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Stable nitrogen isotope studies of the pelagic food web on the Atlantic shelf of the Iberian Peninsula

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Abstract

The structure and variability of pelagic food webs along the north and northwestern shelf of the Iberian Peninsula were analysed using natural abundance of nitrogen stable isotopes of plankton and pelagic consumers. Plankton composition was mainly studied in size-fractionated samples, but also the isotopic signatures of three copepod species, as representative of primary consumers, were considered. Several fish species were included as planktivorous consumers, with special attention to sardine (*Sardina pilchardus*). Finally, top pelagic consumers were represented by the common dolphin (*Delphinus delphis*). The relationship between trophic position and body size implies large variability in the ratio of predator to prey sizes, likely because widespread omnivory and plankton consumption by relatively large predators. Planktivorous species share a common trophic position, suggesting potential competition for food, and low nitrogen isotope enrichment between prey and consumers suggest nutrient limitation and recycling at the base of the food web. Both experimental and field evidences indicate that the muscle of sardine integrates fish diet over seasonal periods and reflects the composition of plankton from large shelf areas. The low mobility of sardines during periods of low population size is consistent with differential isotopic signatures found in shelf zones characterised by upwelling nutrient inputs.

Keywords: stable isotopes, nitrogen, plankton, fish, pelagic, food web, NE Atlantic

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References

1. Introduction

Body size is a powerful predictor of general properties of ecosystems, as most metabolic processes are scaled to size (Kerr & Dickie, 2001). In marine ecosystems trophic interactions are often dependent on size, as primary producers are generally of small size while consumers are progressively larger. Food webs are thus structured in terms of organism size, as demonstrated in plankton (Fry & Quiñones, 1994, Rolff, 2000) and fish communities (Jennings et al., 2002a; Jennings & Mackinson, 2003). Furthermore, the position of a particular fish species in the food web may be more dependent on its individual size than on its taxonomical status (Jennings et al., 2001). One application of size-based analysis has been to describe the structure of benthic food webs and their response to human impacts (France et al., 1998; Jennings et al., 2002b). Food web changes are also expected in the pelagic domain because of the large environmental variability (e.g. Cushing, 1978) and fishing pressure (Pauly et al., 2001). Omnivory and large variability in diet of most consumers, however, complicates the analysis of pelagic food webs from examination of gut-contents (Varela et al., 1990; Olaso et al.,1999; Garrido, 2003). Alternatively, the trophic position of consumers in the food web can be estimated from the accumulation of heavy isotopes in their structural tissues, as lighter isotopes are easily mobilised in metabolic reactions. Nitrogen stable isotopes are often used because they display relatively large variations in most food webs (Wada & Hattori, 1991; Vander Zanden & Rasmussen, 2001). The comparison of isotopic signatures of species with very different generation times is complicated by the different turnover of the isotopes in each species: from days in plankton to months or years in large predators (O'Reilly et al. 2002; Post, 2002). Field studies overcome this difficulty by sampling at spatial scales large enough to cover most of the range of variability in the nutrient sources at the base of the food web (Sholto-Douglas et al., 1991; Jennings et al., 2001, 2002a, b), which is the primary determinant of isotopic enrichment at upper trophic levels (Post, 2002). Such strategy is particularly suited to upwelling ecosystems where there are marked variations in the nutrient sources over relatively short spatial and temporal scales.

Two different types of food webs are expected in the pelagic shelf ecosystem in the Atlantic margin of the Iberian Peninsula. First, the seasonal upwelling from March to October produces significant inputs of nutrients sustaining high values of plankton biomass (Alvarez-Salgado et al., 2002). The upwelling is forced by northern winds that produce the Ekman transport of surface waters offshore. The effects of upwelling in the western shelf (Portugal and Galicia) are higher than those in the northern region (Mar Cantábrico), which receives lower nutrient inputs (Botas et al., 1990). During upwelling, a metazoan food web based on new nutrients from the upwelling (as nitrate) would be expected (Cushing, 1978). On the other hand, a coastal poleward current flows during autumn and winter in an opposite direction to upwelling surface currents (Alvarez-Salgado et al., 2003; Cabanas et al., 2003). Waters inside the poleward current contain less nutrients than waters fertilised by the upwelling and display low plankton biomass (Fernández et al., 1993). In this situation, a microbial food web based on the remineralisation of the organic matter would be expected. As light isotopes are preferentially mobilised in metabolic processes, plankton growing on nutrients remineralised in situ is expected to have a lower proportion of heavy isotopes than plankton growing on new nutrients originated in deep waters, as those supplied by upwelling (e.g. Wada & Hattori, 1991). Both oceanographic and trophic situations can be found during the transition from winter to spring (Calvo-Diaz et al., 2004).

