

Ontogenetic and seasonal changes in the feeding habits and trophic levels of two small pelagic fish species

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ABSTRACT: We investigated ontogenetic and seasonal changes in the feeding ecology of 2 small pelagic fish species, the European anchovy *Engraulis encrasicolus* and the European pilchard *Sardina pilchardus* in the Gulf of Lions (NW Mediterranean). By analysing the stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, we determined the seasonal variation in the food sources and in the trophic level of these species, and we examined dietary shifts during development. The results of these investigations provided estimates of the diets of both species. We compared the values observed during different seasons (summer, autumn and winter) and at different developmental stages (late-larvae, juveniles and adults) for both species, together with the values of potential groups of prey (microplankton, cladocerans, copepods and appendicularians). Late-larvae preferred to feed on microplankton, although differences in the diet appeared after metamorphosis. Cladocerans were usually the preferred prey when available (summer), and appendicularians were the preferred prey in autumn. During the winter, the diets seemed to be more heterogeneous. Different feeding behaviours between the late-larvae of the 2 species were the most likely reason for the slightly different trophic levels found in the present study. This research demonstrates that studies with stable isotopes can furnish an alternative and/or complementary method for determining the diet of small pelagic fishes over extended periods and provides comprehensive knowledge of the functioning of the pelagic ecosystem.

KEY WORDS: *Engraulis encrasicolus* · *Sardina pilchardus* · Stable isotopes · NW Mediterranean · Trophic ecology · Microplankton · Copepods · Appendicularians · Cladocerans

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INTRODUCTION

An understanding of the primary ecological processes in marine ecosystems, such as variation in food sources, trophic transfer through the food web and nutrient cycling, is fundamental for relating ecosystem functioning to management. In this context, the description of the trophic ecology of marine organisms is vital to determine the intrinsic factors that control their distribution, abundance, and, ultimately, their function within the ecosystem. Fishes have developed a wide variety of feeding-related structures (e.g. gill rakers, pyloric caeca, or teeth) and behaviours (e.g. filter feeding vs. particulate

feeding) that may undergo modifications through ontogeny or season (Gerking 1994), with diverse ecological implications. Many studies have addressed the trophic roles of both top and bottom species of the food web, i.e. marine predators and plankton communities, respectively (Hunter & Price 1992, Rice 1995, Pace et al. 1999), and, among these studies, some have focused on a group that is particularly important ecologically: the small pelagic fishes (Bakun 1996, Cury et al. 2000, Palomera et al. 2007).

Small pelagic fish are essential elements of marine ecosystems owing to their significant biomass at intermediate levels of the food web (Cury et al. 2000, Palomera et al. 2007). Pelagic fish play a significant

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role in connecting the lower and upper trophic levels (Cury et al. 2000). Their massive populations, which exert a huge pressure on zooplankton and, at the same time, are the main food for many species, may vary greatly in size under intensive exploitation or following changes in productivity. Therefore, fluctuations in small pelagic populations owing to fishing or natural factors modify the structure and functioning of the marine ecosystem (e.g. Cury et al. 2000, Shannon et al. 2000, Daskalov 2002). The significant abundance and success of pelagic fish in upwelling areas have been attributed to the flexibility of their feeding behaviour (van der Lingen et al. 2009). Pelagic fish have also been identified as important groups in many ecosystems (Libralato et al. 2006), e.g. in upwelling areas, where they exhibit a 'wasp-waist' flow control (Cury et al. 2000). Therefore, the interactions among different populations of small pelagic fishes can also be strongly influenced by regime climatic shifts and can have strong impacts on fisheries (e.g. Klyashtorin 1998, Rodríguez-Sánchez et al. 2002).

Low trophic-level species are directly influenced by the remarkable environmental differences between seasons (Calbet et al. 2001). The populations of small pelagic fish may be affected by any environmental change that influences the plankton community, which is the basis of the diet of these fishes. In fact, the seasonal unevenness of oceanographic parameters (i.e. salinity, fluorescence and, most importantly, temperature) and of river runoff has been shown to have important effects on the biology and viability of these fish populations (Lloret et al. 2001, 2004). The early life stages (i.e. larvae and juveniles) are especially sensitive to such effects (Govoni 2005, Ruiz et al. 2006, Costalago et al. 2011). Several species of small pelagic fish co-occur in the Mediterranean Sea. Unquestionably, the European anchovy *Engraulis encrasicolus* and the European sardine *Sardina pilchardus* are the most relevant of these species in terms of both biomass and fishery catches (Palomera et al. 2007). These 2 small pelagic fish are also key species at mid-trophic levels in the Mediterranean Sea (Coll & Libralato 2012). *E. encrasicolus* was found to be a key species in the North Adriatic (Coll et al. 2007, Barausse et al. 2009) and in the Aegean Sea (Tsagarakis et al. 2010). On the contrary, in the NW Mediterranean, *S. pilchardus* is among the leading keystone species and is also considered to exhibit 'wasp-waist' trophic control (Coll et al. 2006, Palomera et al. 2007, Navarro et al. 2011). Small pelagic fish are the most vulnerable to any environmental shift among fish species (Coll et al. 2008). At

the same time, they are the main constituents of the diet of several pelagic, demersal and apical species (e.g. Coll et al. 2006, Palomera et al. 2007, Navarro et al. 2009).

A significant number of studies on the ecology of anchovy and sardine in the Mediterranean Sea have been conducted (see reviews by Palomera et al. 2007, Morello & Arneri 2009), but relatively few of these studies address dietary composition. Previous papers that included dietary information focussed on larval stages of *Engraulis encrasicolus* and *Sardina pilchardus* (Conway et al. 1998, Tudela et al. 2002, Catalán et al. 2010, Morote et al. 2010), the adults of *E. encrasicolus* (Tudela & Palomera 1997, Plounevez & Champalbert 2000), or the juveniles and adults of *E. encrasicolus* (Borme et al. 2009). However, none of the previous works, that, in addition, have usually been limited in temporal resolution, included data about sardines, except that of Morote et al. (2010), and they studied only larvae <1.6 cm in total length. A more comprehensive understanding of the trophic dynamics of assemblages of small pelagic fish from a seasonal and ontogenetic perspective is therefore essential to highlight the important role of these populations within the marine ecosystem. The present study intends to provide this necessary knowledge more comprehensively by gathering and analyzing data from different seasons and life stages of these 2 pelagic species for the first time in the Mediterranean.

