

Linking small pelagic dietary shifts with ecosystem changes in the Gulf of Lions

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ABSTRACT: Since 2008, a severe decrease in size and body condition together with a demographic truncation has been observed in the sardine (secondarily in anchovy) population of the Gulf of Lions (NW Mediterranean Sea). In parallel, sprat biomass, which was negligible before, has increased tenfold. All of these changes have strongly affected the regional fisheries. Using trophic and isotopic data from contrasting periods of low versus high growth and condition, we investigated potential changes in diet and interspecific feeding interactions through time. Evidence of resource partitioning was found between sprat and both anchovy and sardine in 2004 and 2005. Since 2010, the isotopic niches of the 3 species have tended to overlap, suggesting higher risk of competition for food resources. Moreover, the wider trophic niche of sprat indicates higher variability in individual diets. Anchovy and sardine diet varied through time, with a high proportion of large copepods or cladocerans in periods of high growth and condition (1994 and 2007, respectively) versus a dominance of small copepods in the present (2011–2012). Furthermore, an important reduction in prey diversity was also identified in the diet of both anchovy and sardine during the most recent period. Our results support the hypothesis that changes in small pelagic fish growth, size and body condition and ultimately biomass could be due to bottom-up control characterized by changes in food availability and increasing potential trophic competition.

KEY WORDS: Trophic ecology · Anchovy · Sardine · Sprat · Dietary overlap · NW Mediterranean

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INTRODUCTION

Small pelagic fish species are characterized worldwide by important temporal fluctuations in their abundance and biomass, generally ruled by environmental changes in marine ecosystems (Schwartzlose et al. 1999, Alheit & Niquen 2004). Bottom-up control, driven by changing ocean conditions, was therefore often proposed as the main hypothesis to explain these regime shifts (Cury & Shannon 2004), but

changes in plankton quantity and quality are difficult to confirm due to a lack of sufficient observations of the planktonic community in both time and space.

In the Gulf of Lions, sardine *Sardina pilchardus*, W. 1792, anchovy *Engraulis encrasicolus*, L. 1758 and sprat *Sprattus sprattus*, L. 1758, the 3 main small pelagic species, act as a critical link between planktonic production and top predators (Bănaru et al. 2013). Additionally, anchovy and sardine support pelagic trawling and purse seine fisheries (between 30 and

50% of the total landings in this area, Bănaru et al. 2013), making them both ecologically and economically essential (Palomera et al. 2007). During the last decade, a decrease in size and body condition was observed for anchovy and sardine, while at the same time, sprat biomass, which had been negligible before, increased tenfold (Van Beveren et al. 2014). These changes made sardine and anchovy commercially less interesting, so that landings dropped dramatically, reaching their lowest values in 150 yr for sardines (Van Beveren et al. 2016). Surprisingly, the recruitment has remained high and these modifications primarily affected the adults, with a disappearance of older age classes, especially for sardine (Van Beveren et al. 2014, Brosset et al. 2015).

Such changes in the small pelagic fish community are far less common than changes in recruitment and might result from selective pressure from fishing or natural predation or modifications in prey availability and/or quality. Yet, fishing pressure does not appear to be the main driver of these changes, as these populations were/are not overfished (GFCM 2014) and exploitation rates have remained low over the last 2 decades (i.e. at around 10 to 20% on average without exceeding 40%, see Van Beveren et al. 2016). Predation pressure from Atlantic Bluefin tuna, the main top predator of the small pelagic fish in this area, has been recently evaluated to be <2% (Van Beveren et al. 2016), indicating that top-down control is unlikely. While predation due to natural predators or fishing remained at low levels for these species, body condition in sardine and anchovy is strongly affected by mesozooplankton abundance (Brosset et al. 2015), pointing to bottom-up control as the most probable source of changes in small pelagic fish in the NW Mediterranean. Unfortunately, plankton records are scarce in the Gulf of Lions, and no time series is currently available to test for such bottom-up control.

Stomach content analysis and stable isotope analysis (SIA) are 2 of the main approaches for investigating feeding habits and trophic interactions (Darnaude et al. 2004, Post et al. 2007). Stomach content analysis documents recently consumed food items and permits a quantitative and qualitative snapshot of the diet (Hyslop 1980). SIA is complementary to stomach content analysis and facilitates an integrated measure of the assimilated food over the previous months depending on the variability of prey and their stable isotope ratios, the fractionation and the isotopic turnover. Values of $\delta^{15}\text{N}$ may be related to the trophic level of an individual, while the $\delta^{13}\text{C}$ ratio indicates the primary production sources, i.e. the different feeding environments (coastal/oceanic, pelagic/

benthic), used by consumers (Vander Zanden & Rasmussen 1999, 2001). Combining stomach content analysis and SIA has become an effective tool to investigate changes in trophic structure. Hence, their joint use contributes to the further understanding of how an ecosystem may be affected by changes in inter-specific interactions (Caut et al. 2006).

Several studies have provided important information on the feeding habits and diets of these 3 species in the Gulf of Lions (Plounevez & Champalbert 2000, Costalago et al. 2012, Costalago & Palomera 2014, Pethybridge et al. 2014, Le Bourg et al. 2015). However, they were usually limited to 1 species or a given period, so that the investigation of potential temporal changes over the last 15 to 20 yr is still missing. The aim of our study was to investigate the hypothesis that recent changes observed in anchovy, sardine and sprat populations in the Gulf of Lions might result from diet changes. In this study, we investigated changes in the feeding habits between periods of contrasting growth and condition (before and after 2008) defined by Van Beveren et al. (2014). In particular, isotopic interspecific overlap, trophic niche width and prey type were examined through time. Given that sardine, sprat and anchovy strongly co-occur in terms of spatial distribution in the Gulf of Lions (Saraux et al. 2014), trophic overlap between species might result in food competition if resources become limited (Hardin 1960). Moreover, the niche width, i.e. an index of prey diversity estimated through the range of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values or Shannon's diversity, may be used to determine how generalist a population might be in terms of diet and feeding areas (Newsome et al. 2007). Finally, prey species composition might have a strong effect on the energy intake of predators (Beaugrand et al. 2003, Blanchard et al. 2012).

MATERIALS AND METHODS

Study area

The Gulf of Lions (42° 26'–43° 40' N, 3° 00'–5° 27' E; Fig. 1) is located in the northwestern Mediterranean Sea and is characterized by a large continental shelf (Millot 1990). Shallow waters between 0 and 200 m associated with Rhône River discharge and coastal upwelling due to northern winds support high productivity, making it one of the most productive areas of the Mediterranean Sea (Minas & Minas 1989, Lefevre et al. 1997). A decreasing trend in the concentration of nutrients occurs from east to west and from

