerhead turtles (*Caretta caretta*) tracked from the Adriatic Sea. *Marine Biology* 159:1527–1535. DOI:10.1007/s00227-012-1937-2.
- Casale P, Mazaris AD, Freggi D, Vallini C, Argano R. 2009. Growth rates and age at adult size of loggerhead sea turtles (*Caretta caretta*) in the Mediterranean Sea, estimated through capture-mark-recapture records. *Scientia Marina* 73:589–595. DOI:10.3989/scimar.2009.73n3.

- Ceriani SA, Roth JD, Sasso CR, McClellan CM, James MC, Haas HL, Smolowitz RJ, Evans DR, Addison DS, Bagley DA, Ehrhart LM, Weishampe JF. 2014. Modeling and mapping isotopic patterns in the Northwest Atlantic derived from loggerhead sea turtles. *Ecosphere* 5:art122–art122. DOI:10.1890/ES14-00230.1.
- Copat C, Bella F, Castaing M, Fallico R, Sciacca S, Ferrante M. 2012. Heavy metals concentrations in fish from Sicily (Mediterranean Sea) and evaluation of possible health risks to consumers. *Bulletin of Environmental Contamination and Toxicology* 88:78–83. DOI:10.1007/s00128-011-0433-6.
- D'Anna G, Badalamenti F, Riggio S. 1999. Traditional and experimental floating fish aggregating devices in the Gulf of Castellammare (NW Sicily): Results from catches and visual observations. *Scientia Marina* 63:209–218. DOI:10.3989/scimar.1999.63n3-4.
- D'Ilio S, Mattei D, Blasi MF, Alimonti A, Bogianni S. 2011. The occurrence of chemical elements and POPs in loggerhead turtles (*Caretta caretta*): An overview. *Marine Pollution Bulletin* 62:1606–1615. DOI:10.1016/j.marpolbul.2011.05.022.
- DeNiro M, Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495–506. DOI:10.1016/0016-7037(78)90199-0.
- Dodd Jr. CK. 1988. Synopsis of the biological data on the loggerhead sea turtle *Caretta caretta* (Linnaeus 1758). U.S. Fish and Wildlife Service Biological Report 88:110.
- Favalli M, Karátson D, Mazzuoli R, Pareschi MT, Ventura G. 2005. Volcanic geomorphology and tectonics of the Aeolian archipelago (Southern Italy) based on integrated DEM data. *Bulletin of Volcanology* 68:157–170. DOI:10.1007/s00445-005-0429-3.
- France RL. 1995a. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnology and Oceanography* 40:1310–1313. DOI:10.4319/lo.1995.40.7.1310.
- France RL. 1995b. Carbon-13 enrichment in benthic compared to planktonic algae: Food web implications. *Marine Ecology Progress Series* 124:307–312. DOI:10.3354/meps124307.
- Fry B, Sherr EB. 1984. $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* 27:13–47.
- Godley B, Thompson D, Waldron S, Furness RW. 1998. The trophic status of marine turtles as determined by stable isotope analysis. *Marine Ecology Progress Series* 166:277–284. DOI:10.3354/meps166277.
- Godley BJ, Thompson DR, Furness RW. 1999. Do heavy metal concentrations pose a threat to marine turtles from the Mediterranean Sea? *Marine Pollution Bulletin* 38:497–502. DOI:10.1016/S0025-326X(98)00184-2.
- Goodman Hall A, Avens L, McNeill JB, Wallace B, Goshe LR. 2015. Inferring long-term foraging trends of individual juvenile loggerhead sea turtles using stable isotopes. *Marine Ecology Progress Series* 537:265–276. DOI:10.3354/meps11452.
- Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaentologia Electronica* 4:9.
- Hatase H, Sato K, Yamaguchi M, Takahashi K, Tsukamoto K. 2006. Individual variation in feeding habitat use by adult female green sea turtles (*Chelonia mydas*): Are they obligately neritic herbivores? *Oecologia* 149:52–64. DOI:10.1007/s00442-006-0431-2.
- Hatase H, Takai N, Matsuzawa Y, Sakamoto W, Omuta K, Goto K, Arai N, Fujiwara T. 2002. Size-related differences in feeding habitat use of adult female loggerhead turtles *Caretta caretta* around Japan determined by stable isotope analyses and satellite telemetry. *Marine Ecology Progress Series* 233:273–281. DOI:10.3354/meps233273.
- Hawkes LA, Broderick AC, Coyne MS, Godfrey MH, Lopez-Jurado L-F, Lopez-Suarez P, Godley BJ. 2006. Phenotypically linked dichotomy in sea turtle foraging requires multiple conservation approaches. *Current Biology* 16:990–995. DOI:10.1016/j.cub.2006.03.063.
