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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.

Keywords : Trophic ecology, Anchovy, Sardine, Sprat, Dietary overlap, NW Mediterranean

1. INTRODUCTION

Small pelagic fish species are characterized worldwide by important temporal fluctuations of 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 argued as the main hypothesis to explain these regime shifts (Cury & Shannon 2004), but changes in plankton quantity and quality is 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 three 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 years 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% in average without exceeding 40%, see Van Beveren et al. 2016). Predation pressure from Atlantic Bluefin tunas, the main top predator of those small pelagic fish in this area, has been recently evaluated to be < 2% (Van Beveren et al. *submitted*), indicating that a top-down control is unlikely. While predation due to natural predator or fishing remained at low levels for these species, body condition in sardine and anchovy has been shown to be strongly affected by mesozooplankton abundance (Brosset et al. 2015), advocating for a bottom-up control as the most probable source of small pelagic fish changes 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 a bottom-up control.

Stomach content analyses and stable isotope analyses (SIA) are two of the main approaches for investigating feeding habits and trophic interactions (Darnaude et al. 2004, Post et al. 2007). Stomach content analyses document recently consumed food items and permit a quantitative and qualitative snapshot of the diet (Hyslop 1980). SIA are complementary to stomach content analyses and allow obtaining an integrated measure of the assimilated food over the previous months depending on the variability of prey and their stable isotope ratios, the fractioning and the isotopic turnover. Values of δ^{15}N may be related to the trophic level of an individual, while the δ^{13}C ratio indicates the primary production sources, that is, the different feeding environments (coastal/oceanic, pelagic/benthic) used by consumers (Vander Zanden & Rasmussen 1999, 2001). Combining stomach content analyses 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 interspecific interactions (Caut et al. 2006).

Several studies have provided important information on feeding habits and diets of these 3 species in the Gulf of Lions (Plounevez & Champalbert 2000; Costalago et al 2012; Costalago and Palomera 2014; Pethybridge et al. 2014; Le Bourg et al 2015). However, they were usually limited to one species or a given period, so that the investigation of potential temporal changes along the last 15 to 20 years is still missing. The aim of this study was to investigate the hypothesis that recent changes observed in anchovy, sardine and sprat populations from the Gulf of Lions might result from diet changes. In this study, we propose to investigate changes in the feeding habits between periods of contrasted 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. Indeed, knowing that sardines, sprats and anchovies 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 δ^{15}N and δ^{13}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).

2. MATERIAL AND METHODS

2.1 Study area

The Gulf of Lions (42°26'-43°40'N and 3°00'-5°27'E; Fig. 1) is located in the North Western Mediterranean Sea and is characterized by a large continental shelf (Millot 1990). Shallow waters between 0 and 200 meters 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 exists from East to West and from 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 inside 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, as well as 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).

2.2 Sample collection

Fish and zooplankton samples were collected during MERLUMED and 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 µm mesh size) at each trawl station (4 stations in 2004 and 2005 and 10 in 2014, respectively). Zooplankton samples were stored in frozen sea water to be used for SIA as bulk. Using a combination of previously published data (Costalago et al. 2012, Le Bourg et al. 2015) and new samples that were analyzed for this study, stable isotope values were available for June and July of 2004, 2005, 2008, and 2010-2014 (except for sprat in 2008 which was not sampled). Stomach content data were available in 2011-2012 for both anchovy and sardine (previously published data; n = 118 & 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 et al. 2014) and in 1994 for anchovy (n=50, unpublished).

2.3 Stable isotope analyses

Fish isotope analyses were conducted on a piece of ~1 cm^3 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 during 48h and grounded into a fine powder before being encapsulated in a tin cup and sent for SIA analysis to the LIENSs laboratory

(La Rochelle, France). An acidification step was necessary for zooplankton samples to remove any ^{13}C-enriched carbonates (DeNiro & Epstein 1978). A subsample was acidified with 1% of HCl, rinsed with distilled water and dried to determine the δ^{13}C ratio while an untreated subsample was used for δ^{15}N analysis. Three zooplankton replicates were performed from each sampled site for both δ^{13}C and δ^{15}N. A continuous-flow isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific) coupled to an elemental analyzer (Flash EA1112, Thermo Scientific) was used to perform stable isotope measurements. Results were expressed in parts per thousand (‰) relative to Vienna PeeDee Belemnite and atmospheric N_2 for δ^{13}C and δ^{15}N, respectively, using the equation:

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

where X is ^{13}C or ^{15}N and R is the isotope ratio ^{13}C/^{12}C or ^{15}N/^{14}N, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicated a precision of 0.2‰ for both δ^{13}C and δ^{15}N 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 δ^{13}C 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.

2.4 Stomach content analyses

A similar protocol was applied on all analyses of stomach contents regardless of the year (see details in Costalago and Palomera, 2014; Le Bourg *et al.*, 2015). Briefly, fish were frozen at -20°C immediately after being caught to stop digestive processes. Then, the gut was removed and stored into alcohol (95%) at 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 preys 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 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 literature (see Le Bourg *et al.*, 2015).

Prey importance was assessed using the percentage of occurrence (%O, proportion of stomachs where the prey species was found), percentage in number (%N, ratio between the number of one prey species and the total number of prey), percentage in dry weight (%DW, ratio between the weight of one 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), necessary to compute the %IRI, which allows an integrated comparison between dietary items of the same species (Hyslop 1980, Cortés 1997):

$$\%\mathrm{IRI} = \frac{\mathrm{IRI}}{\sum_{a=1}^{n} \mathrm{IRI}} \text{ x } 100$$

where IRI = %O x (%N + %DW) and n is the number of prey species. Because of potential disparities in species or groups of species names between 1994 and 2011-2012, we carefully linked all prey names to current species name. Due to a lack of correspondence between periods, few species were not retained. However these species/groups of species, all had very low %IRI (< 0.2%), so that their absence could 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-Wienner diversity index: $-\sum_{i=1}^{q} pi \ln(pi)$ and *pi* is the proportion of IRI (Hill 1973, Medina et al. 2015).

2.5 Data analyses

Determinants of isotopic values

To investigate the potential effect of species, year, fish length, latitude, longitude and coastal/offshore gradient on $\delta^{13}C$ and $\delta^{15}N$ 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 explanatory variable, and each final group is characterized by its mean isotope values. Two separate CARTs were applied for fish $\delta^{13}C$ and $\delta^{15}N$ values. As less stations and years were sampled for zooplankton, we only tested annual differences in $\delta^{13}C$ and $\delta^{15}N$ to look for interannual variability (2004, 2005 and 2014) using one-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 analyze 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 & Villéger (2015) to obtain an informative assessment of isotopic overlap. Then, niche overlaps between the different species were determined using SEA_c. Niche overlap varied between 0 (no overlap) and 1 (one species is included in the other species stable isotope space). Additionally to niche overlap, the size of standard ellipses was also compared between species and between years for each species. To do that, 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 (e.g *p*, the proportion of ellipses in 2011 that were lower than 2012, see Jackson et al. (2011) for more details). We considered two 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 to make robust comparisons among data sets comprising different sample sizes (Syväranta 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 in function of prey using a correlation matrix. For each predator species, only prey constituting > 2% of %IRI in at least one of our data subsets were considered for the analysis, except for non-identified 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 (representing each 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 (%DW) 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 ± 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.