All previous studies on the trophic ecology of these small pelagic fish species have been based on the direct analysis of stomach contents (Tudela & Palomera 1997, Conway et al. 1998, Plounevez & Champalbert 2000, Borme et al. 2009, Morote et al. 2010). This approach involves implicit methodological errors because it cannot accurately quantify the importance of prey items that are readily digested and because it does not identify the prey items that are actually assimilated following ingestion. The stable isotope approach can augment conventional means of dietary analysis because stable isotopes reflect time-integrated dietary records and present a perspective on trophic dynamics that involves a more substantial time period than the analysis of stomach contents can provide (Polunin & Pinnegar 2008). Therefore, stable isotope analysis yields information that cannot always be obtained from direct observation and can support hypotheses about the developmental changes in the feeding strategies of a species and about species interactions because these hypotheses are based on data about assimilated food rather than ingested food. In fish, the stable isotope values of muscular tissue integrate dietary information

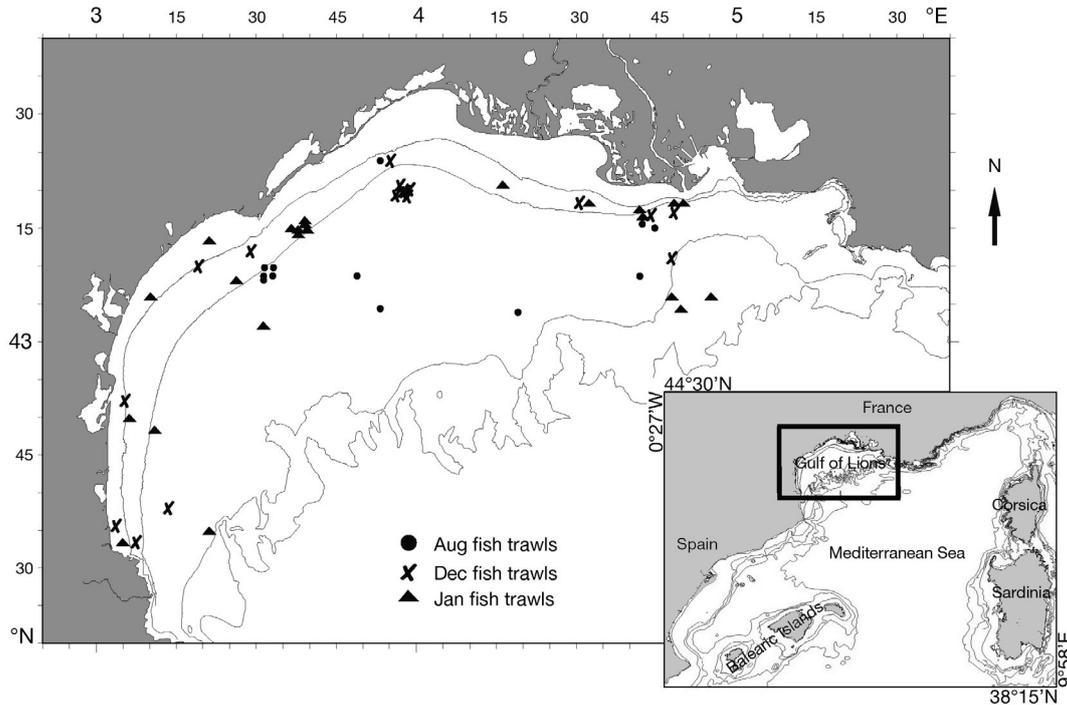


Fig. 1. Study area (Gulf of Lions, NW Mediterranean), indicating fish and plankton sampling locations. The bathymetric lines correspond to depths of 20, 50, 100 and 200 m

between 40 and 80 d prior to sampling (Bode et al. 2007, Buchheister & Latour 2010). Stable isotopes of nitrogen ($\delta^{15}\text{N}$) are indicators of trophic positions because consumers are predictably enriched in $\delta^{15}\text{N}$ relative to their food (Post 2002, Vanderklift & Ponsard 2003). Stable carbon isotope values ($\delta^{13}\text{C}$) give information on primary production and are useful for tracing the origin of the prey consumed (Vander Zanden et al. 1999, Pinnegar & Polunin 2000). Furthermore, by combining stable isotope values for consumers and their prey, powerful isotopic mixing models can be applied to obtain estimates of the relative contribution of each potential prey item to the diet of the consumer (e.g. stable isotope analysis in R [SIAR] isotopic mixing model; Parnell et al. 2010). These models add useful information to investigations of food selectivity and can be used to complement data from stomach content analysis (Peterson 1999, Lin et al. 2007, Tripp-Valdez & Arreguín-Sánchez 2009).

Flaherty & Ben-David (2010) pointed out that there are limitations in the use of isotopic mixing models because of the important differences in isotope values that can be found if there is spatial heterogeneity, so that habitat-derived variation in consumers' isotopes would be mistaken as diet variation in resource isotope values. Since populations of *Engraulis encrasicolus* and *Sardina pilchardus* in the Gulf of Lions are widespread and homogeneously distributed over

the whole of the continental shelf and therefore feeding on the same available resources, we could assume that this limitation would not be of great influence in our case.

The present study uses $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as indicators of the trophic relationships between *Engraulis encrasicolus* and *Sardina pilchardus* for different age groups (late-larvae, juveniles and adults) during different seasons (summer, autumn and winter) and therefore under different environmental conditions. The study investigates seasonal and ontogenetic changes in the food sources and trophic levels of these species, and it examines possible dietary shifts during development. These analyses yield estimates of the diet composition of both species. All of these findings are important because they can supply the knowledge needed to fill current information gaps existing in both experimental and direct observational studies and allow improved management of fish stocks.