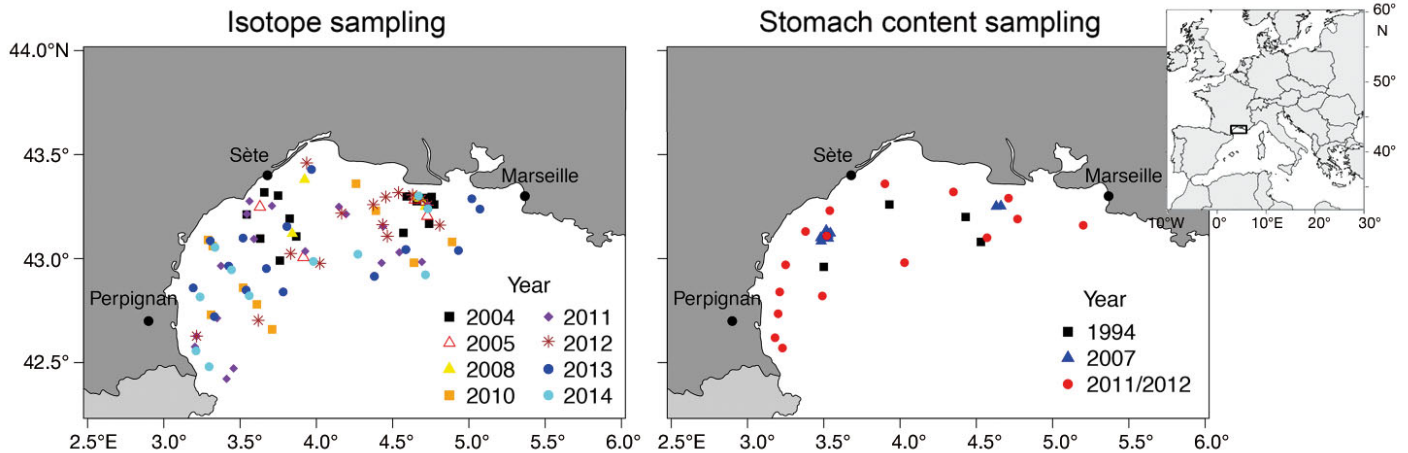


Fig. 1. Sampling sites in the Gulf of Lions (NW Mediterranean Sea). Locations for isotope sampling and stomach content sampling are shown

coastal to deeper waters (Bănaru et al. 2013). The particulate organic matter and phyto- and zooplankton communities at the base of the food webs show inter annual, seasonal and spatial variations in terms of composition and stable isotope ratios in this area (Darnaude et al. 2004, Bănaru et al. 2014, Espinasse et al. 2014). These variations may be related to the terrestrial and anthropogenic river inputs, to currents and wind forcing influencing the hydrography of the area and to changes in the phyto- and zooplankton communities in terms of species composition and size classes (Rau et al. 1990, Harmelin-Vivien et al. 2008, Bănaru et al. 2014).

Sample collection

Fish and zooplankton samples were collected during Pelagic Mediterranean (PELMED) scientific surveys during the summer under a similar protocol. Fish were sampled with a pelagic trawl with a small-mesh cod-end (mesh length 5 mm, ISO 1107) and towed at an approximate speed of 4 knots over 30 min periods. All specimens were selected randomly from hauls, and their standard length was determined to the nearest mm, as well as their mass (to the nearest g) and sex. Numerous stations were sampled each year in the Gulf of Lions (i.e. inshore/offshore, west and east) to cover the complete area (Fig. 1) and to avoid bias of interannual differences due to spatial heterogeneity. In 2004, 2005 and 2014, zooplankton was sampled using a vertical WP2 net (200 μ m mesh size) at each trawl station (4 stations in 2004 and 2005 and 10 in 2014). Zooplankton samples were stored in frozen sea water to be used in bulk for SIA. Using a combination of

previously published data (Costalago et al. 2012, Le Bourg et al. 2015) and new samples that were analysed for this study, stable isotope values were available for June and July of 2004, 2005, 2008 and 2010 to 2014 (except for sprat in 2008 which was not sampled). Stomach content data were available in 2011 and 2012 for both anchovy and sardine (previously published data; $n = 118$ and 104 for sardine and anchovy, respectively; Le Bourg et al. 2015) as well as prior to population changes: in 2007 for sardine ($n = 156$; Costalago & Palomera 2014) and in 1994 for anchovy ($n = 50$, this study).

SIA

Fish isotope analyses were conducted on a ca. 1 cm^3 piece of white muscle that had been kept frozen at -80°C (Sweeting et al. 2005). Both fish white muscle and zooplankton samples were freeze-dried for 48 h and ground into a fine powder before being encapsulated in a tin cup and sent to the LIENSs laboratory (La Rochelle, France) for SIA analysis. An acidification step was necessary for zooplankton samples to remove any ^{13}C -enriched carbonates (DeNiro & Epstein 1978). A subsample was acidified with 1% HCl, rinsed with distilled water and dried to determine the $\delta^{13}\text{C}$ ratio; an untreated subsample was used for $\delta^{15}\text{N}$ analysis. Three zooplankton replicates were performed from each sampled site for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. A continuous-flow isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific) coupled to an elemental analyser (Flash EA1112, Thermo Scientific) was used to perform stable isotope measurements. Results were expressed in parts per thousand (‰) relative to Vienna PeeDee Belemnite and atmos-

pheric N₂ for δ¹³C and δ¹⁵N, respectively, using the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3 \quad (1)$$

where X is ¹³C or ¹⁵N and R is the isotope ratio ¹³C:¹²C or ¹⁵N:¹⁴N. Replicate measurements of internal laboratory standards (acetanilide) indicated a precision of 0.2‰ for both δ¹³C and δ¹⁵N values.

Variation in lipid content among fish species can introduce a bias in carbon stable isotope analyses (Post et al. 2007). Therefore, the potential effect of lipids on δ¹³C values of fish samples was corrected by applying the procedure of Post et al. (2007) when the C:N ratio was >3.5 (which was mainly the case for sprat). Thus, the time and uncertainty due to lipid extraction were reduced. For all years, fish length ranges were kept similar (from ~10 to 15 cm, Table 1) to avoid any bias due to ontogenetic changes.

Stomach content analyses

A similar protocol was applied to all analyses of stomach contents regardless of the year (see details in Costalago & Palomera 2014, Le Bourg et al. 2015). Briefly, fish were frozen at -20°C immediately after being caught to stop digestive processes. The gut was then removed and stored in 95% alcohol in the laboratory. Each stomach was carefully opened and all prey species were placed in a Petri dish and identified and quantified under a stereo microscope to the lowest possible taxonomic level, depending on the digestion state. Unidentified prey were excluded from the statistical analyses. A single difference in protocol occurred, as fish were analysed individually in all years except for sardines in 2007, for which fish

Table 1. Standard length and number of samples analysed for stable isotopes of anchovy *Engraulis encrasicolus*, sardine *Sardina pilchardus* and sprat *Sprattus sprattus*. NA: data not available

Year	Mean ± SD length (mm)					
	Anchovy		Sardine		Sprat	
	n	n	n	n	n	n
2004	113 ± 16	65	131 ± 22	83	106 ± 6	14
2005	121 ± 15	29	129 ± 18	25	114 ± 8	8
2008	115 ± 4	15	136 ± 4	17	NA	NA
2010	110 ± 4	32	100 ± 5	33	87 ± 8	32
2011	103 ± 19	61	117 ± 14	51	96 ± 8	57
2012	97 ± 5	36	111 ± 13	38	95 ± 8	50
2013	108 ± 17	43	104 ± 18	38	103 ± 8	39
2014	111 ± 14	49	121 ± 15	42	94 ± 9	36

of a given station were pooled together, i.e. between 16 and 20 stomachs were pooled together. In that case, the associated sardine length corresponded to the mean length of all individuals from a given sample. Prey dry weight was derived from the literature (see Le Bourg et al. 2015).

Prey importance was assessed using the percentage of occurrence (%O, proportion of stomachs in which a given prey species was found), percentage in number (%N, ratio between the number of a given prey species and the total number of prey), percentage in dry weight (%W, ratio between the weight of a given prey species and the total weight of prey). These percentages were then combined to calculate the index of relative importance (IRI, Pinkas et al. 1971), which is necessary to compute the %IRI; this allows an integrated comparison between dietary items of the same species (Hyslop 1980, Cortés 1997):

$$\%IRI = \frac{IRI}{\sum_{a=1}^n IRI} \times 100 \quad (2)$$

where $IRI = \%O \times (\%N + \%W)$, a is a specific prey category, and n is the number of prey species. Because of potential disparities in the names of species or groups of species between 1994 and 2011–2012, we carefully linked all prey names to current species names. Due to a lack of correspondence between periods, several species were not retained. However, these species/groups of species all had very low %IRI (<0.2%), so that their absence would not significantly affect the computation of %IRI and trophic niche width (see below).