3. RESULTS

3.1 Factors influencing isotope values

Fish size, 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 δ^{13}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 δ^{13}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 δ^{13}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 δ^{13}C values, with no differences between the three species studied. Between 2004 and 2014, the δ^{13}C isotope values of all three 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 δ^{15}N signal was accounted for by year and species variables (Fig 3b). In 2005 and 2011 to 2013, δ^{15}N values were lower for anchovy and sprat in comparison to sardine (Fig 3b). In contrast, during all others years (2004, 2008, 2010 and 2014), sardine and sprat had more similar δ^{15}N values than anchovy. Only in anchovy in certain years did other variables affect isotope values. Indeed, 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 δ^{15}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 (δ^{15}N range), with no clear temporal trend (Fig 2).

Similarly to fish, the δ^{13}C values of the bulk of zooplankton were higher in 2004 and 2005 in comparison to 2014 (ANOVA, $p < 0.001$) while δ^{15}N values remained stable over time (ANOVA, $p > 0.05$; Fig.A.1).

3.2 Temporal changes in isotopic niche overlap

Figure 4 displays the bayesian ellipse areas (SEA_C) that represent the isotopic niche of the three small pelagic species in a scaled isotopic niche space. Two patterns were observed: firstly, sprat was segregated from sardine and anchovy during the two earliest years (2004 and 2005, Fig 4); second, the overlap of sprat isotopic niche on sardine's 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 the one from sardine. 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).

3.3 Temporal changes in isotopic niche width

The niche width of the three 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: $P < 0.01$) to then decrease again in 2010 (SEA_B: $P < 0.01$) and finally remain 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 three species (SEA_B: $P > 0.05$, Fig 2.). On the contrary, during 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.). Thus, sprat always had a larger or equivalent isotopic niche width when compared to anchovy or sardine. In 2008, when only two species were available, sardine SEA_B was significantly larger than the anchovy one (SEA_B: $P < 0.001$).

3.4 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 and 4). The anchovy diet in 1994 was characterized by the dominance of *Acartia clausi* (18.23 %IRI), *Microsetella* spp (13.52 %IRI), Clauso/Paracalanidae (13.22 %IRI), *Oncaea* spp. (12.67 %IRI) and Euphausiacaea larvae (7.31 %IRI)

(Table 3). Copepods also dominated the anchovy diet in 2011-2012, but anchovy fed almost only on two species: *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, see Table 4).

In the PCA performed on the dry weight (%DW) of the different prey of anchovy, the first (F1) and second (F2) component represented respectively 35% and 25% of the total variance (Fig 5). F1 represented a gradient whereby *Oncaea* spp was opposed mainly to *Acartia clausi* and Clauso/Paracalanidae species. F2 represented a gradient opposing *Microsetella* spp to *Acartia 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 *Acartia 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, F1 and F2 represented 44% and 18% of the total variance respectively (Fig 6.). *Microsetella* spp was opposed to *Oncaea* spp, *Corycaeus* spp and cladocerans on F1 axis, while the second axis showed a clear separation between the cladocerans and other prey species. The two 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.

4. DISCUSSION

Data availability does not allow us to describe the trophic ecology along the last 20 years, but we can contrast the present situation to data collected before the drastic changes in demography occurred. The comparison of small pelagic trophic data: (i) showed wider isotopic niche in sprat compared to sardine and anchovy; (ii) tended to confirm the hypothesis of changes in the anchovy and sardine diets and (iii) 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 $\delta^{13}C$. Assuming that wider sprat isotopic niche reflects higher variability in individual diets (Matthews & Mazumder 2004), sprat intraspecific 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 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 bottom-up processes were the most likely drivers (Van Beveren et al. 2014). Being a generalist feeder with low intraspecific 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 niche through time. The carbon isotopic composition of fish muscle is mainly related to the ingested preys (Hobson 1999). As previously stated, strong changes in δ^{13}C values of small pelagic fish and zooplankton were observed along years. 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 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 δ^{15}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 between 2004 and 2013, although the largest shrinkage occurred between 2004 and 2005. Nevertheless, this considerable change in isotopic niche area between the two 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 of 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%, respectively). 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 has been 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 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 relative importance index (%IRI) and 65% in relative dry weight (%DW). This contrasted with 2011-2012 years when they represented barely more than 5% of the relative importance index and 15% in relative dry weight (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 Coryceidae, *Microsetella* spp. and *Oncaea* spp.), which increased threefold. 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 practically absent in recent years, 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 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 to build a sufficient fat to survive an energetically demanding winter, especially so for sardines which reproduce at that moment. This might thus explain the apparent adult overmortality 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 three 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 an 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. Indeed, there was clear isotopic niche segregation between sprat and the two other species in 2004 and 2005, indicating that sprat could have exploited different species groups from anchovy and sardine during these still prosperous years. On the contrary, 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 stable isotope analyses are in accordance with stomach content analyses, which also provided evidence for potential strong competition between sprat, sardine and anchovy (Le Bourg et al. 2015). So, this study underlined the importance of new food sharing in the context of the recent increase of the sprat population. The special case of 2013 might be caused by very limited food resources (quantitatively or/and qualitatively), forcing the three 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 diet with time. This result was in agreement with observed and modeled changes in the composition and the phenology of zooplankton in Mediterranean areas surrounding the Gulf of Lions (Balearic Sea: Auger et al., 2014; Puelles and Molinero, 2008; Ligurian Sea: Molinero et al., 2008, 2005). Recent low prey diversity and small prey sizes suggest that recent environmental changes affected the planktonic production. This study, despite some missing values in some years, showed a decreasing trend in δ^{13}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 nano phytoplankton) had lower δ^{13}C values than micro phytoplankton. Interestingly, possible reduction in δ^{13}C values for zooplankton between 2004/2005 and 2010 correspond to the period of the 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 a bottom-up control.

CONCLUSION

This study supports the hypothesis that changes in small pelagic fish growth, size and body condition could be due to a bottom-up control characterized by changes in food availability or/and increasing potential trophic competition, two other factors that might influence the feeding success and the 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 an insight in the trophic dynamics of the pelagic ecosystem, possibly allowing a more efficient monitoring of marine food-web evolution.