Large populations of planktivorous fish, such as the sardine (*Sardina pilchardus*) show ample fluctuations in size and distribution over the Atlantic Iberian shelf (Carrera & Porteiro, 2003; ICES, 2005). Upwelling intensity was shown to affect both positive and negatively sardine recruitment in this area (Dickson et al., 1988; Roy et al., 1995) but the main direct effect was due to the transport of eggs and larvae offshore by northern winds (Guisande et al., 2001). In this way, strong upwelling during the recruitment season would decrease the probability of survival of sardine larvae as they are dispersed to outer shelf and oceanic zones. In contrast, southern winds favour the progress of the poleward current, which tend to accumulate fish larvae near the coast where plankton biomass and production are high (Fernández et al., 1993). At high population sizes, sardines spawning and distribution areas extend over the whole continental shelf and the adults display feeding migrations to the upwelling area off Galicia, while at low population sizes a reduction in the mobility of adult sardines between the Mar Cantábrico and Galicia was expected (Carrera & Porteiro, 2003). In the latter case, sardines will remain in the same areas where they were hatched.

The objective of the study was to determine the trophic structure of the pelagic ecosystem on the Atlantic shelf of the Iberian Peninsula and its spatial and interannual variability. For this purpose, the natural abundance of nitrogen stable isotopes was measured in plankton and representative nektonic species. Special attention was dedicated to the case of the sardine, as a key pelagic species. Previous studies have explored the applicability of stable isotopes to dilucidate the complexity of the pelagic food web (Bode et al., 2003, 2006), the diet of sardines (Bode et al., 2004a) and the succession of zooplankton (Bode & Alvarez-Ossorio, 2004) in this region.

2. Materials and Methods

2.1. Survey cruises

Samples of plankton and pelagic fish were collected mostly during the annual surveys of pelagic fishes in the Atlantic coast of the Iberian Peninsula between 1998 and 2004 (Table 1). The main objective of these cruises was to estimate the size of the spawning populations of the small pelagic fish species. Details on the methods for estimating fish biomass and geographic coverage can be found in ICES (2005). Each of the surveys covered at least the northern and northwestern shelves and included plankton and sardines. For comparative purposes, the study area was divided into several zones (Fig. 1). Zone I included the Portuguese shelf south of Cabo da Rocha (ca. 39 °N). Zone II extended over the northern Portuguese and Galician shelves, entering into the southern Bay of Biscay up to 8 °W. This zone was further divided in two subzones IIa and IIb, north and south of Cape Finisterre (ca. 43 °N), respectively. Finally, zone III included all samples in the southern Bay of Biscay (Mar Cantábrico) between 8 °W and 46 °N. The studied area corresponds approximately to ICES fishery areas VIIIc and IXa (ICES 2005). General oceanographic conditions for the sampling years were summarized by the upwelling and poleward index values. The former indicated the strength of the upwelling due to North-East winds (Alvarez-salgado et al., 2002) and the latter the strength of the winter poleward flow of shelf water, generally associated to downwelling conditions (Alvarez-Salgado et al., 2003; Cabanas et al., 2003). The upwelling index was computed from geostrophic wind at a 2ºx2º cell centred at 42º 30’ N, 12º 30’ W by the Pacific Environmental Laboratory (PFEL, http://www.pfeg.noaa.gov/). Monthly upwelling index values were averaged between March and October (upwelling season) for the year preceding each sampling cruise. The poleward index was computed as the upwelling index but in this case the cell was centred at 43º N, 11º W and the averaged period was from October to December (Alvarez-Salgado et al., 2003; Cabanas et al., 2003).