The trophic niche width was measured for each period, using the exponential of Shannon's entropy, $N_1 = \exp(H)$, where H is the Shannon-Wiener diversity index calculated as $-\sum_{i=1}^q p_i \ln(p_i)$, where i is a specific prey category, q is the number of prey species, and p_i is the proportion of IRI (Hill 1973, Medina et al. 2015).

Data analyses

Determinants of isotopic values

To investigate the potential effect of species, year, fish length, latitude, longitude and coastal/offshore gradient on δ¹³C and δ¹⁵N values, we used the classification and regression tree (CART) approach of Breiman et al. (1984). Decision trees were built by recursively partitioning our dataset into increasingly homogeneous subgroups of isotope values. Each split is defined by a simple rule based on a single explana-

tory variable, and each final group is characterized by its mean isotope values. Two separate CARTs were applied for fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. As fewer stations and years were sampled for zooplankton, we only tested annual differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to look for interannual variability (2004, 2005 and 2014) using 1-way analyses of variance (ANOVA).

Isotopic niches

Standard ellipse areas corrected for small sample size (SEA_c , Jackson et al. 2011), which define the isotopic niche space of 40% of typical individuals within the group based on bivariate normal distributions, were calculated as a measure of the mean core population isotopic niche in order to analyse resource partitioning among species over time. However, because both the shape and size of the area filled by the points influence this estimate, we first scaled isotope values as recommended by Cucherousset & Vil  ger (2015) to obtain an informative assessment of isotopic overlap. Niche overlaps between the different species were then determined using SEA_c . Niche overlap varied between 0 (no overlap) and 1 (1 species is included in the other species' stable isotope space). In addition to niche overlap, the size of standard ellipses was also compared between species and between years for each species. To accomplish this, Bayesian standard ellipse areas (SEA_B) were calculated, which allowed for robust statistical comparisons. In addition, the probability of Bayesian ellipses, which were larger or smaller relative to the compared group, was computed (p , the proportion of ellipses in 2011 that were lower than 2012, see Jackson et al. 2011 for more details). We considered 2 SEA_B to be significantly different when more than 95% of the posterior estimates of one group were smaller than those of another group (Turner et al. 2010). Estimation via Bayesian inference allowed for robust comparisons among data sets comprising different sample sizes (Syv  ranta et al. 2013).

Temporal variation in stomach contents

To describe the temporal variations in stomach contents of anchovy and sardine, we applied a principal component analysis (PCA), which is a multivariate analysis of individuals' stomach contents as a function of prey using a correlation matrix. For each predator species, only prey constituting >2% of %IRI in at least 1 of our data subsets were considered for

the analysis, except for unidentified copepods. Empty stomachs were excluded from this analysis. To deal with different sampling strategies for sardine stomach contents between 2007 and 2011/2012, the 8 stations of 2007 (each representing between 16 and 20 individuals) were weighted by the number of corresponding individuals in the PCA analysis. The PCAs were performed on the dry weight (%W) of the different prey ingested for each individual. Similar results were obtained when PCAs were performed on the proportion of frequency (%N), showing the robustness of these results.

All statistical analyses were performed with R version 3.0.2. Values are indicated as mean \pm standard error (SE), and all statistical tests were performed at a significance level of 0.05. All data were tested for normality and heteroscedasticity using Kolmogorov-Smirnov and Levene's tests.

RESULTS

Factors influencing isotope values

Fish size and nitrogen and carbon stable isotope ratios were determined for 330 anchovies, 327 sardines and 236 sprats (Table 1, Fig. 2). Using a CART analysis, 85% of the variability in the $\delta^{13}\text{C}$ values were significantly explained by the variables year and species, whereas spatial locations of capture inside the Gulf of Lions (latitude and longitude), fish length and the coastal vs offshore gradient were not retained (Fig. 3a). The year variable had the greatest effect, and $\delta^{13}\text{C}$ values were mainly split accordingly (Fig. 3a). First, the maxima for 2004 and 2005 were separated from all other years. In this small group, a secondary partition separated high $\delta^{13}\text{C}$ sprat values from anchovy and sardine values. From all remaining years (2008 to 2014), the years 2010 and 2011 showed the most negative values, especially for sprat (Fig. 3a). Years 2008, 2012, 2013 and 2014 had intermediate $\delta^{13}\text{C}$ values, with no differences among the 3 species studied. Between 2004 and 2014, the $\delta^{13}\text{C}$ isotope values of all 3 species decreased progressively, but less for sardine and anchovy (-2‰) than for sprat (-3‰ ; Fig. 2).

According to the CART, 39% of the variability for the $\delta^{15}\text{N}$ signal was accounted for by year and species variables (Fig. 3b). In 2005 and 2011 to 2013, $\delta^{15}\text{N}$ values were lower for anchovy and sprat in comparison to sardine (Fig. 3b). In contrast, during all other years (2004, 2008, 2010 and 2014), sardine and sprat had more similar $\delta^{15}\text{N}$ values than anchovy.

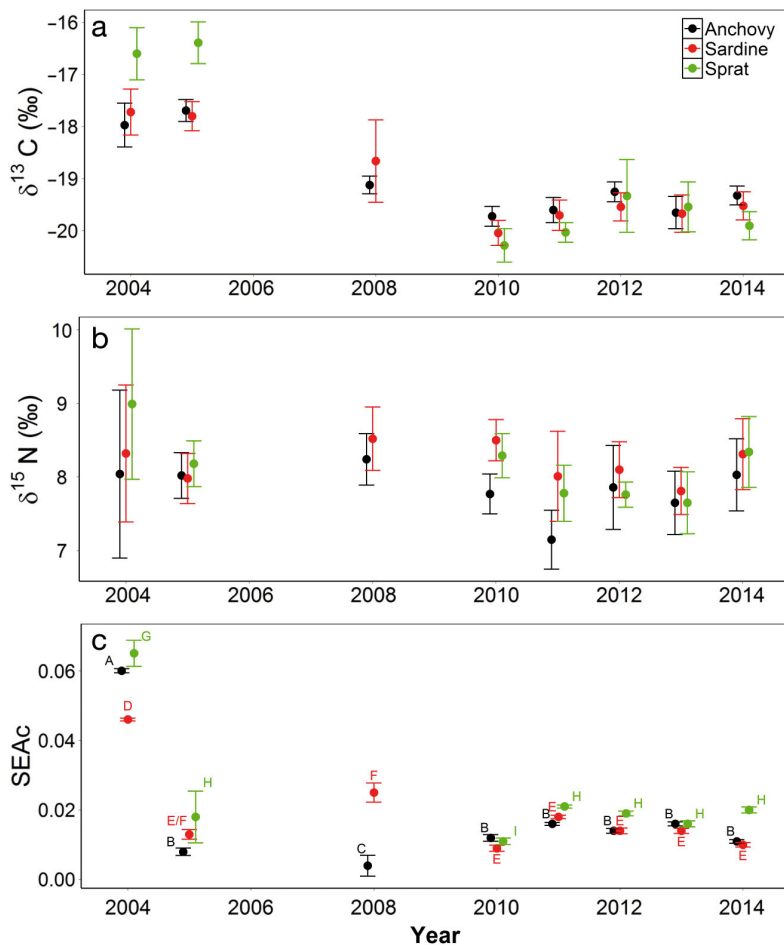


Fig. 2. Summary of (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ values (mean \pm SD, in ‰), and (c) trophic niche size of sardine *Sardina pilchardus*, anchovy *Engraulis encrasicolus* and sprat *Sprattus sprattus* over time in the Gulf of Lions. SEA_C : standard ellipse area corrected for small sample sizes, indicated in $\% ^2$. The Bayesian area estimates of the standard ellipse metrics (SEA_B) that present significant differences between years for each species are indicated by different superscripts (A–C for anchovy, D–F for sardine and G–I for sprat)

Only in anchovy in certain years did other variables affect isotope values. Anchovy values in 2004, 2008, 2010 and 2014 also depended on the sampling location, in particular the longitude, with lower values in the east. Moreover, in the western part during those years, anchovy $\delta^{15}\text{N}$ values increased with size (Fig. 3b). However, even if year was significant in the CART analysis (Fig. 3b), during our study period only a slight difference in trophic levels was observed for any species ($\delta^{15}\text{N}$ range), with no clear temporal trend (Fig. 2).