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References

Alheit J, Niquen M (2004) Regime shifts in the Humboldt Current ecosystem. Progress in Oceanography 60:201–222

André G, Garreau P, Garnier V, Fraunié P (2005) Modelled variability of the sea surface circulation in the North-western Mediterranean Sea and in the Gulf of Lions. Ocean Dynamics 55:294–308

Auger PA, Ulses C, Estournel C, Stemmann L, Somot S, Diaz F (2014) Interannual control of plankton communities by deep winter mixing and prey/predator interactions in the NW Mediterranean: Results from a 30-year 3D modeling study. Progress in Oceanography 124:12–27

Ayón P, Swartzman G, Espinoza P, Bertrand A (2011) Long-term changes in zooplankton size distribution in the Peruvian Humboldt Current System: conditions favouring sardine or anchovy. Mar Ecol Prog Ser 422:211–222

Bănaru D, Carlotti F, Barani A, Grégori G, Neffati N, Harmelin-Vivien M (2014) Seasonal variation of stable isotope ratios of size-fractionated zooplankton in the Bay of Marseille (NW Mediterranean Sea). J Plankton Res 36:145–156

Bănaru D, Mellon-Duval C, Roos D, Bigot J-L, Souplet A, Jadaud A, Beaubrun P, Fromentin J-M (2013) Trophic structure in the Gulf of Lions marine ecosystem (north-western Mediterranean Sea) and fishing impacts. Journal of Marine Systems 111–112:45–68

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology 73:1007–1012

Beaugrand G, Brander KM, Alistair Lindley J, Souissi S, Reid PC (2003) Plankton effect on cod recruitment in the North Sea. Nature 426:661–664

Blanchard JL, Jennings S, Holmes R, Harle J, Merino G, Allen JI, Holt J, Dulvy NK, Barange M (2012) Potential consequences of climate change for primary production and fish production in large marine ecosystems. Phil Trans R Soc B 367:2979–2989

Borme D, Tirelli V, Brandt S, Fonda Umani S, Arneri E (2009) Diet of *Engraulis encrasicolus* in the northern Adriatic Sea (Mediterranean): ontogenetic changes and feeding selectivity. Marine Ecology Progress Series 392:193–209

Breiman L, Friedman JH, Olshen RA, Stone CJ (1984) Classification and Regression Trees. Chapman and Hall, London

Brosset P, Ménard F, Fromentin J, Bonhommeau S, Ulses C, Bourdeix J, Bigot J, Van Beveren E, Roos D, Saraux C (2015) Influence of environmental variability and age on small pelagic fish body condition in the Gulf of Lions. Marine Ecology Progress Series

Brosset P, Menard F, Fromentin J, Bonhommeau S, Ulses C, Bourdeix J, Bigot J, Van Beveren E, Roos D, Saraux C (2015) Influence of environmental variability and age on the body condition of small pelagic fish in the Gulf of Lions. Mar Ecol Prog Ser 529:219–231

Caut S, Roemer GW, Donlan CJ, Courchamp F (2006) Coupling stable isotopes with bioenergetics to estimate interspecific interactions. Ecological Applications 16:1893–1900

Cortés E (1997) A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. Can J Fish Aquat Sci 54:726–738

Costalago D, Navarro J, Alvarez-Calleja I, Palomera I (2012) Ontogenetic and seasonal changes in the feeding habits and trophic levels of two small pelagic fish species. Mar Ecol Prog Ser 460:169–181

Costalago D, Palomera I (2014) Feeding of European pilchard (Sardina pilchardus) in the northwestern Mediterranean: from late larvae to adults. Scientia Marina 78:41–54

Cucherousset J, Villéger S (2015) Quantifying the multiple facets of isotopic diversity: New metrics for stable isotope ecology. Ecological Indicators 56:152–160

Cury P, Shannon L (2004) Regime shifts in upwelling ecosystems: observed changes and possible mechanisms in the northern and southern Benguela. Progress in Oceanography 60:223–243

Darnaude AM, Salen-Picard C, Polunin NVC, Harmelin-Vivien ML (2004) Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138:325–332

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506

Dumont HJ, Velde IV de, Dumont S (1975) The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 19:75–97

Espinasse B, Harmelin-Vivien M, Tiano M, Guilloux L, Carlotti F (2014) Patterns of variations in C and N stable isotope ratios in size-fractionated zooplankton in the Gulf of Lion, NW Mediterranean Sea. J Plankton Res 36:1204–1215

GFCM (2014) Report of the working group on stock assessment of small pelagic species, p39.

Hardin G (1960) The competitive exclusion principle. Science 131:1292–1297

Harmelin-Vivien M, Loizeau V, Mellon C, Beker B, Arlhac D, Bodiguel X, Ferraton F, Hermand R, Philippon X, Salen-Picard C (2008) Comparison of C and N stable isotope ratios between surface particulate organic matter and microphytoplankton in the Gulf of Lions (NW Mediterranean). Continental Shelf Research 28:1911–1919

Hill MO (1973) Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology 54:427–432

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326

Hyslop EJ (1980) Stomach contents analysis—a review of methods and their application. Journal of Fish Biology 17:411–429

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80:595–602

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42–48

Le Bourg B, Bănaru D, Saraux C, Nowaczyk A, Le Luherne E, Jadaud A, Bigot JL, Richard P (2015) Trophic niche overlap of sprat and commercial small pelagic teleosts in the Gulf of Lions (NW Mediterranean Sea). Journal of Sea Research 103:138–146

Lefevre D, Minas HJ, Minas M, Robinson C, Le B. Williams PJ, Woodward EMS (1997) Review of gross community production, primary production, net community production and dark community respiration in the Gulf of Lions. Deep Sea Research Part II: Topical Studies in Oceanography 44:801–832

Matthews B, Mazumder A (2004) A critical evaluation of intrapopulation variation of δ13C and isotopic evidence of individual specialization. Oecologia 140:361–371

Medina A, Goñi N, Arrizabalaga H, Varela JL (2015) Feeding patterns of age-0 bluefin tuna in the western Mediterranean inferred from stomach-content and isotope analyses. Marine Ecology Progress Series 527:193–204

Millot C (1990) The Gulf of Lions' hydrodynamics. Continental Shelf Research 10:885–894

Minas M, Minas H. (1989) Primary production in the Gulf of Lions with considerations to the Rhone River inputs. Water Pollution Research Reports 32(5):112–125

Molinero JC, Ibanez F, Nival P, Buecher E, Souissi S (2005) North Atlantic climate and northwestern Mediterranean plankton variability. Limnology and Oceanography 50:1213–1220

Molinero JC, Ibanez F, Souissi S, Buecher E, Dallot S, Nival P (2008) Climate control on the long-term anomalous changes of zooplankton communities in the Northwestern Mediterranean. Global Change Biology 14:11–26

Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Frontiers in Ecology and the Environment 5:429–436

Nikolioudakis N, Isari S, Pitta P, Somarakis S (2012) Diet of sardine Sardina pilchardus: an "end-to-end" field study. Mar Ecol Prog Ser 453:173–188

Palomera I, Olivar MP, Salat J, Sabatés A, Coll M, García A, Morales-Nin B (2007) Small pelagic fish in the NW Mediterranean Sea: An ecological review. Progress in Oceanography 74:377–396

Pethybridge H, Bodin N, ArsenaultPernet EJ, Bourdeix JH, Brisset B, Bigot JL, Roos D, Peter M (2014) Temporal and inter-specific variations in forage fish feeding conditions in the NW Mediterranean: lipid content and fatty acid compositional changes. Mar Ecol Prog Ser 512:39–54

Pinkas L, Oliphant M., Iverson I (1971) Food Habits of Albacore, Bluefin Tuna, and Bonito In California Waters.