2.2. Plankton

Plankton samples were generally collected during the night using a conical net from 100 m depth to the surface at stations distributed over the shelf up to the shelf break (Fig. 1). The size of the mesh was 20 μm for most cruises and 40 μm for PELACUS 0303 and 0304. Most samples were subsequently fractionated through sieves of 40, 80, 200, 500, 1000 and 2000 μm and each fraction was carefully washed with filtered seawater, transferred to glass-fibre filters and stored frozen. The plankton retained by the 2000 μm sieve (generally large salps) was not used in this study. In addition, water from the surface was prefiltered through a sieve of 20 μm mesh-size and subsequently filtered through Whatman glass-fibre GF/F filters to characterise seston < 20 μm. These filters were stored frozen and processed as the filters with net plankton samples.

The natural abundance of ^{15}N in selected copepods, which were assumed to be representative of primary consumers, was analysed in samples collected at 43° 40' N, 5° 35' W off Gijón (NW Spain) between March 2001 and May 2004. Adult copepods of genera *Acartia, Calanus* and *Centropages* (mostly *A. clausi*, *C. helgolandicus* and *C. typicus*, respectively) were selected from samples collected with vertical hauls of a WP_2 type net (200 μm mesh-size) between 100 m depth and the surface.

2.3. Planktivorous and top consumers

Fish samples were collected by means of a pelagic trawl, in the case of annual fish surveys, or purse seine nets, in the case of commercial landings of sardine. Squids (*Allotheuthis* spp.) were occasionally collected by the pelagic trawl. Individuals of each species was measured (± 5 mm), weighted (± 0.2 g) and dissected to obtain portions of white muscle, which were stored frozen for isotopic determinations. Samples of the muscle of the common dolphin (*Delphinus delphis*) were obtained from individuals stranded on the coast (Bode et al., 2003).

2.4. Stable isotope determinations

Natural abundance of ^{15}N ($\delta^{15}N$, ‰) was measured using an isotope-ratio mass spectrometer (Finnigan Matt Delta Plus) coupled to an elemental analyser (Carlo Erba CHNSO 1108) after oven drying plankton (50 °C, 24 h) or freeze-drying muscle samples in the case of large organisms. The determinations were calibrated against atmospheric nitrogen. Precision (± 1 se) of triplicate $\delta^{15}N$ determinations was better than 0.03‰ (Bode et al. 2003, 2004a; Bode & Alvarez-Ossorio, 2004).

2.5. Experiments with sardines in captivity

The permanence of the isotopic signature in sardine muscle tissues was studied by rearing wild sardines in the aquaculture facilities of IEO Vigo for several months. Several hundred sardines were captured from the coast using a commercial fishing vessel in July 2003 and carefully transferred to tanks in the laboratory. The sardines were fed with a commercial food (Gemma 0.3, Skretting), composed of particles of 0.3-0.6 mm in diameter. After conditioning the sardines for six months to the artificial food, the experiment started in February 2004 and was organised in two steps. First, an initial group of 50 sardines (mean ± se length = 20.0 ± 0.2 cm) was maintained without food for 30 days. Thereafter, food was provided for an additional 50 days period. A control group of 50 sardines was kept in a separate tank and fed regularly. The tanks were under shadow nets and filled with coastal water filtered by gravity through a 2 mm mesh and subsequently through gravel and sand particles. Seston (> 20 µm) entering the tanks was < 1 mg l^{-1}. The temperature of the tanks was similar to that of the coastal waters (mean ± se = 13.6 ± 0.1 °C, n = 66). At intervals between one and 10 days, three individuals from each control or experimental tank were sacrificed to obtain samples of white muscle for isotopic determinations. Samples of the food were also analysed.