Similarly to fish, the $\delta^{13}\text{C}$ values of the bulk of zooplankton were higher in 2004 and 2005 in comparison to 2014 (ANOVA, $p < 0.001$), while $\delta^{15}\text{N}$ values remained stable over time (ANOVA, $p > 0.05$; see Fig. A1 in the Appendix).

Temporal changes in isotopic niche overlap

Fig. 4 displays the Bayesian ellipse areas (SEA_C) that represent the isotopic niche of the 3 small pelagic species in a scaled isotopic niche space. Two patterns were observed: firstly, sprat was segregated from sardine and anchovy during the 2 earliest years (2004 and 2005, Fig. 4); second, the overlap of the sprat isotopic niche on that of sardine was present until 2014 (Table 2). Sprat also overlapped strongly with anchovy in 2012 and 2013, but no clear temporal trend was evidenced (Table 2). In contrast, the trophic niche overlap of sardine and anchovy was high in 2004 and 2005 (Table 2). In 2008, the total anchovy niche even fell within that of sardine (100% overlap). However, during the subsequent years (2010–2014), overlap was much lower except for 2013 (at least halved compared to before 2010, Table 2). For each species, the isotopic location of the SEA_C differed among years (Fig. 4).

Temporal changes in isotopic niche width

The niche width of the 3 species varied notably between years (Fig. 2). The anchovy isotopic niche width decreased between 2004 and 2008 (SEA_B : $p < 0.001$) and then increased to remain steady at middle values (SEA_B : $p > 0.05$, Fig. 2). The sardine isotopic niche width also decreased between 2004 and 2005 (SEA_B : $p < 0.001$), but increased between 2005 and 2008 (SEA_B : $p < 0.01$) and then decreased again in 2010 (SEA_B : $p < 0.01$) and finally remained steady until 2014 (SEA_B : $p > 0.05$, Fig. 2). The isotopic niche width of sprat decreased between 2004 and 2010 (SEA_B : $p < 0.01$) and subsequently increased and stabilized from 2011 to 2014 (SEA_B : $p > 0.05$, Fig. 2). In 2010 and 2013, the SEA_B width was similar between all 3 species (SEA_B : $p > 0.05$, Fig. 2), whereas during the years 2004, 2005, 2011, 2012 and 2014, sprat always had a larger SEA_B than sardine and anchovy (SEA_B : $p < 0.05$, Fig. 2). In 2008, when only 2 species were available, sardine SEA_B was significantly larger than that of anchovy (SEA_B : $p < 0.001$).

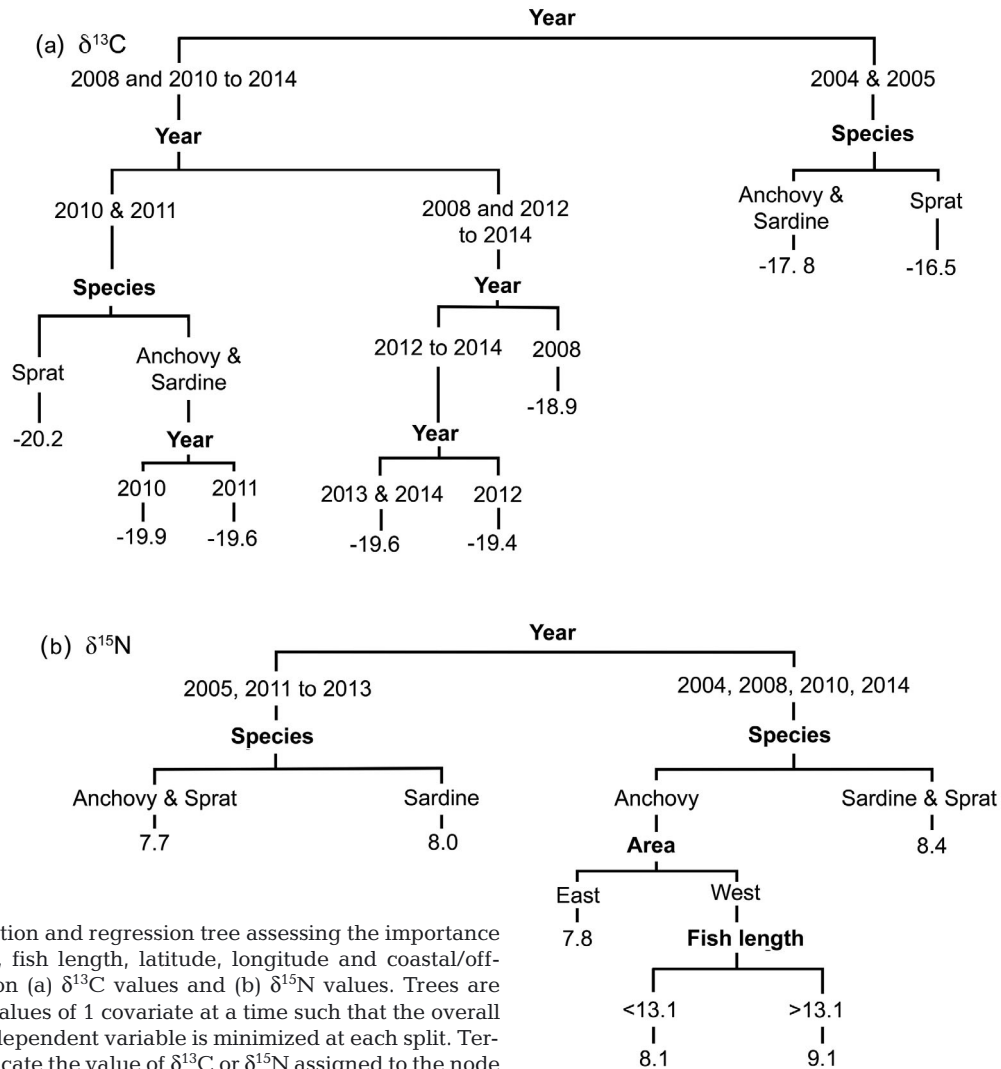


Fig. 3. Classification and regression tree assessing the importance of year, species, fish length, latitude, longitude and coastal/off-shore gradient on (a) $\delta^{13}\text{C}$ values and (b) $\delta^{15}\text{N}$ values. Trees are split off on the values of 1 covariate at a time such that the overall variance in the dependent variable is minimized at each split. Terminal nodes indicate the value of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ assigned to the node

Table 2. Standard ellipse overlaps for each combination of species. The values represent the percentage of overlap between the standard ellipse areas in the scaled stable isotope space. Each number in the cell refers to the percentage of overlap of the area of the species indicated first (e.g. 68% is the percentage of ellipses of sardine *Sardina pilchardus* that overlapped with ellipses of anchovy *Engraulis encrasicolus*, 52% is the percentage of ellipses of anchovy that overlapped with ellipses of sardine). NA: not applicable