Plounevez S, Champalbert G (2000) Diet, feeding behaviour and trophic activity of the anchovy (*Engraulis encrasicolus*, L.) in the Gulf of Lions (Mediterranean Sea). Oceanologica Acta 23:175–192

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189

Puelles MLF de, Molinero JC (2008) Decadal changes in hydrographic and ecological time-series in the Balearic Sea (western Mediterranean), identifying links between climate and zooplankton. ICES J Mar Sci 65:311–317

Rau GH, Teyssie JL, Rassoulzadegan F, Fowler SW (1990) C-13/C-12 AND N-15/N-14 VARIATIONS AMONG SIZE-FRACTIONATED MARINE PARTICLES-IMPLICATIONS FOR THEIR ORIGIN AND TROPHIC RELATIONSHIPS. Marine Ecology Progress Series 59:33–38

Sánchez-Gómez S (2013) Advances in the knowledge of condition and energy budgets along the life cycle of two small pelagic fishes from the Western Mediterranean.

Saraux C, Fromentin J-M, Bigot J-L, Bourdeix J-H, Morfin M, Roos D, Van Beveren E, Bez N (2014) Spatial Structure and Distribution of Small Pelagic Fish in the Northwestern Mediterranean Sea. PLoS ONE 9:e111211

Schwartzlose RA, Alheit J, Bakun A, Baumgartner TR, Cloete R, Crawford RJM, Fletcher WJ, Green-Ruiz Y, Hagen E, Kawasaki T, Lluch-Belda D, Lluch-Cota SE, MacCall AD, Matsuura Y, Nevárez-Martínez MO, Parrish RH, Roy C, Serra R, Shust KV, Ward MN, Zuzunaga JZ (1999) Worldwide large-scale fluctuations of sardine and anchovy populations. South African Journal of Marine Science 21:289–347

Shannon LJ, Field JG, Moloney CL (2004) Simulating anchovy–sardine regime shifts in the southern Benguela ecosystem. Ecological Modelling 172:269–281

Strady E, Harmelin-Vivien M, Chiffoleau JF, Veron A, Tronczynski J, Radakovitch O (2015) 210 Po and 210 Pb trophic transfer within the phytoplankton–zooplankton–anchovy/sardine food web: a case study from the Gulf of Lion (NW Mediterranean Sea). Journal of environmental radioactivity 143:141–151

Sweeting CJ, Jennings S, Polunin NVC (2005) Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. Functional Ecology 19:777–784

Syväranta J, Lensu A, Marjomäki TJ, Oksanen S, Jones RI (2013) An Empirical Evaluation of the Utility of Convex Hull and Standard Ellipse Areas for Assessing Population Niche Widths from Stable Isotope Data. PLoS ONE 8:e56094

Tudela S, Palomera I (1997) Trophic ecology of the European anchovy *Engraulis encrasicolus* in the Catalan Sea (northwest Mediterranean). Marine Ecology Progress Series 160:121–134

Turner TF, Collyer ML, Krabbenhoft TJ (2010) A general hypothesis-testing framework for stable isotope ratios in ecological studies. Ecology 91:2227–2233

Van Beveren E, Bonhommeau S, Fromentin J-M, Bigot J-L, Bourdeix J-H, Brosset P, Roos D, Saraux C (2014) Rapid changes in growth, condition, size and age of small pelagic fish in the Mediterranean. Mar Biol:1–14

Van Beveren E, Fromentin J-M, Rouyer T, Bonhommeau S, Brosset P, Saraux C (2016) The fisheries history of small pelagics in the Northern Mediterranean. ICES J Mar Sci

Vander Zanden MJ, Rasmussen JB (1999) Primary consumer δ13c and δ15n and the trophic position of aquatic consumers. Ecology 80:1395–1404

Vander Zanden MJ, Rasmussen JB (2001) Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. Limnology and oceanography 46:2061–2066

Vijverberg J, Frank TH (1976) The chemical composition and energy contents of copepods and cladocerans in relation to their size. Freshwater Biology 6:333–345

Wiegand MD (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. Rev Fish Biol Fisheries 6:259–286

Zarubin M, Farstey V, Wold A, Falk-Petersen S, Genin A (2014) Intraspecific Differences in Lipid Content of Calanoid Copepods across Fine-Scale Depth Ranges within the Photic Layer. PLoS ONE 9:e92935

Table 1. Mean standard length (in mm ± standard deviation, sd) and number of samples analysed (n) for stable isotopes of *Engraulis encrasicolus*, *Sardina pilchardus* and *Sprattus sprattus.*

Year	*Engraulis encrasicolus* Standard length (mm)		*Sardina pilchardus* Standard length (mm)		*Sprattus sprattus* Standard length (mm)	
	Mean ± sd	n	Mean ± sd	n	Mean ± sd	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

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 that are overlapped with the ellipses of the anchovy while 52% is the percentage of the ellipses of anchovy that are overlapped with sardine).

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

Table 3. Summary of stomach content analysis for *E. encrasicolus*. %N: Percent number; %W: percent estimated dry weight; %O: percent frequency of occurrence; %IRI: percent Index of Relative Importance; Exp(H): trophic niche width determinate with stomach content.

Prey type		**1994**				**2011 and 2012**			
		%N	%W	%O	%IRI	%N	%W	%O	%IRI
Crustacea									
Copepods									
	Acartia clausi	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
	Centropages typicus	9.76	4.82	50	6.21	3.60	3.64	21.15	1.81
	Corycaeus spp.	1.72	2.05	55.26	1.78	4.45	23.81	43.27	14.46
	Clytemnestra	0.02	<0.01	2.63	<0.01	0.08	<0.01	1.92	<0.01
	Candacia spp.	4.00	8.15	44.74	4.63	0.90	1.10	10.58	0.25
	Oithona spp.	1.48	0.07	42.11	0.55	0.12	<0.01	5.77	<0.01
	Oncaea spp.	15.30	0.39	94.74	12.67	20.15	15.43	65.38	27.52
	Microsetella spp.	16.08	0.22	97.37	13.52	26.38	13.34	74.04	34.78
	Euterpina acutifrons	1.25	0.36	94.74	1.30	2.01	0.13	36.54	0.89
	Temora stylifera	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	Diatoms	-	-	-	-	9.21	9.60	3.85	0.86
Chaetognatha									
	Chaetognatha	0.05	0.01	2.63	<0.01	-	-	-	-
Larvae									
Crustacae	Euphausiacae	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	Gasteropodae	<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 egg's	0.15	0.09	47.37	0.10	1.1	0.49	16.35	0.31
	Other fish egg's	0.24	0.42	50	0.28	0.14	<0.01	2.88	<0.01
		Exp(H) = 10.84				Exp(H) = 5.57			

Table 4. Summary of stomach content analysis for *S. pilchardus*. %N: Percent number; %W: percent estimated dry weight; %O: percent frequency of occurrence; %IRI: percent Index of Relative Importance; Exp(*H*): trophic niche width determinate with stomach content.