MATERIALS AND METHODS

Study area and sample collection

The present study was conducted in the Gulf of Lions (Fig. 1), one of the most productive areas of the NW Mediterranean (Salat 1996). In terms of biomass,

it is also the most important area of the Mediterranean for small pelagic fish species (Barangé et al. 2009). During 3 different oceanographic cruises on board the RV 'L'Europe' (IFREMER, France), we collected late-larvae, juveniles and adults of *Engraulis encrasicolus* and *Sardina pilchardus*. The standard length ranges considered to classify the individuals within these groups were: for late-larvae 2.0–3.5 cm for *E. encrasicolus* and 2.5–4.0 cm for *S. pilchardus*, for juveniles 3.6–8.5 cm for *E. encrasicolus* and 4.1–10.5 cm for *S. pilchardus* and for adults 8.6–12 cm for *E. encrasicolus* and 10.6–14 cm for *S. pilchardus*. All individuals were considered as adults when they reached the minimum length at first maturity observed during the cruises. The first cruise was conducted during autumn (12 to 21 December 2007), the second cruise was conducted during summer (21 to 29 July 2008) and the third was conducted during winter (11 to 27 January 2009). All specimens were caught with a pelagic trawling net equipped with a small-mesh codend (mesh length: 5 mm; ISO 1107) and towed at an average speed of 3.6 knots over a 30 to 40 min period. The samples were immediately frozen (-20°C) after sorting on board.

During each season, plankton samples were collected at the same sites where the pelagic trawls were made. Sampling was done at 16 plankton stations in summer, 15 in autumn and 13 in winter using a standard WP2 net with a mesh size of 200 μm and a scaled-down WP2 net with a mesh size of 53 μm . The WP2 net sample was sieved through a 3000 μm plankton mesh to obtain the 200 to 3000 μm mesozooplankton fraction and the scaled-down WP2 net was sieved through a 200 μm plankton mesh to obtain the 53 to 200 μm microplankton fraction. All plankton samples were split with a Motoda plankton splitter (Motoda 1959). One-half of each sample was preserved in buffered 4% formaldehyde-seawater solution for subsequent qualitative analyses of plankton community composition, whereas the other half was frozen (-20°C) on board for biomass measurements and stable isotopic determination. Qualitative analysis of plankton was performed in the laboratory, and individuals were identified to the lowest taxonomical level possible under a stereomicroscope (Leica MZ12) with a magnification of up to 100 \times . The mesozooplankton samples were analysed in aliquots representing about 10% of the sample and repeated until counting at least 400 copepods in each; additional subsamples were also taken for any other abundant organism (i.e. cladocerans during summer). Microplankton samples were subsampled differently: 1 to 2% of the original volume was analysed to estimate

the presence of nauplii, dinoflagellates, ciliates and diatoms; small copepods (mainly copepodites) were analysed in volumes sufficient to count at least 400 individuals. The number of individuals of each identified taxon and abundances (ind. m^{-3}) were calculated.

Stable isotope analysis

A portion of dorsal muscle (without skin) was extracted from each individual (late-larvae, juveniles and adults). Muscle has been defined as the most appropriate tissue to analyse stable isotope in fish (Sweeting et al. 2005). Plankton samples from each season were defrosted in the laboratory, pooled together and sorted into different potential prey groups (microplankton, copepods and appendicularians in all the seasons, and also cladocerans in summer) which were selected according to previous studies of stomach contents (Morote et al. 2010 for larvae, D. Costalago unpubl. data for juveniles, Plounevez & Champalbert 2000 for adults).

All fish and plankton samples were freeze-dried, powdered, and 0.9 to 1.0 mg of each sample was packed into tin capsules. The samples were then oxidised with CuO and $\text{CO}_3\text{O}_4/\text{Ag}$ at approximately 900°C in a Flash EA 1112 Elemental Analyser coupled with a pyrolyser TC-EA and a gas bench through a ConFlo III Finnigan MAT interface. NO_x was reduced with Cu at 680°C . The combustion products N_2 and CO_2 were introduced into a Delta C Finnigan MAT mass spectrometer through an MgClO_4 drying column. The isotope ratio mass spectrometry facility at the Serveis Científic-Tècnics of the University of Barcelona (Spain) applies international standards, generally run for every 12 samples: IAEA CH7 (87% of C), IAEA CH6 (42% of C) and USGS 24 (100% of C) for $\delta^{13}\text{C}$ and IAEA N1 and IAEA N2 (with 21% of N) and IAEA NO_3 (13.8% of N) for $\delta^{15}\text{N}$.

The $\delta^{13}\text{C}$ values were corrected for the effect of lipids both in fish and prey samples following Logan et al. (2008). This procedure reduces the time and uncertainty associated with lipid extraction procedures, and it improves the estimates of dietary proportions derived from stable-isotope mixing models (Phillips & Gregg 2001, Logan et al. 2008).

Isotopic mixing model

To estimate the diet composition at each age (late-larvae, juveniles and adults) during each season (summer, autumn and winter) we applied a Bayesian

model in SIAR 4.1.1 (Stable Isotope Analysis in R 2.12.2). This model runs under the free software R (R Development Core Team 2009). The model allows the inclusion of sources of uncertainty. In particular, the variability in the isotope signatures (mean and standard deviation) of prey species can be incorporated into the model (Parnell et al. 2010). SIAR uses Markov-chain Monte Carlo modelling, takes data on animal stable isotopes and fits a Bayesian model of the diet habits based on a Gaussian likelihood function with a Dirichlet prior mixture distribution for the mean.

The model also assumes that each target value (i.e. the stable isotope data for each individual) comes from a Gaussian distribution with an unknown mean and standard deviation. The structure of the mean is a weighted combination of the food sources' isotopic values. The standard deviation depends on the uncertainty in the fractionation corrections and the natural variability among target individuals within a defined group. We used the isotopic discrimination of $1.01 \pm 0.17\text{‰}$ for C and $3.56 \pm 0.17\text{‰}$ for N as the average of discrimination factors estimated for muscle analyses of different marine fish species provided in Caut et al. (2009).

Trophic level

To estimate the trophic levels (TL) of the different individuals we used the equation:

$$TL_{\text{consumer}} = TL_{\text{basal}} + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{prey}}) / \Delta\delta^{15}\text{N} \quad (1)$$

TL_{consumer} refers to the mean TL of each individual fish. $\delta^{15}\text{N}_{\text{prey}}$ and $\delta^{15}\text{N}_{\text{consumer}}$ were, respectively, the isotopic values of microplankton and individual fish obtained in the present study (in each season). We applied a basal trophic level (TL_{basal}) of 1.5, assuming that the microplankton (mostly composed by phytoplankton) showed a trophic level between 1 of primary producers and 2 of micro- and mesozooplankton (Coll et al. 2006). The values of the isotopic discrimination factor for N ($\Delta\delta^{15}\text{N}$) were the same used for the isotopic mixing model (estimated from Caut et al. 2009).