Year	Sardine anchovy	Anchovy sardine	Anchovy sprat	Sprat anchovy	Sardine sprat	Sprat sardine
2004	68	52	0	0	0	0
2005	58	89	0	0	0	0
2008	100	15	NA	NA	NA	NA
2010	0	0	0	0	50	41
2011	12	14	8	6	41	35
2012	14	13	69	52	43	30
2013	69	63	65	65	54	49
2014	32	27	9	5	70	32

Size, area and temporal variations in diet composition

The diet of sardine and anchovy was mainly zooplanktivorous, with some traces of phytoplankton consumption (diatoms) in 2011/2012 for both species (see Tables 3 & 4). The anchovy diet in 1994 was characterized by the dominance of *Acartia clausi* (18.23% IRI), *Microsetella* spp. (13.52% IRI), individuals belonging to the genera *Clausocalanus* and *Paracalanus* (hereafter referred to as Clauso/Paracalanidae; 13.22% IRI), *Oncaea* spp. (12.67% IRI) and Euphausiacea larvae (7.31% IRI) (Table 3). Copepods also dominated the anchovy diet in 2011/2012, but anchovy fed almost exclusively on 2 gen-

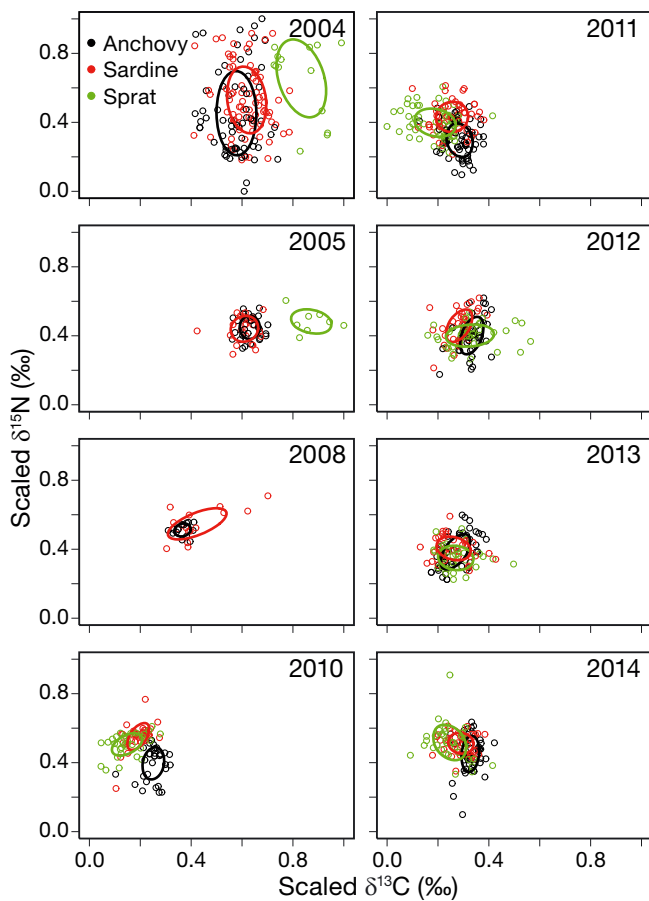


Fig. 4. Species scaled isotopic niche space between 2004 and 2014, represented by solid bold lines based on the area of the standard ellipses corrected for small sample sizes (SEAc). Stable carbon and nitrogen isotope values are from the muscle of adult anchovy *Engraulis encrasicolus*, sardine *Sardina pilchardus* and sprat *Sprattus sprattus*

era: *Microsetella* spp. (34.78% IRI) and *Oncaea* spp. (27.52% IRI). In 2007, sardine diet was composed of copepods (60.31% IRI, mainly *Euterpina acutifrons*, *Microsetella* spp. and *Temora stylifera*) and cladocerans (31.48% IRI). In contrast, cladocerans were almost absent in 2011/2012, while *Microsetella* spp. (29.34% IRI), *Oncaea* spp. (24.55% IRI) and *Corycaeus* spp. (21.64% IRI) dominated the sardine diet (Table 4). For both sardine and anchovy, we also found a strong increase in the proportion of Corycaeidae during years 2011 and 2012.

The trophic niche width, measured with the exponential of Shannon's entropy, was highest in the earliest period studied for both species (10.84 in 1994 versus 5.57 in 2011/2012 for anchovy, Table 3; and 8.54 in 2007 versus 6.46 in 2011/2012 for sardine, Table 4).

In the PCA performed on the dry weight (%W) of the different prey of anchovy, the first (Dim1) and second (Dim2) component represented 35% and 25% of the total variance, respectively (Fig. 5). Dim1 represented a gradient whereby *Oncaea* spp. was opposed mainly to *Acartia clausi* and Clauso/Paracalanidae species. Dim2 represented a gradient with *Microsetella* spp. opposed to *A. clausi* and Clauso/Paracalanidae species in anchovy diet. The majority of anchovy from 1994 had negative values on both axes, while the majority of anchovy from 2011/2012 had positive values. Thus, we deduced an increasing proportion in dry weight of *Microsetella* spp. and *Oncaea* spp. and a decreasing proportion of *A. clausi* and Clauso/Paracalanidae species in the anchovy diet from 1994 to 2011/2012 (Fig. 5). In the PCA carried out on the sardine data, Dim1 and Dim2 represented 44% and 18% of the total variance, respectively (Fig. 6). *Microsetella* spp. was opposed to *Oncaea* spp., *Corycaeus* spp. and cladocerans on the Dim1 axis, while the second axis showed a clear separation between the cladocerans and other prey species. The 2 time periods were opposed on the second axis, reflecting a decreasing gradient of cladocerans and an increasing proportion of *Oncaea* spp., *Corycaeus* spp. and *Microsetella* spp. in the sardine diet from 2007 to 2011/2012. Fish length (4 length classes of 1.5 cm from 10 to 16 cm) as well as sampling area (3 areas; west, central and east) were plotted on the PCA, but both factors displayed no trend.

DISCUSSION

Data availability does not allow us to describe the trophic ecology over the last 20 yr, but we can contrast the present situation to data collected before the drastic changes in demography occurred. The comparison of small pelagic trophic data (1) showed a wider isotopic niche in sprat compared to sardine and anchovy, (2) tended to confirm the hypothesis of changes in the anchovy and sardine diets, and (3) suggested a recent increase in the trophic overlap between the different small pelagic fish species of the Gulf of Lions.

Using SIA and isotope-derived metrics to study interspecific trophic differences, we showed that sprat had a larger isotopic niche width (in 2004, 2005, 2011, 2012 and 2014) than sardine and anchovy or at least equivalent (in 2010 and 2013), mainly due to the broad range of $\delta^{13}\text{C}$. Assuming that the wider sprat isotopic niche reflects higher variability in individual diets (Matthews & Mazumder 2004), sprat intraspeci-

Table 3. Summary of stomach content analysis for anchovy *Engraulis encrasicolus*. %N: percent by number; %W: percent estimated dry weight; %O: percent frequency of occurrence; %IRI: percent index of relative importance; Exp(*H*): trophic niche width based on stomach content analysis. Individuals belonging to the genera *Clausocalanus* and *Paracalanus* are grouped as 'Clauso-Paracalanidae'. -: taxon not found