Prey type			**2007**				**2011 and 2012**			
			%N	%W	%O	%IRI	%N	%W	%O	%IRI
Crustacea										
	Copepods									
		Acartia clausi	-	-	-	-	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
		Centropages typicus	-	-	-	-	1.51	1.55	0.13	0.49
		Corycaeus spp.	4.09	4.11	75.00	4.28	8.61	30.10	0.46	21.64
		Clytemnestra	-	-	-	-	0.43	0.16	0.03	0.02
		Candacia spp.	0.16	18.27	12.50	1.60	0.13	1.04	0.03	0.04
		Oithona spp.	-	-	-	-	0.16	0.04	0.04	<0.01
		Oncaea spp.	11.58	0.61	100	8.48	17.61	12.27	0.68	24.55
		Microsetella spp.	6.31	11.11	87.50	10.60	24.65	10.09	0.70	29.34
		Euterpina acutifrons	3.82	19.27	62.50	10.03	9.12	4.43	0.36	5.87
		Temora stylifera	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	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										
	Crustacae	Euphausiacae	-	-	-	-	-	-	-	-
		Decapoda	0.54	2.63	37.5	0.83	0.09	0.76	0.05	0.05
	Mollusca	Gasteropodae	-	-	-	-	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 egg’s	-	-	-	-	5.40	6.87	0.11	1.68
		Other fish egg’s	-	-	-	-	2.80	0.83	0.21	0.91
			Exp(*H*) = 8.54				Exp(*H*) = 6.46			

Figure captions:

Figure 1. Sampling sites in the Gulf of Lions (Northwestern Mediterranean Sea). The left panel indicates isotope sampling locations and the right one stomach content sampling locations.

Figure 2. Summary of a) δ^{13}C, b) δ^{15}N values (mean, s.d., in ‰) and c) trophic niche size of the 3 species over time in the Gulf of Lions. SEA_C (SEA_C: standard ellipse area) are indicated in ‰². The Bayesian area estimate of the standard ellipse metrics (SEA_B) that present differences between years for each species are indicated by superscripts.

Figure 3. Classification and regression tree assessing the importance of year, species, fish length, latitude, longitude and coastal/offshore gradient on (A) δ^{13}C values and (B) δ^{15}N values. Trees are split off on the values of one covariate at a time such that the overall variance in the dependent variable is minimized at each split. Terminal nodes indicate the value of assigned δ^{13}C or δ^{15}N to the node.

Figure 4. Species scaled isotopic niche space between 2004 and 2014, represented by solid bold lines based on the area of the standard ellipses corrected to small samples sizes (SEAc). Stable carbon and nitrogen isotope values are from the muscle of adult anchovy, sardine and sprat.

Figure 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; Onca: *Oncaea* spp; Cand: *Candacia* spp; Acar: *Acartia clausi*; Euph: Euphausiacae; Cent: *Centropages typicus*. Barycenters of the years (Y) are added as supplementary variables.

Figure 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. Barycenters of the years (Y) are added as supplementary variables.

Figure 1.

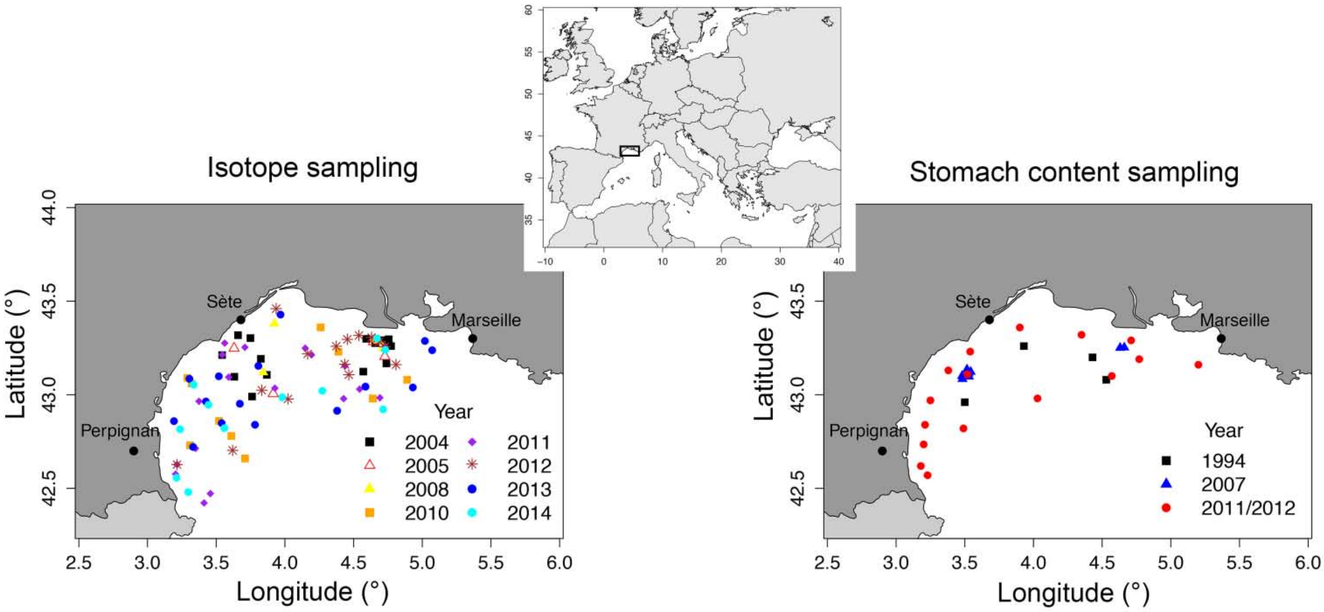

Isotope sampling
Stomach content sampling
Latitude (°)
Longitude (°)
Sète
Marseille
Perpignan
Year
2004
2005
2008
2010
2011
2012
2013
2014
1994
2007
2011/2012
42.5
43.0
43.5
44.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
-10
0
10
20
30
40
35
40
45
50
55
60

Figure 2.