Selectivity index

The output data of the SIAR model, together with the relative composition of the functional groups of plankton in the environment, were used to estimate

the Ivlev diet selectivity index (Ivlev 1961, Krebs 1989) for each case analyzed. The value of the index was calculated with the equation:

$$(r_i - p_i) / (r_i + p_i) \quad (2)$$

where r_i is the proportion of prey item i calculated from the SIAR model and p_i is the proportion of prey item i available from the marine environment.

Statistical analysis

ANOVA tests were used to examine the differences in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among species and ages in each season (summer, autumn and winter). Post hoc comparisons for observed means were performed with a Tukey test. The assumptions of ANOVA were checked with a Kolmogorov-Smirnov test for normality and a Levene test for homogeneity of variances. All analyses were performed with SPSS v.19 statistical software. A significance level of $p < 0.05$ was used for all tests unless otherwise stated.

RESULTS

Plankton composition

The microplankton samples were composed primarily of phytoplankton (mainly diatoms; to a lesser extent, dinoflagellates, such as *Ceratium* spp. and *Peridinium* spp.; and tintinnids), together with high numbers of copepod nauplii during the summer and winter and small copepods, especially *Oncaea* spp., throughout the year and, during the winter, *Paracalanus parvus*. Mesozooplankton was dominated by copepods (mainly calanoids). The plankton community also included a relatively less important number of appendicularians during the autumn and winter (Fig. 2). Cladocerans occurred during summer but not during other seasons (Fig. 2).

Isotopic differences

Sardina pilchardus showed lower $\delta^{15}\text{N}$ values in summer and the highest $\delta^{15}\text{N}$ values in autumn, except for late-larvae (winter), which had significantly lower $\delta^{15}\text{N}$ values than in any other age group and season (Table 1, Fig. 3). Regarding *S. pilchardus* $\delta^{13}\text{C}$ values (juveniles and adults), there were no significant differences ($p = 0.25$) between autumn and winter seasons (Table 2), while summer $\delta^{13}\text{C}$ val-

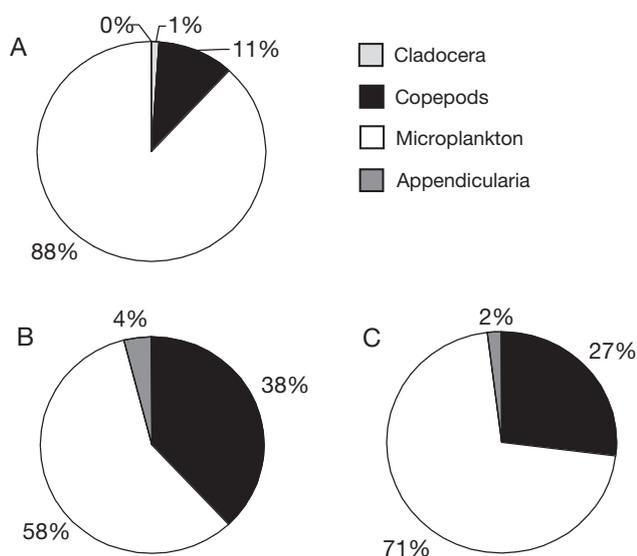


Fig. 2. Composition of plankton community in the Gulf of Lions during (A) summer, (B) autumn and (C) winter showing proportions of the 4 dietary functional groups defined in the present study

ues were statistically different from the other 2 seasons (Tables 1 & 2, Fig. 3). *S. pilchardus* late-larvae (winter) also showed significantly lower values of $\delta^{13}\text{C}$ compared to adults and juveniles (Table 2). *S. pilchardus* values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ did not differ in autumn between adults and juveniles ($p = 0.870$ for $\delta^{15}\text{N}$, $p = 0.213$ for $\delta^{13}\text{C}$; Table 1). For both *S. pilchardus* age groups (juveniles and adults) analysed together, $\delta^{13}\text{C}$ appeared to differ between the summer and other seasons, and $\delta^{15}\text{N}$ differed between winter and the other seasons (Table 1, Fig. 3).

Engraulis encrasicolus $\delta^{15}\text{N}$ isotopic values in both autumn and winter displayed marked differences between adults and juveniles (Table 1, Fig. 3). *E. encrasicolus* late-larvae (summer) had significantly higher values of $\delta^{15}\text{N}$ than juveniles (Table 1, Fig. 3). No differences in $\delta^{15}\text{N}$ values were observed among seasons for adult *E. encrasicolus*, whereas $\delta^{15}\text{N}$ values were statistically different for juveniles during autumn than in other seasons. When adults and juveniles were considered together, significant differences in $\delta^{15}\text{N}$ values were found among the 3 periods (Table 1, Fig. 3). *E. encrasicolus* $\delta^{13}\text{C}$ values exhibited substantial

differences during the summer for both juveniles and adults. *E. encrasicolus* late-larvae (summer) had higher $\delta^{13}\text{C}$ values than adults.

The comparisons between the 2 species showed that, during the summer, the $\delta^{15}\text{N}$ values of *Sardina pilchardus* and *Engraulis encrasicolus* adults differed significantly. During the autumn, only differences between adults' $\delta^{15}\text{N}$ values were significant between species. During the winter, however, both the adults and juveniles exhibited significant species differences in $\delta^{15}\text{N}$ values. The $\delta^{13}\text{C}$ values of the juveniles also differed between the species in winter and summer (Tables 1 & 2, Fig. 3). A comparison of the late-larvae (*E. encrasicolus* in summer and *S. pilchardus* in winter) found statistically significant differences in the values of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Table 2).

Concerning types of prey, appendicularians showed higher $\delta^{15}\text{N}$ values than other types in all seasons (Table 3, Fig. 3). Excluding summer, microplankton presented the lowest $\delta^{15}\text{N}$ values. The $\delta^{13}\text{C}$ values are generally variable between seasons (Fig. 3).