Prey type	1994				2011 and 2012			
	%N	%W	%O	%IRI	%N	%W	%O	%IRI
Crustacea								
Copepods								
<i>Acartia clausi</i>	6.60	30.36	57.90	18.23	2.64	7.98	18.27	2.29
Clauso-Paracalanidae	13.54	19.49	52.63	13.22	6.36	3.68	15.38	1.83
<i>Centropages typicus</i>	9.76	4.82	50	6.21	3.60	3.64	21.15	1.81
<i>Corycaeus</i> spp.	1.72	2.05	55.26	1.78	4.45	23.81	43.27	14.46
<i>Clytemnestra</i>	0.02	<0.01	2.63	<0.01	0.08	<0.01	1.92	<0.01
<i>Candacia</i> spp.	4.00	8.15	44.74	4.63	0.90	1.10	10.58	0.25
<i>Oithona</i> spp.	1.48	0.07	42.11	0.55	0.12	<0.01	5.77	<0.01
<i>Oncaea</i> spp.	15.30	0.39	94.74	12.67	20.15	15.43	65.38	27.52
<i>Microsetella</i> spp.	16.08	0.22	97.37	13.52	26.38	13.34	74.04	34.78
<i>Euterpina acutifrons</i>	1.25	0.36	94.74	1.30	2.01	0.13	36.54	0.89
<i>Temora stylifera</i>	1.22	0.41	42.11	0.59	0.02	<0.01	0.96	<0.01
Unidentified copepods	23.26	0.78	71.05	14.55	15.36	18.69	32.69	13.16
Cladoceran	2.79	5.36	73.68	3.37	0.33	<0.01	9.62	0.04
Ostracod	0.29	0.03	44.73	0.08	1.29	0.29	22.12	0.41
Tunicata								
Appendicularia								
	-	-	-	-	0.12	<0.01	0.96	<0.01
Protists								
Diatoms								
	-	-	-	-	9.21	9.60	3.85	0.86
Chaetognatha								
Chaetognatha								
	0.05	0.01	2.63	<0.01	-	-	-	-
Larvae								
Crustacea								
Euphausiacea	0.21	26.96	31.57	7.31	2.34	3.31	7.69	0.45
Decapoda	0.35	0.19	34.21	0.16	1.89	1.47	11.54	0.46
Mollusca								
Gastropodae								
	<0.01	0.03	5.26	<0.01	1.98	0.04	10.58	0.25
Bivalve								
	0.02	0.30	42.11	0.11	1.68	0.29	23.08	0.54
Eggs								
Anchovy								
	0.15	0.09	47.37	0.10	1.1	0.49	16.35	0.31
Other fish								
	0.24	0.42	50	0.28	0.14	<0.01	2.88	<0.01
	Exp(<i>H</i>) = 10.84				Exp(<i>H</i>) = 5.57			

fic trophic competition may be lower than those of other species. Additionally, stomach content analyses tended to indicate a slightly more diversified sprat diet (Le Bourg et al. 2015). These results are particularly interesting from a population dynamics viewpoint. Indeed, sprat biomass has considerably increased since 2008, while the ratio of biomass: abundance of both sardine and anchovy has considerably declined (GFCM 2015). Previous studies have shown that those changes were mainly driven by reduced growth, age truncation and condition, and bot-

tom-up processes were the most likely drivers (Van Beveren et al. 2014). Being a generalist feeder with low intra-specific food competition might thus have conferred important advantages to sprat over sardine and anchovy, especially to adapt to environmental changes. This might also explain why sprat body condition was more stable, while sardine and anchovy condition exhibited stronger ups and downs, possibly in reaction to prey variability (Van Beveren et al. 2014).

This study also depicts a considerable reduction in both sardine and anchovy isotopic niches through time. The carbon isotopic composition of fish muscle is mainly related to the ingested prey items (Hobson 1999). As previously stated, strong changes in $\delta^{13}\text{C}$ values of small pelagic fish and zooplankton were observed over time. Differences between years in fish stable isotope values may be due to inter-annual differences in the planktonic community and/or the organic matter, the latter being at the basis of the food web. The Gulf of Lions is further known as a highly variable area in water circulation, productivity and stable isotope ratios of the plankton community (André et al. 2005, Espinasse et al. 2014), which could add variability in

fish isotopic signature. Yet, similarly to previous findings obtained with a different methodology (e.g. fatty acid profiles, Pethybridge et al. 2014), we showed that isotopic values did not differ between sampling areas except for $\delta^{15}\text{N}$ in anchovy during some years, underlining the weak influence of the sampling area on the isotopic values at the scale of the present study. This allows us to reasonably assume that differences were not due to sampling bias, even if the sampling locations varied from year to year. A reduction of the anchovy isotopic niche was observed

Table 4. Summary of stomach content analysis for sardine *Sardina pilchardus*. Abbreviations as in Table 3

Prey type	2007				2011 and 2012			
	%N	%W	%O	%IRI	%N	%W	%O	%IRI
Crustacea								
Copepods								
<i>Acartia clausi</i>	–	–	–	–	3.58	8.14	0.12	1.74
Clauso-Paracalanidae	3.26	6.78	62.50	4.36	7.07	5.05	0.41	5.95
<i>Centropages typicus</i>	–	–	–	–	1.51	1.55	0.13	0.49
<i>Corycaeus</i> spp.	4.09	4.11	75.00	4.28	8.61	30.10	0.46	21.64
<i>Clytemnestra</i>	–	–	–	–	0.43	0.16	0.03	0.02
<i>Candacia</i> spp.	0.16	18.27	12.50	1.60	0.13	1.04	0.03	0.04
<i>Oithona</i> spp.	–	–	–	–	0.16	0.04	0.04	<0.01
<i>Oncaea</i> spp.	11.58	0.61	100	8.48	17.61	12.27	0.68	24.55
<i>Microsetella</i> spp.	6.31	11.11	87.50	10.60	24.65	10.09	0.70	29.34
<i>Euterpina acutifrons</i>	3.82	19.27	62.50	10.03	9.12	4.43	0.36	5.87
<i>Temora stylifera</i>	2.01	30.95	50.00	11.46	–	–	–	–
Unidentified copepods	10.73	2.92	100.00	9.50	9.43	6.06	0.25	4.77
Cladoceran	43.64	1.63	100	31.48	1.08	0.70	0.11	0.24
Ostracod	–	–	–	–	1.09	1.55	0.12	0.39
Tunicata								
Appendicularia	0.08	0.06	12.50	0.02	–	–	–	–
Protists								
Diatoms	8.39	0.79	75.00	4.79	5.85	9.00	0.11	2.03
Chaetognatha								
Chaetognatha	–	–	–	–	<0.01	<0.01	<0.01	<0.01
Larvae								
Crustacea								
Euphausiacea	–	–	–	–	–	–	–	–
Decapoda	0.54	2.63	37.5	0.83	0.09	0.76	0.05	0.05
Mollusca								
Gastropodae	–	–	–	–	0.12	<0.01	0.02	<0.01
Bivalve	1.29	0.19	50.00	0.51	1.09	1.04	0.09	0.24
Eggs								
Anchovy	–	–	–	–	5.40	6.87	0.11	1.68
Other fish	–	–	–	–	2.80	0.83	0.21	0.91
	Exp(H) = 8.54				Exp(H) = 6.46			

between 2004 and 2013, although the largest shrinkage occurred between 2004 and 2005. Nevertheless, this considerable change in isotopic niche area between the 2 years has to be considered with caution, due to the lower sampling size in 2005 in comparison to 2004, which reduces the precision of the estimated mean niche width (see Syväranta et al. 2013). The largest reduction in sardine isotopic niche was observed between 2008 and 2013, concomitantly with the decrease in sardine size, condition and biomass.