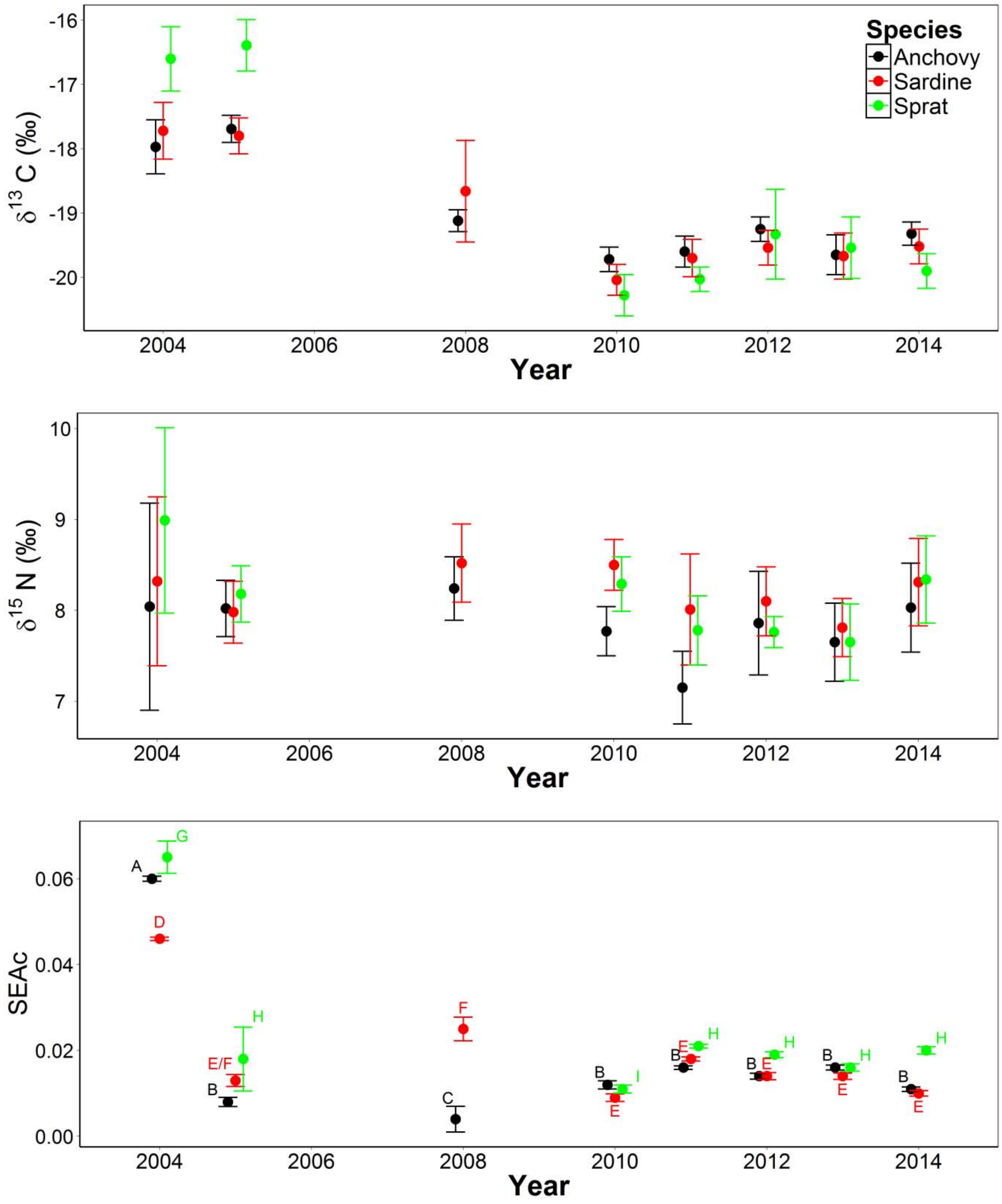

Species
Anchovy
Sardine
Sprat
δ^{13} C (‰)
-16
-17
-18
-19
-20
Year
2004
2006
2008
2010
2012
2014
δ^{15} N (‰)
10
9
8
7
Year
2004
2006
2008
2010
2012
2014
SEAc
0.06
0.04
0.02
0.00
A
G
D
H
E/F
B
F
C
B
I
E
B
F
H
B
E
H
B
H
E
B
H
E
Year
2004
2006
2008
2010
2012
2014

Figure 3.