- Hutchinson J, Simmonds M. 1991. A review of the effects of pollution on marine turtles. Greenpeace International Ecotoxicology Project:16.
- Jennings S, Reñones O, Morales-Nin B, Polunin N, Moranta J, Coll J. 1997. Spatial variation in the ^{15}N and ^{13}C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: Implications for the study of trophic pathways. *Marine Ecology Progress Series* 146:109–116. DOI:10.3354/meps146109.
- Kelly JF. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78:1–27. DOI:10.1139/z99-165.
- Laurent L, Casale P, Bradai MN, Godley BJ, Gerosa G, Broderick AC, Schroth W, Schierwater B, Levy AM, Freggi D. 1998. Molecular resolution of marine turtle stock composition in fishery bycatch: A case study in the Mediterranean. *Molecular Ecology* 7:1529–1542. DOI:10.1046/j.1365-294x.1998.00471.x.
- Laurent L, Lescure J, Excoffier L, Bowen B, Domingo M, Hadjichristophorou M, Kornaraki L, Trabuchet G. 1993. Genetic studies of relationship between Mediterranean and Atlantic populations of loggerhead turtle *Caretta caretta* with mitochondrial marker. *Comptes Rendus De l'Academie Des Sciences. Serie III, Sciences De La Vie* 316:1233–1239.
- Lepoint G, Nyssen F, Gobert S, Dauby P, Bouquegneau JM. 2000. Relative impact of a seagrass bed and its adjacent epilithic algal community in consumer diets. *Marine Biology* 136:513–518. DOI:10.1007/s002270050711.
- Lowe TE, Ryder JM, Carragher JF, Wells RMG. 1993. Flesh quality in snapper, *Parasauratus*, affected by capture stress. *Journal of Food Science* 58:770–773. DOI:10.1111/j.1365-2621.1993.tb09355.x.
- Luschi P, Casale P. 2014. Movement patterns of marine turtles in the Mediterranean Sea: A review. *Italian Journal of Zoology* 81:478–495. DOI:10.1080/11250003.2014.963714.
- Mattei D, Veschetti E, D'Ilio S, Blasi MF. 2015. Mapping elements distribution in carapace of *Caretta caretta*: A strategy for biomonitoring contamination in sea turtles? *Marine Pollution Bulletin* 98:341–348. DOI:10.1016/j.marpolbul.2015.06.001.
- McCauley SJ, Bjørndal KA. 1999. Conservation implications of dietary dilution from debris ingestion: Sublethal effects in post-hatchling loggerhead sea turtles. *Conservation Biology* 13:925–929. DOI:10.1046/j.1523-1739.1999.98264.x.
- McClellan CM, Braun-McNeill J, Avens L, Wallace B, Read AJ. 2010. Stable isotopes confirm a foraging dichotomy in juvenile loggerhead sea turtles. *Journal of Experimental Marine Biology and Ecology* 387:44–51. DOI:10.1016/j.jembe.2010.02.020.
- McClellan CM, Read AJ. 2007. Complexity and variation in loggerhead sea turtle life history. *Biology Letters* 3:592–594. DOI:10.1098/rsbl.2007.0355.
- McKinney RA, Oczkowski AJ, Prezioso J, Hyde KJW. 2010. Spatial variability of nitrogen isotope ratios of particulate material from Northwest Atlantic continental shelf waters. *Estuarine Coastal and Shelf Science* 89:287–293. DOI:10.1016/j.ecss.2010.08.004.
- Michener RH, Schell DM. 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener R,

- editors. Stable isotopes in ecology and environmental science. Oxford, England: Blackwell Scientific Publications. pp. 138–157.
- Mills CE. 2001. Jellyfish blooms: Are populations increasing globally in response to changing ocean conditions? *Hydrobiologia* 451:55–68. DOI:10.1023/A:1011888006302.
- Minagawa M, Wada E. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica Et Cosmochimica Acta* 48:1135–1140. DOI:10.1016/0016-7037(84)90204-7.
- Moore JW, Semmens BX. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters* 11:470–480. DOI:10.1111/j.1461-0248.2008.01163.x.
- Musick JA, Limpus CJ. 1997. Habitat utilization and migration in juvenile sea turtles. In: Lutz PL, Musick JA, editors. The biology of sea turtles. Boca Raton, FL: CRC Press. pp. 137–163.
- Nelms SE, Duncan EM, Broderick AC, Galloway TS, Godfrey MH, Hamann M, Lindeque PK, Godley BJ. 2015. Plastic and marine turtles: A review and call for research. *ICES Journal of Marine Science* 73:165–181. DOI:10.1093/icesjms/fsv165.