2.6. Trophic positions

Trophic positions (TP) of consumers in the pelagic food web were estimated as in Vander-Zanden & Rassmusen (2001):

$$TP = 2 + (\delta^{15}N_c - \delta^{15}N_{hz}) / 3.4$$

where $\delta^{15}N_c$ and $\delta^{15}N_{hz}$ are mean $\delta^{15}N$ values of a given consumer and of herbivorous zooplankton, respectively, the latter used as the reference baseline value (TP = 2). The value of $\delta^{15}N_{hz}$ was estimated from the measurements in the 20-200 µm fraction of plankton and the mean isotopic fractionation value between adjacent trophic levels was 3.4‰ (Vander Zanden & Rasmussen, 2001).

The relationship between isotopic composition and the individual size of organisms was studied by linear regression of $\delta^{15}N$ and $\log_2$ weight size-classes (Jennings et al., 2001, 2002b). Size-fractionated plankton samples were assigned to individual weight classes determined from allometric equations (Rodríguez & Mullin, 1986; Bode et al., 1998, 2003) by assuming that the individual length was the geometric mean of the mesh-size limits for each fraction. The nominal lower and upper limits of the weight classes considered between plankton and dolphins were 1.86 ng and 131 kg, respectively.

3. Trophic structure

3.1. The trophic position of plankton

The distribution of $\delta^{15}N$ in plankton showed high variability and low mean enrichment between size-classes (Fig. 2a). Indeed, mean $\delta^{15}N$ of plankton between 40 and 500 µm was remarkably constant. In this way, significant differences ($P < 0.05$) were only found between four size-class groups < 20, 20-500, 500-1000 and 1000-2000 µm. The average enrichment in $\delta^{15}N$ between these significant groups was 0.52, 0.80 and 0.39‰, respectively.

All selected copepod genera had similar variations in $\delta^{15}N$. Significant differences only appeared between mean values of *Calanus* (6.26‰) and *Centropages* (5.42‰), while *Acartia* (mean $\delta^{15}N$ = 5.87‰) had the largest range of values encompassing those of the other genera.

The mean value for all species (5.9 ± 1.3‰), however, is not significantly different from the mean of the 200-500 μm plankton size-class (Table 2). This further justifies the use of the latter as a representative mean value of zooplankton primary consumers in the pelagic food web when computing trophic positions.

3.2. The trophic position of planktivorous fish

As found for copepods, planktivorous fish displayed similar variations in $\delta^{15}N$ in all species (Table 2). Interestingly, sardines and anchovies (*Engraulis encrasicolus*) showed almost equivalent trophic positions, despite the reported consumption of phytoplankton by the former (Varela et al., 1990; Garrido, 2003) and the exclusive dependence on zooplankton by the latter (Plounevez & Champalbert, 1999). Only *Trachurus trachurus* had mean values significantly higher than those from other species.

S. pilchardus had a mean $\delta^{15}N$ similar to most planktivores (Table 2). Earlier studies reported a significant negative correlation between $\delta^{15}N$ and size for sardines ≥ 18 cm collected in Galicia (Bode et al., 2003). In this study, however, and considering a large number of measurements (Fig. 3), correlations were not significant (ANOVA, $P > 0.05$), indicating large variability in diet also for larger sardines. Taking into account the mean $\delta^{15}N$, adult sardines have a trophic position of 3.5 trophic levels, i.e. that of a secondary consumer (Table 2). Sardine larvae were only slightly below in mean trophic position (3.1 trophic levels) but these differences were lower than the error in the estimations (ca. 0.5 trophic levels).

As a consequence of the similarity in mean $\delta^{15}N$ values, planktivorous fish species were in a narrow range between 3.4 and 3.7 trophic levels (Table 2). Taking into account the standard deviation of these estimates, all species analysed can be considered as equivalent in terms of their trophic role in the food web. For comparison, we also computed the trophic position of the squids *Allotheuthis* and dolphins. The former shared with planktivorous fish the position of a secondary consumer, while the position of dolphins indicates that the studied pelagic food web would have less than five trophic levels.