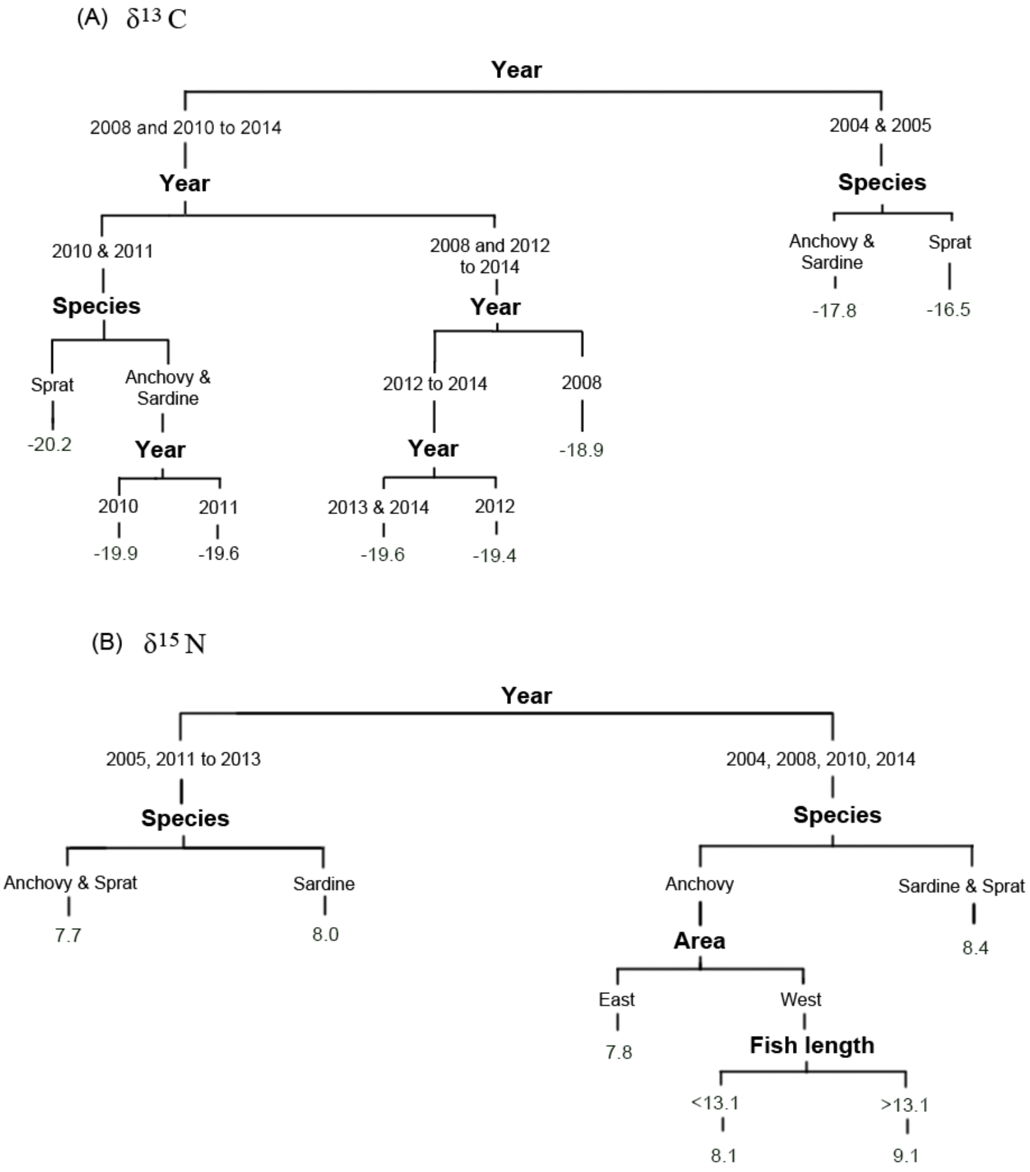
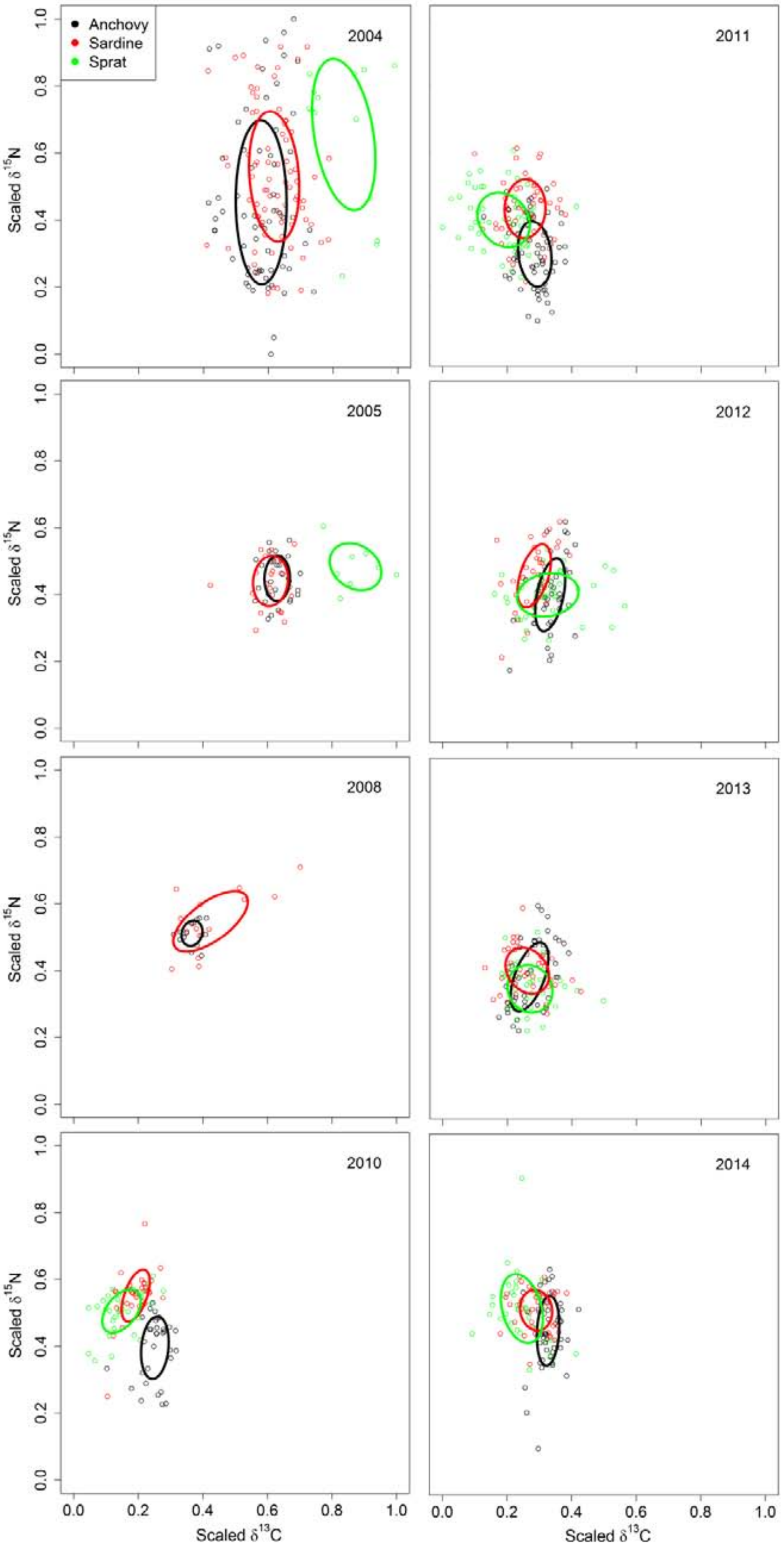
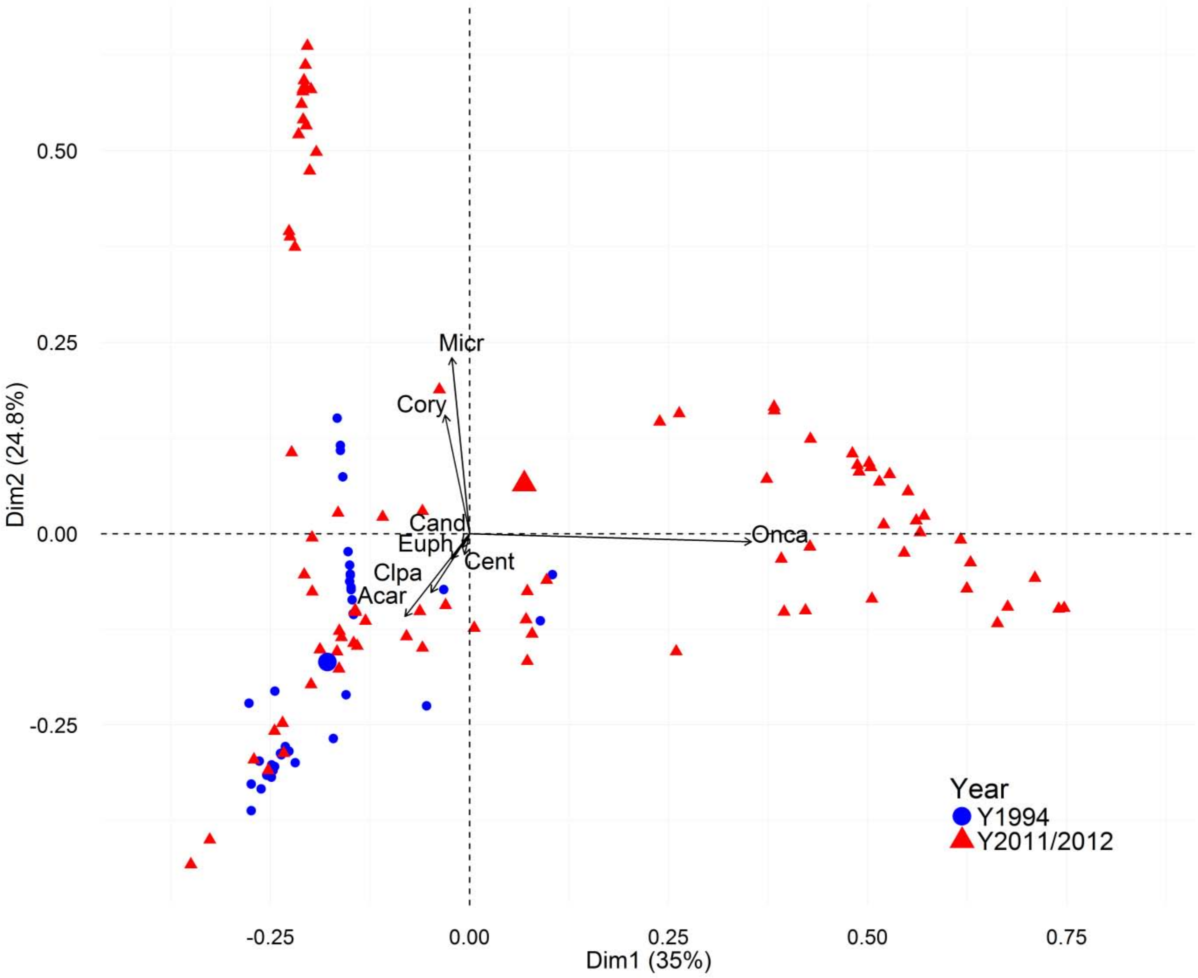