- Pajuelo M, Bjorndal K, Alfaro-Shigueto J, Seminoff J, Mangel J, Bolten A. 2010. Stable isotope variation in loggerhead turtles reveals Pacific-Atlantic oceanographic differences. *Marine Ecology Progress Series* 417:277–285. DOI:10.3354/meps08804.
- Pajuelo M, Bjorndal K, Reich K, Arendt M, Bolten A. 2012a. Distribution of foraging habitats of male loggerhead turtles (*Caretta caretta*) as revealed by stable isotopes and satellite telemetry. *Marine Biology* 159:1255–1267. DOI:10.1007/s00227-012-1906-9.
- Pajuelo M, Bjorndal KA, Reich KJ, Vander Zanden HB, Hawkes LA, Bolten AB. 2012b. Assignment of nesting loggerhead turtles to their foraging areas in the Northwest Atlantic using stable isotopes. *Ecosphere* 3:1–18. DOI:10.1890/ES12-00220.1.
- Payo-Payo A, Ruiz B, Cardona L, Borrell A. 2013. Effect of tissue decomposition on stable isotope signatures of striped dolphins *Stenella coeruleoalba* and loggerhead sea turtles *Caretta caretta*. *Aquatic Biology* 18:141–147. DOI:10.3354/ab00497.
- Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293–320. DOI:10.1146/annurev.es.18.110187.001453.
- Phillips DL, Gregg JW. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia* 127:171–179. DOI:10.1007/s004420000578.
- Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ. 2014. Best practices for use of stable isotope mixing models in food-web studies. *Canadian Journal of Zoology* 92:823–835. DOI:10.1139/cjz-2014-0127.
- Pinnegar JK, Polunin NVC. 1999. Differential fractionation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among fish tissues: Implications for the study of trophic interactions. *Functional Ecology* 13:225–231. DOI:10.1046/j.1365-2435.1999.00301.x.
- Pinnegar JK, Polunin NVC. 2000. Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia* 122:399–409. DOI:10.1007/s004420050046.
- Piovano S, Clusa M, Carreras C, Giacomina C, Pascual M, Cardona L. 2011. Different growth rates between loggerhead sea turtles (*Caretta caretta*) of Mediterranean and Atlantic origin in the Mediterranean Sea. *Marine Biology* 158:2577–2587. DOI:10.1007/s00227-011-1759-7.
- Polunin A, Morales-Nin B, Pawsey W, Cartes J, Pinnegar JK, Moranta J. 2001. Feeding relationships in Mediterranean bathyal assemblages elucidated by carbon and nitrogen stable-isotope data. *Marine Ecology Progress Series* 220:13–23. DOI:10.3354/meps220013.
- Ponsard S, Amlou M. 1999. Effects of several preservation methods on the isotopic content of *Drosophila* samples. *Comptes Rendus l'Académie Des Sciences - Ser III - Sciences De La Vie* 322:35–41.
- Post D. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* 83:703–718. DOI:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.
- Post D, Layman C, Arrington A, Takimoto G, Quattrochi J, Montaña C. 2007. Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152:179–189. DOI:10.1007/s00442-006-0630-x.
- Reich KJ, Bjorndal KA, Bolten AB. 2007. The 'lost years' of green turtles: Using stable isotopes to study cryptic life stages. *Biology Letters* 3:712–714. DOI:10.1098/rsbl.2007.0394.
- Reich KJ, Bjorndal KA, Frick MG, Witherington BE, Johnson C, Bolten AB. 2010. Polymodal foraging in adult female loggerheads (*Caretta caretta*). *Marine Biology* 157:113–121. DOI:10.1007/s00227-009-1300-4.
- Reich KJ, Bjorndal KA, Martínez Del RC. 2008. Effects of growth and tissue type on the kinetics of ^{13}C and ^{15}N incorporation in a rapidly growing ectotherm. *Oecologia* 155:651–663. DOI:10.1007/s00442-007-0949-y.
- Revelles M, Cardona L, Aguilar A, Borrell A, Fernández G, San Félix M. 2007a. Stable C and N isotope concentration in several tissues of the loggerhead sea turtle *Caretta caretta* from the western Mediterranean and dietary implications. *Scientia Marina* 71:87–93. DOI:10.3989/scimar.2007.71n1.
- Revelles M, Cardona L, Aguilar A, Fernández G. 2007b. The diet of pelagic loggerhead sea turtles (*Caretta caretta*) off the Balearic archipelago (western Mediterranean): Relevance of long-line baits. *Journal of the Marine Biological Association of the UK* 87:805–813. DOI:10.1017/S0025315407054707.