3.3. The pelagic food web

All food web components can be arranged according to their individual size to reveal the size-structure of the food web (Fig. 4). The obtained relationship reveal a general size dependence of trophic position in this pelagic ecosystem, when examples of primary producers and top consumers are included. This general relationship, however, may be different when only parts of the food web are considered. For instance, the slope of the log-linear relationships between $\delta^{15}N$ and size for plankton or fish is much smaller that the overall slope and statistically not significant. One of the applications of this type of relationship is the estimation of a mean ratio between the size of predators and prey for the log-linear slope, by assuming that $\delta^{15}N$ values are representative of each size-class and a mean trophic fractionation between trophic levels of 3.4‰, as $2^{3.4/\mathrm{slope}}$. Despite the fact that we sampled individual fish species, their isotopic composition was quite similar in a relatively large range of sizes (Table 2, Fig. 4), thus supporting the assumption of representative sampling. In this way, the average predator-prey body-size ratio for the entire food web would be ca. 2×10^5:1.

4. Spatial and temporal variability

4.1. Variability of stable isotope composition in plankton

Considering all cruises pooled (Table 3), samples from all plankton classes larger than 80 μm in shelf waters were significantly enriched in ^{15}N (< 200 m depth) relative to shelf-break samples (> 200 m depth). The difference between means was ca. 1‰ (0.6 to 1.1‰). Small plankton (including phytoplankton), however, was not enriched. This implies the uncoupling between zooplankton and phytoplankton in the coast and the presence of meroplanktonic larvae in the former. In contrast, there were no significant differences in mean $\delta^{15}N$ of < 20 μm (mostly phytoplankton) or > 200 μm plankton classes (mostly zooplankton) between geographic shelf areas in 2004 (Fig. 5).

Due to the different geographic coverage of cruises it was not possible to include in a single statistical analysis all space and time variability factors. However, the 200-500 μm size-class was sampled all years in zones IIa, IIb and III, thus allowing for the exploration of possible interactions between spatial and interannual variability. The results of a two-way ANOVA, however, indicate significant effects of both between zone and between years variability factors, but no significant interaction between them (Table 4). This suggests that the spatial differences were consistent all years, with the plankton of the southern zone IIa more enriched than in northern zones, even though all zones were influenced by upwelling (Fig. 6a). Taking into account interannual differences in $\delta^{15}N$ (Fig. 6b), samples can be arranged in three periods of maximum (years 2001 and 2003), intermediate (years 2000 and 2004) and minimum isotopic enrichment (years 1999 and 2002).

4.2. Variability of stable isotope composition in sardine

Seasonal differences in sardine $\delta^{15}N$ appeared to be small. Mean values of sardines of zone II sampled in March-April and June 2002 were not significant (Mann Whitney test, $P > 0.05$) and the same result was found for 2004 cruises. Furthermore, our experimental feeding studies revealed that the sardines maintained $\delta^{15}N$ values in the muscle for up to 80 days, even when fastening for relatively long periods (30 days). There were no significant correlations between sardine variables (length, weight, nitrogen content or $\delta^{15}N$) and time, and most of the differences between the values measured in the control and treatment groups were not significant (Table 5). In both groups, mortality was < 10%. Only sardines in the treatment group reached a higher mean weight than those in the control group when the former returned to normal feeding after fasting. Despite the variations induced in feeding, the isotopic signatures of sardines remained within a narrow range of values (mean ± sd = 11.3 ± 0.3‰) during the experiment. Taking into account the $\delta^{15}N$ value of the food (mean ± sd = 9.7 ± 0.1‰, n = 5), the isotopic enrichment between sardine muscle and the consumed food was 1.6 ± 0.1‰. This value is much smaller than the mean value of 3.4‰ generally assumed as the enrichment between adjacent trophic levels.

The large geographical coverage of cruises in 2004, allowed the study of variability in sardine $\delta^{15}N$ over the entire shelf. In this case, the mean value for zone II was significantly higher than those of adjacent zones (Fig. 7a). However, when using data from other years, and besides significant variability between years and sampling zones, there was significant interaction between both factors (Table 6). This interaction implies that, in contrast with plankton, the geographical location of maximum and minimum $\delta^{15}N$ values in sardine varied with the sampling year, at least for zones II and III between 1998 and 2001. High $\delta^{15}N$ values correspond to years 1998 and 1999, particularly in zones IIa and III (Fig. 7b). During 2001, however, all zones had similarly low values.