Table 1. *Engraulis encrasicolus*, *Sardina pilchardus*. Sample size, mean \pm SD of standard length (SL), stable isotope values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) and trophic level (TL) of different age groups of *E. encrasicolus* and *S. pilchardus* (adult, juvenile and late-larva) during summer, autumn and winter in the Gulf of Lions

	n	SL (cm)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	TL
<i>E. encrasicolus</i>					
Summer					
Adult	15	11.57 \pm 0.44	-19.56 \pm 0.16	8.23 \pm 0.34	2.42 \pm 0.09
Juvenile	15	6.37 \pm 0.85	-18.26 \pm 0.41	7.78 \pm 0.41	2.27 \pm 0.12
Late-larva	15	2.15 \pm 0.16	-18.38 \pm 0.11	8.14 \pm 0.32	2.39 \pm 0.09
Autumn					
Adult	14	10.58 \pm 0.18	-17.83 \pm 0.32	8.16 \pm 0.48	2.90 \pm 0.13
Juvenile	15	8.11 \pm 0.49	-17.67 \pm 0.29	8.53 \pm 0.45	3.05 \pm 0.12
Winter					
Adult	7	8.80 \pm 0.23	-17.42 \pm 0.22	8.07 \pm 0.33	2.63 \pm 0.09
Juvenile	15	7.57 \pm 0.17	-17.38 \pm 0.24	7.54 \pm 0.53	2.45 \pm 0.15
<i>S. pilchardus</i>					
Summer					
Adult	17	13.62 \pm 0.43	-19.32 \pm 1.16	8.52 \pm 0.43	2.53 \pm 0.12
Juvenile	15	7.02 \pm 0.20	-17.51 \pm 0.21	7.97 \pm 0.29	2.33 \pm 0.08
Autumn					
Adult	15	12.91 \pm 0.87	-17.68 \pm 0.71	8.91 \pm 0.48	3.22 \pm 0.13
Juvenile	15	9.11 \pm 0.93	-17.97 \pm 0.52	8.95 \pm 0.84	3.24 \pm 0.23
Winter					
Adult	15	11.35 \pm 0.91	-17.64 \pm 0.37	8.69 \pm 0.41	2.87 \pm 0.12
Juvenile	14	9.74 \pm 0.52	-17.77 \pm 0.41	8.88 \pm 0.53	2.95 \pm 0.14
Late-larva	15	3.12 \pm 0.11	-18.10 \pm 0.21	6.74 \pm 0.42	2.19 \pm 0.12

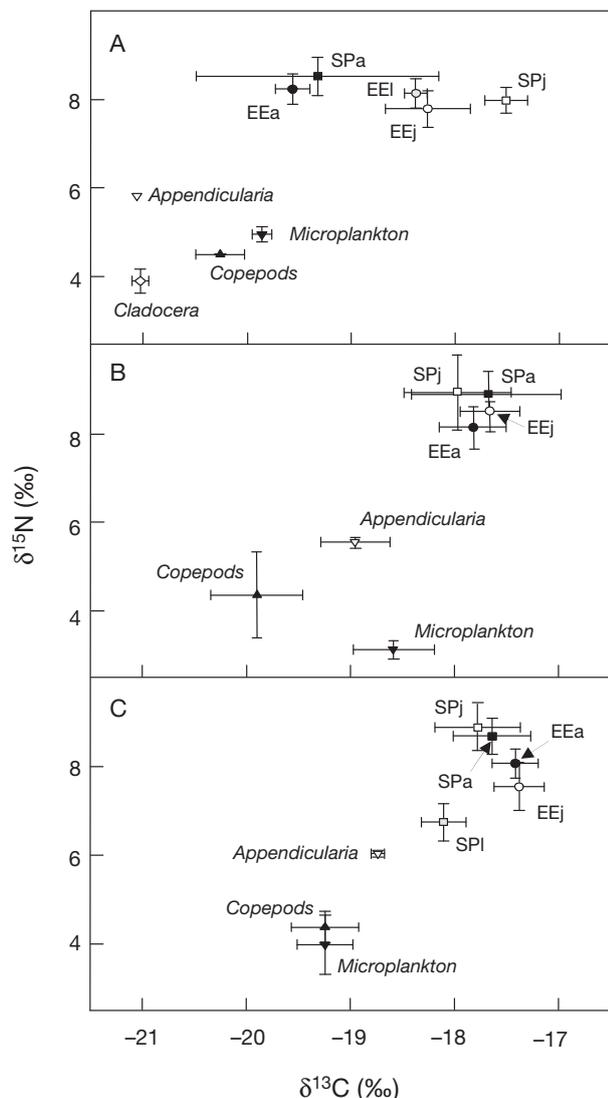


Fig. 3. *Engraulis encrasicolus*, *Sardina pilchardus*. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (mean \pm SD) of *E. encrasicolus* late-larvae (EEI), juveniles (EEj) and adults (EEa), and *S. pilchardus* late-larvae (SPi), juveniles (SPj) and adults (SPa) during (A) summer, (B) autumn and (C) winter. Reference values for the main prey groups (see Fig. 2) in each season are also given

Dietary differences

The diets estimated with the SIAR model (isotopic values in Tables 1 & 3) indicated that during the summer, *Engraulis encrasicolus* adults fed on cladocerans (37.8%) and appendicularians (24.7%), whereas juveniles fed primarily on cladocerans (33.8%) and copepods (35.5%). The juveniles also fed on microplankton (27.1%). The late-larvae fed primarily on microplankton (50.2%) and also on copepods

(35%) (Fig. 4). During the summer, *Sardina pilchardus* adults had a heterogeneous diet, with appendicularians (29.2%) as the main prey. The diet of *S. pilchardus* juveniles was based on cladocerans

Table 2. Summary of the ANOVA results for inter-seasonal (summer, autumn and winter) variation in stable isotopes over species and age of *Engraulis encrasicolus* and *Sardina pilchardus* in the Gulf of Lions. Abbreviations for age combinations in Tukey post hoc test summaries are—L: late-larvae; J: juvenile; A: adults; pairs of means differing significantly ($p = 0.05$) by Tukey test are linked with an 'x'