- Rumolo P, Basilone G, Fanelli E, Barra M, Calabrò M, Genovese S, Gherardia S, Ferreri R, Mazzola S, Bonanno A. 2017. Linking spatial distribution and feeding behavior of Atlantic horse mackerel (*Trachurus trachurus*) in the Strait of Sicily (Central Mediterranean Sea). *Journal of Sea Research* 121:47–58. DOI:10.1016/j.seares.2017.01.002.
- Rumolo P, Bonanno A, Barra M, Fanelli E, Calabrò M, Genovese S, Ferreri R, Mazzola S, Basilone G. 2016. Spatial variations in feeding habits and trophic levels of two small pelagic fish species in the central Mediterranean Sea. *Marine Environmental Research* 115:65–77. DOI:10.1016/j.marenvres.2016.02.004.
- Sarà G, Sarà R. 2007. Feeding habits and trophic levels of bluefin tuna *Thunnus thynnus* of different size classes in the Mediterranean Sea. *Journal of Applied Ichthyology* 23:122–127. DOI:10.1111/jai.2007.23.issue-2.
- Seminoff J, Jones T, Eguchi T. 2006. Stable isotope discrimination ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) between soft tissues of the green sea turtle *Chelonia mydas* and its diet. *Marine Ecology Progress Series* 308:271–278. DOI:10.3354/meps308271.
- Semmens BX, Ward EJ, Moore JW, Darimont CT. 2009. Quantifying inter- and intra-population niche variability using hierarchical Bayesian stable isotope mixing models. *PLoS ONE* 4:e6187. DOI:10.1371/journal.pone.0006187.
- Seney EE, Musick JA. 2007. Historical diet analysis of loggerhead sea turtles (*Caretta caretta*) in Virginia. *Copeia* 2007:478–489. DOI:10.1643/0045-8511(2007)7[478:HDAOLS]2.0.CO;2.
- Snover ML, Hohn AA, Crowder LB, Macko SA. 2010. Combining stable isotopes and skeletal growth marks to detect habitat shifts in

- juvenile loggerhead sea turtles *Caretta caretta*. *Endangered Species Research* 13:25–31. DOI:10.3354/esr00311.
- Stock BC, Semmens BX. 2013. MixSIAR GUI user manual, version 1.0. Available: <http://conserver.iugo-cafe.org/user/brice.semmens/MixSIAR>. Accessed Oct 2014.
- Sweeting CJ, Polunin NVC, Jennings S. 2004. Tissue and fixative dependent shifts of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in preserved ecological material. *Rapid Communication Mass Spectrometry* 18:2587–2592. DOI:10.1002/rcm.1661.
- Sweeting CJ, Polunin NVC, Jennings S. 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry* 20:595–601. DOI:10.1002/(ISSN)1097-0231.
- Thomas SM, Crowther TW. 2015. Predicting rates of isotopic turnover across the animal kingdom: A synthesis of existing data. *Journal of Animal Ecology* 84:861–870. DOI:10.1111/1365-2656.12326.
- Tomás J, Aznar FJ, Raga JA. 2001. Feeding ecology of the loggerhead turtle *Caretta caretta* in the western Mediterranean. *Journal of Zoology* 255:525–532. DOI:10.1017/S0952836901001613.
- Tomás J, Guitart R, Mateo R, Raga JA. 2002. Marine debris ingestion in loggerhead sea turtles, *Caretta caretta*, from the Western Mediterranean. *Marine Pollution Bulletin* 44:211–216. DOI:10.1016/S0025-326X(01)00236-3.
- Vander Zanden HB, Bjorndal KA, Reich KJ, Bolten AB. 2010. Individual specialists in a generalist population: Results from a long-term stable isotope series. *Biology Letters* 6:711–714. DOI:10.1098/rsbl.2010.0124.
- Vizzini S, Sarà G, Michener RH, Mazzola A. 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. *Acta Oecologica* 23:277–285. DOI:10.1016/S1146-609X(02)01156-6.
- Wallace BP, Avens L, Braun-McNeill J, McClellan CM. 2009. The diet composition of immature loggerheads: Insights on trophic niche, growth rates, and fisheries interactions. *Journal of Experimental Marine Biology and Ecology* 373:50–57. DOI:10.1016/j.jembe.2009.03.006.
- Zanden M, Rasmussen J. 1999. Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology* 80:1395–1404. DOI:10.1890/0012-9658(1999)080[1395:PCCANA]2.0.CO;2.
- Zbinden J, Bearhop S, Bradshaw P, Gill B, Margaritoulis D, Newton J, Godley B. 2011. Migratory dichotomy and associated phenotypic variation in marine turtles revealed by satellite tracking and stable isotope analysis. *Marine Ecology Progress Series* 421:291–302. DOI:10.3354/meps08871.