Moreover, stomach contents also confirmed a significant reduction in the trophic niche width of both anchovy and sardine (25% and almost 50%, respec-

tively). Specifically, both species displayed a much narrower diet during recent years (2011/2012) compared to previous time periods (1994 or 2007). This might have resulted from a decrease in the availability of certain prey species, downplaying their role in the diet of the small pelagics, as indicated by temporal differences in composition and size range of consumed prey. The sampling methodology was kept as consistent as possible during the study, especially in terms of season, fishing gear and daylight time. Hence, changes could only be related to variables such as year, area and fish size. However, no size class or sampling area effects were detected during the analyses, so that observed changes are likely to be due to a year effect. For adult anchovy and sardine, copepods were, as in most other studies in the Mediterranean (see Tudela & Palomera 1997, Borme et al. 2009, Nikolioudakis et al. 2012), by far the most important prey item, regardless of the year. However, there was a prey size reduction between both periods. In 1994, large copepods (size >1 mm, *Acartia* spp., *Candacia* spp., *Clauso/Paracalanus* spp. and *Centropages typicus*),

attained almost 50% of the %IRI and 65% in relative dry weight (%W). This contrasted with 2011/2012, when they represented barely more than 5% of the IRI and 15% in %W (Le Bourg et al. 2015). Large copepods in the anchovy diet were replaced in 2011/2012 by small copepods species (size <1 mm, such as *Corycaea* spp., *Microsetella* spp. and *Oncaea* spp.), which increased three-fold. The proportion of small and large copepods in 1994 was in agreement with another study from 1995 and 1996, performed in the same area and during the same period (Plounevez & Champalbert 2000). Marked changes between 1994 and 2011/2012 in the anchovy diet also concerned other prey groups, such as cladocerans that became

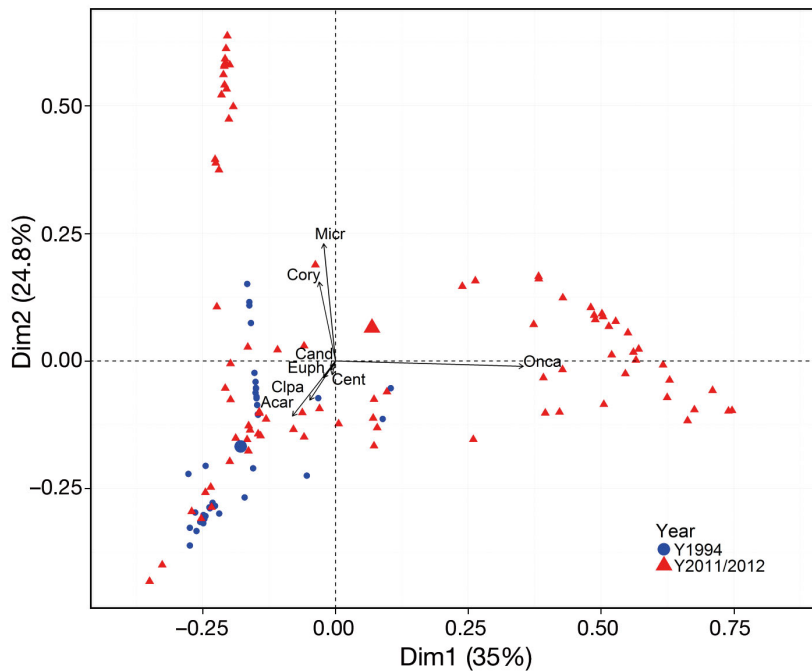


Fig. 5. Principal component analysis of the dry weight percentage of the main anchovy prey in the Gulf of Lions. Cory: *Corycaeus* spp.; Micr: *Microsetella* spp.; Clpa: Clauso-Paracalanidae (individuals belonging to the genera *Clausocalanus* and *Paracalanus*); Onca: *Oncaea* spp.; Cand: *Candacia* spp.; Acar: *Acartia clausi*; Euph: Euphausiacea; Cent: *Centropages typicus*. Barycentres of the years (Y) are added as supplementary variables

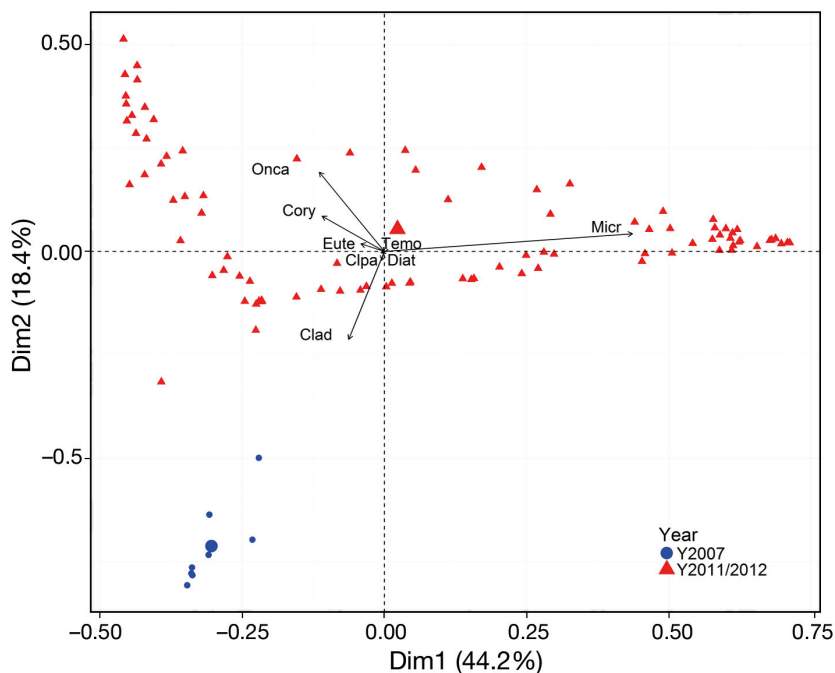


Fig. 6. Principal component analysis of the dry weight percentage of the main sardine prey in the Gulf of Lions. Clad: cladoceran; Temo: *Temora stylifera*; Cory: *Corycaeus* spp.; Micr: *Microsetella* spp.; Eute: *Euterpina acutifrons*; Diat: Diatoms; Clpa: Clauso-Paracalanidae; Onca: *Oncaea* spp. Barycentres of the years (Y) are added as supplementary variables

practically absent in recent years and larvae (mainly Euphausiacea) that also decreased, while diatoms appeared. The decrease of large Euphausiacea larvae, recognized as important anchovy prey in other areas such as the Humboldt Current (Ayón et al. 2011), might have accentuated the deficiency in large prey species.

Similarly, small copepods dominated the sardine diet in recent years, while cladocerans were the dominant prey in 2007. Assuming that larger copepods have a higher energy content (Dumont et al. 1975, Vijverberg & Frank 1976, Zarubin et al. 2014), current nutritional conditions of anchovy and sardine might have thus strongly decreased compared to pre-2008, potentially explaining the strong demographic changes observed in both species after 2008. Furthermore, fish were sampled in July, when adults need to accumulate their fat supply to survive the next winter (Wiegand 1996, Sánchez Gómez 2013). Therefore, the lack of large copepods in their stomachs could reflect difficulties in building sufficient fat reserves to survive an energetically demanding winter, especially for sardines which reproduce at that moment. This might thus explain the apparent high adult mortality rate detected for sardine (less pronounced for anchovy, Van Beveren et al. 2014). However, in order to better understand the impact of the changes in sardine and anchovy diet, a detailed investigation of zooplankton variations in terms of quantity/quality and fish energetics would be required.

Nonetheless, considering the usual importance of large copepods in the diet of anchovy and sardine, these changes probably reflect a potential decrease in their availability, which might result either from an increase in competition between fish species or a decrease in abundance of large copepods. First, the increase in sprat abundance and biomass might have

drawn more intensive interspecific competition. Indeed, all 3 species have been shown to inhabit the same areas and to spatially co-occur in the Gulf of Lions, except for some deeper grounds where only anchovy occurs (Saraux et al. 2014).