(A) δ13 C
Year
2008 and 2010 to 2014
2004 & 2005
Year
Species
2010 & 2011
2008 and 2012 to 2014
Anchovy & Sardine
Sprat
-17.8
-16.5
Species
Year
Sprat
Anchovy & Sardine
2012 to 2014
2008
-20.2
Year
Year
-18.9
2010
2011
2013 & 2014
2012
-19.9
-19.6
-19.6
-19.4
(B) δ15 N
Year
2005, 2011 to 2013
2004, 2008, 2010, 2014
Species
Species
Anchovy & Sprat
Sardine
Anchovy
Sardine & Sprat
7.7
8.0
Area
8.4
East
West
7.8
Fish length
<13.1
>13.1
8.1
9.1

Figure 4.



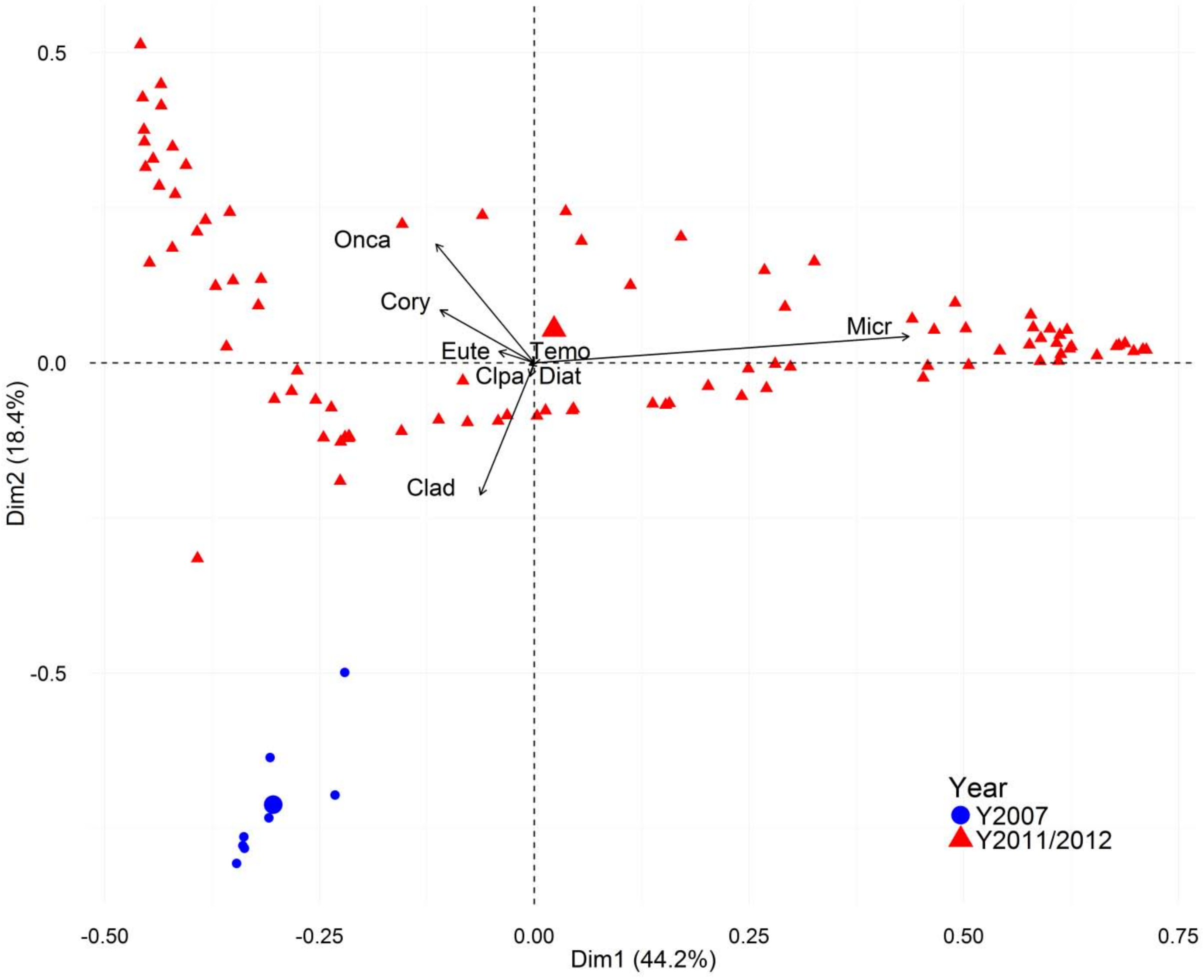
● Anchovy
● Sardine
● Sprat
2004
2011
2005
2012
2008
2013
2010
2014
Scaled δ^{15}N
Scaled δ^{13}C
0.0
0.2
0.4
0.6
0.8
1.0

Figure 5.



0.50
0.25
0.00
-0.25
Dim2 (24.8%)
-0.25
0.00
0.25
0.50
0.75
Dim1 (35%)
Micr
Cory
Cand
Euph
Clpa
Acar
Cent
Onca
Year
Y1994
Y2011/2012

Figure 6.


Onca
Cory
Eute
Temo
Clpa
Diat
Clad
Micr
0.5
0.0
-0.5
Dim2 (18.4%)
-0.50
-0.25
0.00
0.25
0.50
0.75
Dim1 (44.2%)
Year
Y2007
Y2011/2012