4.3. Isotopic enrichment and population size

Considering mean values of $\delta^{15}N$ in zone II, the area most sampled in the study period, there is a mismatch between maximum and minimum values in 200-500 μm plankton compared to sardines (Fig. 8). Years of high $\delta^{15}N$ in sardines, as in 1999, coincided with low values in plankton, and vice versa, maximum $\delta^{15}N$ in plankton were associated to minimum values in sardines, as in 2001 and 2003. Such differences cannot be attributed to differences in the individual size of sardines in the annual samples (mean ± se length = 18 ± 3 cm, n = 347, ANOVA P > 0.05). There is a significant negative linear relationship between mean annual $\delta^{15}N$ of sardine and plankton, indicating that sardines were on average 40% more enriched than plankton (Fig. 9). This relationship was used to estimate the missing values for years 1998 (when no plankton samples for zone II were available) and 2000. The absolute value of enrichment of sardines relative to plankton showed a decrease between 1998 and 2000 followed by a general increase in recent years (Fig.10a). This pattern was parallel to a marked increase in the size of the sardine population since 2000 and a decreasing trend in upwelling intensity (Fig. 10b). In contrast, the poleward index first decreased befrore 2000 but showed alternative high and low values thereafter. Sardine stock and upwelling were significantly correlated ($r = -0.708$, $n = 7$, $P > 0.05$). Mean isotopic enrichment, however, was not significantly correlated with the size of the sardine population ($r = -0.205$, $n = 7$, $P > 0.05$) or the upwelling index ($r = 0.294$, $n = 7$, $P > 0.05$).

5. Discussion

5.1. Size-structure of the food web

Our results on $\delta^{15}N$ indicate that the main trophic compartments of the pelagic ecosystem are weakly structured in terms of size and species composition. First, plankton values were very variable in all sizes considered, and only certain sizes resulted with significant differences. The average enrichment between plankton size classes (between 0.39 and 0.80‰), however, was smaller than the average value of 3.4‰ expected between adjacent trophic levels (e.g. Vander Zanden & Rasmussen, 2001; Post, 2002). Also, there were small differences in $\delta^{15}N$ between the representative primary consumers, the selected copepods, even though they differ considerably in size. Such low enrichment between size-classes confirm our previous reports for part of the study area (Bode et al., 2003) and agrees with reports in other oceanic (Fry & Quiñones, 1994) and coastal areas (Rolff, 2000). A large overlap in the size of organisms at different trophic levels, as the presence of phytoplankton chains that can be larger than protozoan heterotrophs, and widespread omnivory (e.g. Calbet, 2001) can be the cause of the low isotopic enrichment observed.

Second, most planktivorous consumers displayed similar average $\delta^{15}N$ values. This would imply either strong competition for the same prey or spatial and temporal segregation of their populations. While the latter explanation is not supported by the frequent observations of mixed shoals and schools of the main species (e.g. Muiño et al., 2003), the former agrees with the information derived from gut-content studies (Varela et al., 1990; Plounevez & Champalbert, 1999; Olaso et al., 1999; Garrido, 2003). Furthermore, our study revealed that previously reported ontogenic changes in $\delta^{15}N$ values may be not significant when the observations are averaged over large areas and several years. For instance, a decrease of $\delta^{15}N$ with individual size was described for sardines sampled in 1998 and 1999 in zones II and III (Bode et al., 2003, 2004a). This decrease was attributed to the higher efficiency of large sardines in capturing phytoplankton, less enriched in ^{15}N than zooplankton prey. Adding more observations from different areas and years, however, revealed that the decrease was not significant (Fig. 3), as $\delta^{15}N$ content in sardines decreased, and that of mesozooplankton increased, in recent years (Fig. 8). In addition, the average isotopic content of sardine larvae was similar to that of adults. Other species may display different patterns in $\delta^{15}N$ variability with size, as found in *Trachurus trachurus* (Bode et al., 2006).