	Effect	F	df	p	Post hoc
Summer					
$\delta^{15}\text{N}$	Species	6.55	1, 75	<0.001	
	Age	14.83	1, 75	<0.001	AxL
	Species \times Age	0.25	2, 75	0.62	
$\delta^{13}\text{C}$	Species	10.87	1, 75	0.002	
	Age	57.54	2, 75	<0.001	AxL, JxL, A
	Species \times Age	2.89	1, 75	0.11	
Autumn					
$\delta^{15}\text{N}$	Species	14.71	1, 58	<0.001	
	Age	1.85	1, 58	0.18	
	Species \times Age	1.19	1, 58	0.28	
$\delta^{13}\text{C}$	Species	0.38	1, 58	0.54	
	Age	0.27	1, 58	0.61	
	Species \times Age	2.98	1, 58	0.89	
Winter					
$\delta^{15}\text{N}$	Species	51.58	1, 65	<0.001	
	Age	96.81	2, 65	<0.001	LxJ, A
	Species \times Age	7.03	1, 65	0.07	
$\delta^{13}\text{C}$	Species	11.45	1, 65	<0.001	
	Age	8.29	2, 65	<0.001	LxJ, A
	Species \times Age	0.93	1, 65	0.34	

Table 3. Sample size (n), mean \pm SD of stable isotope values ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of prey groups during summer, autumn and winter in the Gulf of Lions

	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Summer			
Cladocera	3	-21.03 \pm 0.08	3.90 \pm 0.27
Copepods	3	-20.26 \pm 0.23	4.50 \pm 0.01
Microplankton	3	-19.85 \pm 0.09	4.95 \pm 0.17
Appendicularia	1	-21.06	5.82
Autumn			
Copepods	3	-19.90 \pm 0.44	4.38 \pm 0.97
Microplankton	4	-18.59 \pm 0.38	3.13 \pm 0.20
Appendicularia	2	-18.96 \pm 0.33	5.55 \pm 0.12
Winter			
Copepods	3	-19.24 \pm 0.32	4.37 \pm 0.36
Microplankton	5	-19.24 \pm 0.27	3.98 \pm 0.67
Appendicularia	3	-18.74 \pm 0.06	6.03 \pm 0.08

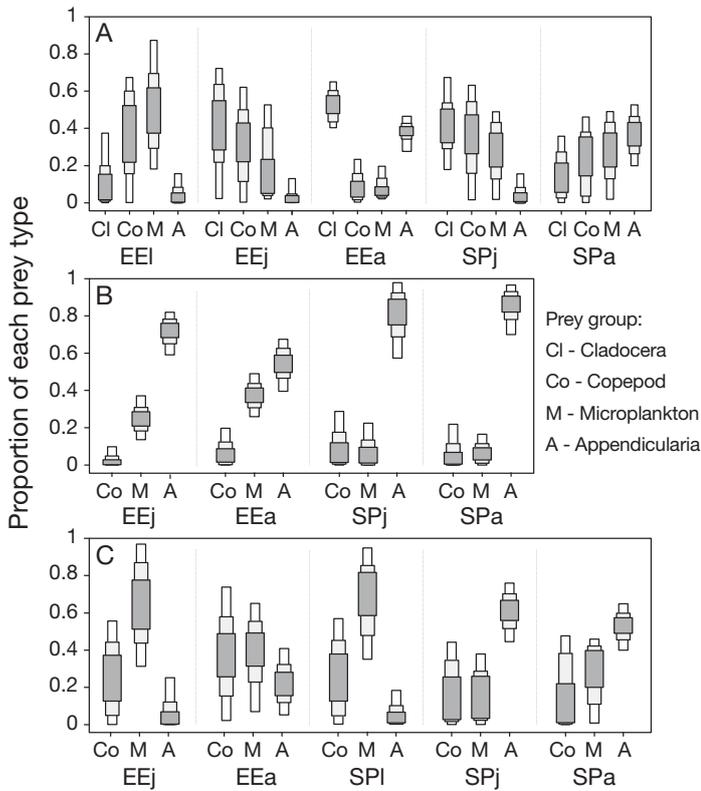


Fig. 4. *Engraulis encrasicolus*, *Sardina pilchardus*. Results of stable isotope analysis in R (95, 75 and 50% credibility intervals) showing estimated prey contributions to the diet of *E. encrasicolus* late-larvae (EEI), juveniles (EEj) and adults (EEa), and *S. pilchardus* late-larvae (SPI), juveniles (SPj) and adults (SPa) from the Gulf of Lions (northwestern Mediterranean) during (A) summer, (B) autumn and (C) winter

(36.8%), copepods (33.5%) and microplankton (25.1%) (Fig. 4).

During the autumn, the diets of adults were similar to the diets of juveniles for both *Sardina pilchardus* and *Engraulis encrasicolus*. The main prey types for *S. pilchardus* were appendicularians (88% in adults and 82.8% in juveniles). Appendicularians were also the primary prey item for *E. encrasicolus* (59.4% in adults and 75.4% in juveniles) (Fig. 4).

Sardina pilchardus adults and juveniles in winter also showed a diet based on appendicularians (49% in adults and 58.1% in juveniles), but the primary prey item of late-larvae was microplankton (56.1%). In the same season *Engraulis encrasicolus* juveniles fed primarily on microplankton (69%), whereas adult *E. encrasicolus* fed primarily on copepods and appendicularians in similar amounts (40.7 and 40.8%, respectively) (Fig. 4).

Prey selectivity

Ivlev's selectivity index showed that during the summer, both cladocerans and appendicularians were the most heavily selected prey (Fig. 5). Positive selection was also exhibited for copepods, while microplankton was negatively selected in all cases (Fig. 5). During autumn, appendicularians were generally preferred. An apparent neutral selection was shown for copepods, and negative selection was shown for microplankton (Fig. 5). During the winter, copepods, followed by appendicularians, were highly selected in nearly all cases. The only exception was *Sardina pilchardus* late-larvae, which did not select appendicularians. Microplankton was not positively selected in any case (Fig. 5).