The hypothesis of increased interspecific competition during the last decade is supported by the changes in the degree of overlap and segregation in the isotopic niches of the fish species. There was clear isotopic niche segregation between sprat and the 2 other species in 2004 and 2005, indicating that sprat could have exploited different species groups from anchovy and sardine during these still prosperous years. However, sprat diet has overlapped significantly with anchovy and/or sardine diet since 2010. Conversely, the overlap between anchovy and sardine was strong in 2004, 2005 and 2008, but was nearly inexistent in 2010, 2011, 2012 and 2014. These SIA are in accordance with stomach content analyses, which also provided evidence for potentially strong competition between sprat, sardine and anchovy (Le Bourg et al. 2015). Thus, this study underlines the importance of new food sharing in the context of the recent increase in the sprat population. The special case of 2013 might have been caused by very limited food resources (quantitatively or/and qualitatively), forcing the 3 species to highly exploit prey species with similar stable isotope ratios (possibly similar prey species).

Second, beyond prey size spectrum changes, we also showed the decreasing richness of small pelagic fish diets over time. This result is in agreement with observed and modelled changes in the composition and the phenology of zooplankton in Mediterranean areas surrounding the Gulf of Lions (Balearic Sea: Fernández de Puelles & Molinero 2008, Auger et al. 2014; Ligurian Sea: Molinero et al. 2005, 2008). Recent low prey diversity and small prey sizes suggest that recent environmental changes affected the planktonic production. Our study, despite some missing values in some years, showed a decreasing trend in $\delta^{13}\text{C}$ values for zooplankton. This is also in agreement with other studies, which underlined similar isotopic values in 2010 and in 2014, and most importantly strictly lower values in 2010 than in 2004 and 2005 (Bănaru et al. 2013, Espinasse et al. 2014, Strady et al. 2015). Although isotopic variations may result from a multitude of drivers, strong differences documented in this study could support important changes in the planktonic community and/or in the organic matter sources. Auger et al. (2014) showed that climatic and environmental variations may induce changes in size structure of the

plankton community, while Rau et al. (1990) showed that small phytoplankton (pico- and nanophytoplankton) had lower $\delta^{13}\text{C}$ values than microphytoplankton. Interestingly, the possible reduction in $\delta^{13}\text{C}$ values for zooplankton between 2004/2005 and 2010 corresponds to the period of drastic changes in small pelagic fish populations (Van Beveren et al. 2014). As already observed in the Benguela ecosystem, where shifts between anchovy and sardine regimes were caused by changes in the availability of mesozooplankton prey mediated through changes in environmental conditions (Shannon et al. 2004), growth and condition of sardine and secondarily anchovy in the Gulf of Lions are also likely to be mediated by bottom-up control.

CONCLUSION

This study supports the hypothesis that changes in small pelagic fish growth, size and body condition could be due to bottom-up control characterized by changes in food availability and/or increasing potential trophic competition, 2 factors that might influence feeding success and energy allocation. Our results illustrate the utility of comparing feeding habits between periods using a combination of short (stomach content) and longer-term (SIA) indicators. This not only helps to understand the current ecosystem fluctuations in the Gulf of Lions, but also provides insight into the trophic dynamics of the pelagic ecosystem, possibly allowing more efficient monitoring of marine food-web evolution.

Acknowledgements. We thank the captain and crew of RV 'L'Europe', as well as all the scientists onboard for their assistance during PELMED surveys. PELMED surveys are co-financed by the EU through the Data Collection Framework. We also thank Isabel Palomera for providing Spanish data, Mireille Harmelin-Vivien for comments on previous works, Gaël Guillou for performing stable isotope analyses and Virginie Marques for artwork help. This research was partly funded through the EcoPelGol project (Study of the Pelagic ecosystem in the Gulf of Lions), financed by France Filière Pêche (FFP) and partly funded through Policy-oriented marine Environmental Research for the Southern European Seas (PERSEUS) financed by FP7 EU project, End-to-end Modelling and Indicators for Biodiversity Scenarios (EMIBIOS) financed by the Fondation pour la Recherche et la Biodiversité (FRB) and Interactions plankton – planctonophages (IPP) financed by WP2 Marine Ecosystems Réponse dans l'Expérience de la Méditerranée (MERMEX). P.B. acknowledges doctoral fellowship support from the French Ministère de l'Éducation Nationale, de la Recherche et de la Technologie.

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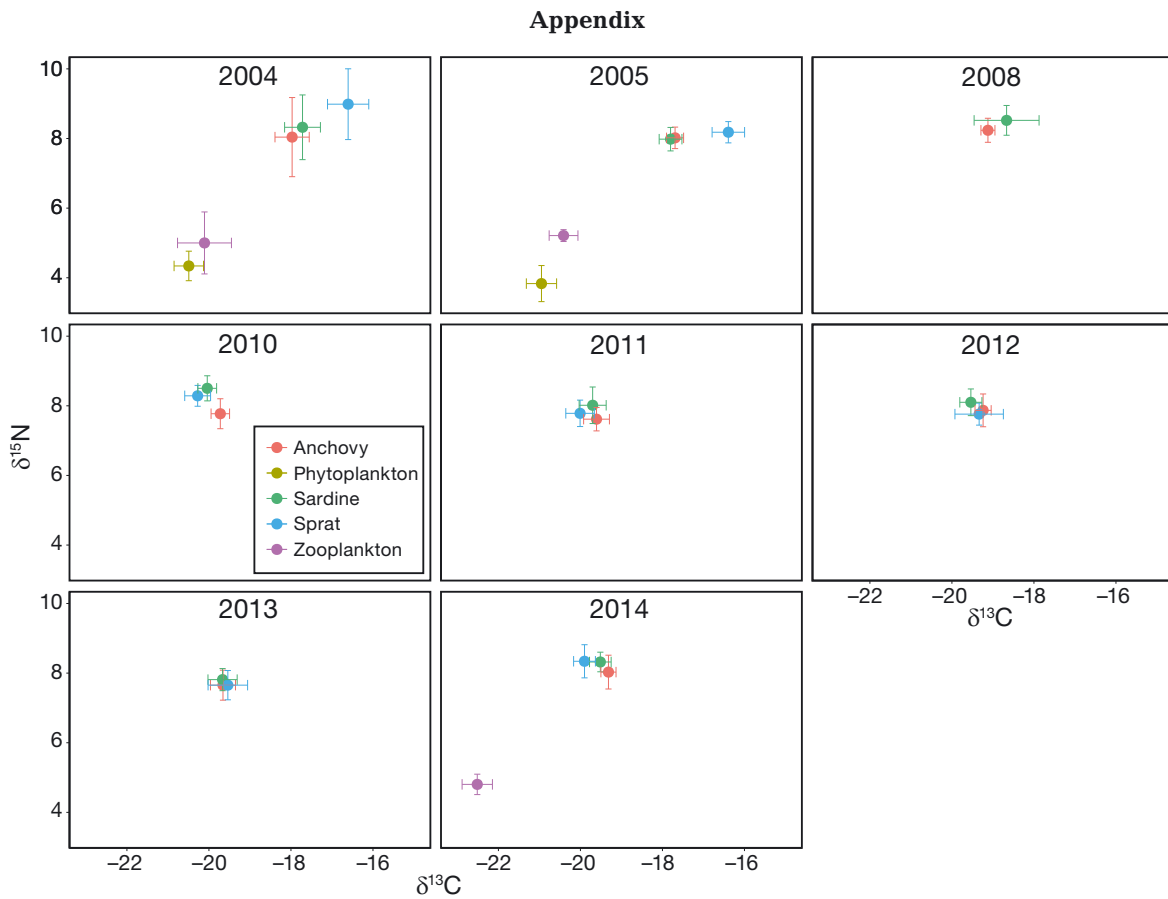


Fig. A1. Dual isotope plot representing the different small pelagic fish species sampled each year and the basal trophic levels (phytoplankton and zooplankton). All of the different groups are represented by mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (\pm SD)

Editorial responsibility: Stylianos Somarakis,
Heraklion, Greece

Submitted: March 29, 2016; Accepted: June 8, 2016
Proofs received from author(s): July 13, 2016