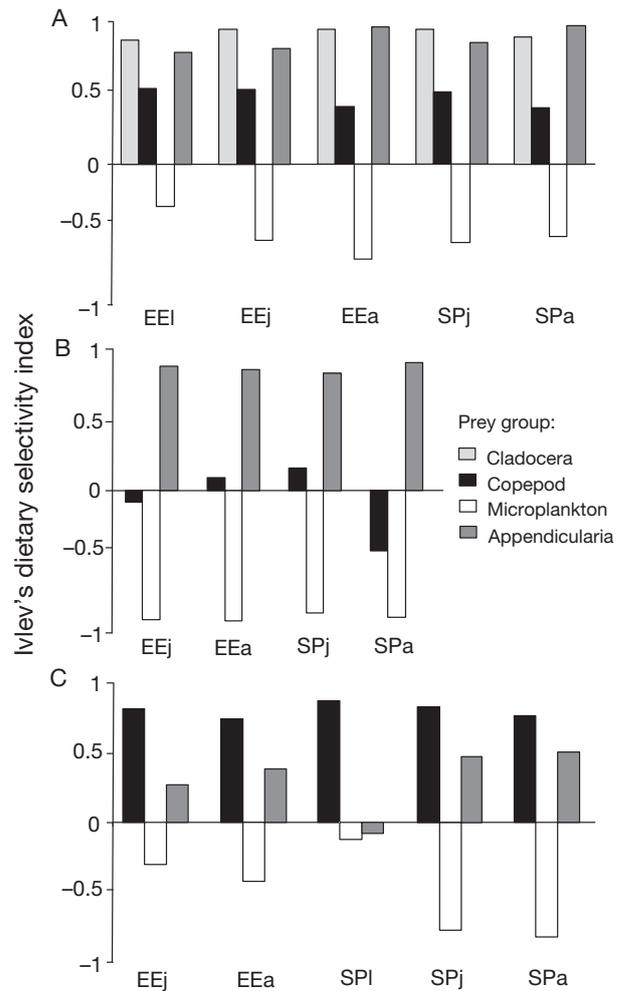


Fig. 5. *Engraulis encrasicolus*, *Sardina pilchardus*. Ivlev's dietary selectivity index for *E. encrasicolus* late-larvae (EEI), juveniles (EEj) and adults (EEa), and *S. pilchardus* late-larvae (SPI), juveniles (SPj) and adults (SPa) during (A) summer, (B) autumn and (C) winter

DISCUSSION

Our study determined the trophic dynamics of anchovy *Engraulis encrasicolus* and sardine *Sardina pilchardus* during different seasons and at different life stages. The results of the study emphasised the feeding plasticity of these species in the Gulf of Lions, as already observed in the Adriatic Sea (Borme et al. 2009). The results showed that the late-larvae of both species feed more abundantly on microplankton than on any other prey and that a remarkable difference in the diet occurred after metamorphosis. If cladocerans were available (i.e. during the summer), they were usually the preferred prey. The high selectivity for appendicularians in autumn seemed to explain the high trophic levels found for this season because appendicularians are the prey group with the highest $\delta^{15}\text{N}$ values. These high $\delta^{15}\text{N}$ values of appendicularians could be due to the retention of zooplankton organisms of high $\delta^{15}\text{N}$ values, such as carnivorous copepods or even small larvae, on their houses (Deibel & Lee 1992); this is in accordance with the data of Hobson et al. (2002), who obtained a higher value of stable-nitrogen isotope and a higher trophic level in appendicularians than in mixed zooplankton samples.

The difference in trophic levels between *Engraulis encrasicolus* and *Sardina pilchardus* late-larvae resulted from their distinct feeding behaviour. *E. encrasicolus* late-larvae, which showed values of $\delta^{15}\text{N}$ similar to those in the other stages, generally fed on copepods and microplankton, a diet similar to that of *S. pilchardus* late-larvae. However, despite the high proportion of these types of prey in the sea during summer, *E. encrasicolus* late-larvae have been found to feed preferentially on appendicularians and cladocerans if these food items are available. Morote et al. (2010) found that *E. encrasicolus* larvae <15 mm standard length (SL) fed primarily on copepods and cladocerans (see also Tudela et al. 2002), whereas *S. pilchardus* ate a relatively high number of protists along with copepod nauplii. Other studies of *S. pilchardus* larvae >13 mm SL reported a diet based on phytoplankton (Rasoanarivo et al. 1991). Given the mean SL of the larvae examined in the current study, it is probable that they did not retain the isotopic signal from parental feeding activity (Pepin & Dower 2007). In view of these considerations and the results cited, it seems reasonable to suppose that *S. pilchardus* late-larvae, whose diet is more herbivorous, would exhibit a lower trophic level than that of *E. encrasicolus* late-larvae. This difference is even clearer from the selectivity indices, which indicate

that *E. encrasicolus* late-larvae tend to feed on prey with higher $\delta^{15}\text{N}$ values rather than on the microplankton that represent a primary constituent of the diet of *S. pilchardus* late-larvae.

The observed differences between ages during the winter and summer for *Engraulis encrasicolus* and *Sardina pilchardus* reflected a change in the diet across the ontogenetic development of the fishes. We did not find differences during autumn, because no late-larvae of any species were collected during that period. These findings suggested the hypothesis that the diet shift occurred primarily at the time of metamorphosis (Lindsay et al. 1998), whereas juveniles and adults maintained similar diets. However, *E. encrasicolus* during summer exhibited clear differences between juveniles and the other 2 stages. Lindsay et al. (1998) also found a drastic change in $\delta^{15}\text{N}$ in Japanese anchovy *E. japonicus* as individuals grew from 15 to 30 mm SL and another change between 30 and 70 mm SL. These size ranges are almost coincident with the sizes of the juveniles in our study (6.37 ± 0.85 cm of SL). Therefore, dietary changes may also occur after metamorphosis.

The most frequently cited explanation for the ontogenetic dietary shift refers to the development of the feeding apparatus of the fishes (June & Carlson 1971, King & Macleod 1976, MacNeill & Brandt 1990, Gerking 1994). According to these authors, larvae become able to filter-feed when the development of their gill rakers is complete. Thus, differences in the diet are related to the minimum prey sizes that are efficiently retained by the feeding apparatus.

In contrast, Tanaka et al. (2006) analysed the stomach contents and gill-raker morphology of 3 species of planktivorous pelagic fishes. These authors found that the differences in the diets of these species were explained by differences in feeding behaviour (filter-feeding vs. particulate feeding) rather than by differences in morphology. This conclusion supports our results for juveniles and adults of *E. encrasicolus* during summer. These fish, caught at different locations and different times, are considered to have a fully developed filtering apparatus. Consequently, the differences in the diet indicated here by stable isotopes could depend on shifts in feeding habits mediated by food density. For example, the fish could shift between filter-feeding and particulate feeding, depending on the concentrations of different prey items (Bulgakova 1996).

Previous studies based on stomach contents analysis argued that *Engraulis encrasicolus* in the Gulf of Lion is mainly zooplanktivorous (Tudela & Palomera 1997, Plounevez & Champalbert 2000, Morote et al.

2010, Costalago et al. 2011), whereas *Sardina pilchardus* also feeds significantly on microheterotrophs (Rasoanarivo et al. 1991, Morote et al. 2010). These observations, together with our results, led us to hypothesise that both *E. encrasicolus* and *S. pilchardus* are omnivorous all through their life cycles. However, our results demonstrated a slightly higher trophic level for *S. pilchardus* in all seasons and stages, except late-larvae, a similar pattern to that described for the Atlantic coast of the Iberian Peninsula (Bode et al. 2007). This discrepancy was explained by van der Lingen (1998) and Bode et al. (2006), who demonstrated that sardines primarily obtain protein nitrogen from zooplankton rather than from phytoplankton. This argument is also supported by the observation that herbivores generally have higher $\delta^{15}\text{N}$ variability (Mill et al. 2007). Moreover, the differences between the present study and those by Coll et al. (2006) and Navarro et al. (2011), both focused on the Catalan Sea, could be considered a consequence of the diet data used by those authors. In particular, they based their results on anchovy diet data reported by Tudela & Palomera (1997) and on sardine diet data from the eastern Mediterranean (Demirhindi 1961), resulting in a higher trophic level in *E. encrasicolus* than in sardine (3.05 and 2.97, respectively). Alternatively, some differences in the diets of *E. encrasicolus* and *S. pilchardus* between areas may also have influenced these results.

A comparison of the trophic levels found in our study with values from upwelling areas shows that adults of both anchovy and sardine in the Gulf of Lions normally exhibited trophic levels similar to the trophic levels found for homologous species in regions with upwelling. Bode et al. (2007) found trophic levels of 3.5 for *Sardina pilchardus* and of 3.4 for *Engraulis encrasicolus* for the northern Iberian Atlantic coast. Miller et al. (2010) found trophic levels of 2.9 for *S. sagax* and of 3.1 for *E. mordax* for the California Current. Moreover, the trophic level of 2.9 derived for *S. sagax* in the southern Benguela Current is lower than the value for *S. pilchardus* in the Gulf of Lions (van der Lingen & Miller 2011). In this context, Miller et al. (2011) showed that fish from less productive areas exhibited relatively higher trophic levels than those from more productive areas. Therefore, the food web of areas with relatively low average primary production, like the Gulf of Lions, is more linear than the food web in zones with upwelling (e.g. the Galician coast, where the trophic structure is more intricate and ramified) (Agostini & Bakun 2002). A more linear food web implies that individuals, in this case

small pelagic fish in the Mediterranean sea, are more dependent on the adjacent lower trophic level than those small pelagic fish from more highly productive areas (Miller et al. 2011), a pattern that could be interpreted as a bottom-up ecosystem structure in the Mediterranean. However, in view of the high biomass of the mid-trophic level small pelagic fish in the northwestern Mediterranean, this structure is actually closer to a 'wasp-waist' system, as found in the Adriatic Sea (Coll et al. 2007).

The trophic dynamics of zooplankton and small pelagic fish occupy the most significant position within marine pelagic food webs (Shannon et al. 2009). In the Gulf of Lions, a substantial amount of seasonal variability affecting the lower trophic levels has been widely reported (Molinero et al. 2005). These temporal fluctuations reflect an important feature of the area—the effect of the Rhone River. The Rhone is the primary source of the runoff entering the Mediterranean. The mean annual flow of the river is $1700 \text{ m}^3 \text{ s}^{-1}$, and its catchment area is $98\,000 \text{ km}^2$ (Darnaude et al. 2004). In fact, correlations between river discharge and marine pelagic fish abundance in the northwestern Mediterranean have been extensively studied (García & Palomera 1996, Lloret et al. 2001, 2004) and are generally explained by the enhancement of planktonic production produced by the input of nutrients from rivers. We found significant differences in the $\delta^{13}\text{C}$ signatures of fishes among seasons in all cases. These results demonstrate the importance of seasonal variability for the structure of the food web.

Lindsay et al. (1998) also found that, in central Japan, rivers may supply the coastal trophic web with different $\delta^{15}\text{N}$ signatures depending on the season. In agreement with this result, our study showed that this heavy nitrogen isotope was generally more abundant in both food sources and fish samples during autumn. The difference may reflect the higher amount of rainfall during autumn in this region. Moreover, Odum (1985) suggested that the augmented contributions of flows to detritus could serve as a marker of disruption in energy transport from lower to higher trophic levels, conferring higher signature to $\delta^{15}\text{N}$ than expected with a less direct transmission to predators. With this in mind, it can be argued that nutrients derived from the Rhone River in autumn tend to have heavier N. Correspondingly, we showed that $\delta^{15}\text{N}$ values in summer were generally lower for the 2 fish species than in the other 2 studied seasons, probably because summer is the driest season and the river discharges are lower.

In conclusion, the present study shows that adults of *Engraulis encrasicolus* and *Sardina pilchardus* generally prey over larger plankton than juveniles and late-larvae. We also illustrate the importance of appendicularians in the diet of both species, especially when cladocerans are not available, and prove that stable isotope analysis is an essential tool for complement dietary studies based on direct observations of stomach contents, because appendicularians are likely easily digested (Capitanio et al. 2005) and could have been underestimated in some cases (Capitanio et al. 1997, D. Costalago unpubl. data). Moreover, based on our output data from the SIAR model and the data we gathered from the plankton samples, we also showed an innovative manner of calculating dietary selectivity through Ivlev's selectivity index. To our knowledge, this approach has never previously been attempted, and we believe that, with limitations and future improvements, it can be useful for drawing a more comprehensive and accurate picture of trophic dynamics.

Assessing the isotopic values of different types of prey in different seasons is therefore essential in understanding whether isotopic seasonal variations of pelagic fish are caused by changes in diet or by variations of basal isotopic levels due to environmental oscillations. Anthropogenic alterations in the ecosystem, such as overfishing, eutrophication, or climate change often entail alterations in the trophic structure of the communities; therefore, the above-discussed findings may be useful in future management scenarios that would take into account an ecosystem approach to fisheries in the northwestern Mediterranean.

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