Strong size-structure, however, was found across all compartments, particularly when top predators along with phytoplankton were included. A similar result was previously reported for demersal fish communities, where species composition was not the main source of variability in $\delta^{15}N$ (Jennings et al. 2001). Further studies including benthic (Jennings et al., 2002b) and whole shallow water, coastal communities (Jennings et al., 2002a) illustrated the applicability of the slope of the size-trophic position relationship as a measure of ecosystem structure. In our study, considering only pelagic components, we found a slope lower that those reported for other studies. A smaller slope implies a larger average predator: prey body-size ratio which can be explained by the dominance of large consumers of small prey (planktivorous fish and mammals) in pelagic ecosystems in contrast with benthic communities where most predators are only slightly higher than their prey (Cushing, 1978). For instance, values of this ratio in studies including benthic components are in the order of 10^2 to 10^3 (Jennings et al., 2001, 2002a, b) compared to 10^5 in our study. These values, however, are only mean values for the trophic continuum in a given ecosystem. Actual predator: prey size ratios may vary for each predator between 10^2 and 10^{10}, if we consider sardines feeding on phytoplankton or dolphins feeding on sardines, respectively (Bode et al., 2006). Still, the relationship between trophic position and size may be a general indicator of changes in the pelagic food web over long periods or areas (e.g. decrease of predators) providing representative samples of plankton and top consumers are included.

5.2. Variability of trophic positions

When considering individual species, average trophic positions derived from $\delta^{15}N$ data were rather homogeneous. Assuming that copepods are the primary consumers, planktivores varied only between 3.4 and 3.7 trophic levels, while the top predator was only at 4.2 trophic levels. This result is in agreement with previous assumptions indicating that pelagic food webs in upwelling areas were relatively short due to the prevalence of plankton consumers (Ryther, 1969). Trophic positions computed using gut-content analysis (e.g. http://www.fishbase.org) were almost coincident with our estimations, with the exception of sardine, which was generally considered as a partial herbivore due to the presence of phytoplankton in their guts (Bode et al., 2006). Isotopic analysis of muscle samples in sardine, however, showed that most of nitrogen was provided by zooplanktonic prey, while phytoplankton contributed to a small fraction of carbon (Bode et al., 2004a).

The general coincidence between gut-content and isotopic estimates seems to confirm the validity of the assumption of a constant isotopic enrichment of 3.4‰ between adjacent trophic levels, as used in our study. However, several studies showed that, despite most compilations converge towards the mean value of 3.4‰, there is a large variability in enrichment between predators and prey (Vander Zanden & Rasmussen, 2001; Post, 2002; McCutchan et al., 2003). In this way, average isotopic enrichment between plankton size-classes in our study varied between 0.4 and 0.8‰, and similar values can be found between the size-class typically composed of phytoplankton (< 20 μm) and copepod species. Also, our experiment with sardines in captivity revealed an enrichment of only 1.6‰ between food and muscle. In contrast, isotopic enrichment values close to 3.4‰ were reported for predatory fish (e.g. Vander Zanden & Rasmussen, 2001). Nitrogen limitation and the remineralisation of organic matter in microbial food webs, significant in the study area despite the upwelling (Alvarez-Salgado et al., 2002; Bode et al., 2004b), may be responsible for the low enrichment between plankton size-classes. Rapid consumption of substrates would prevent the discrimination of light versus heavy nitrogen isotopes and the composition of substrates and products (and similarly the composition of prey and predator) would converge. Other studies also report low enrichment between zooplankton classes (Fry & Quiñones, 1994) and particularly in plankton fractions < 20 μm (Rau et al., 1990; Rolff, 2000). The isotopic enrichment between planktivorous fish and the top predator in our study was between 2 and 3‰, revealing a larger isotopic discrimination than in the case of plankton, likely because of the higher nitrogen content of their diet (Adams & Sterner, 2000).

In any case, average trophic positions derived from $\delta^{15}N$ are of low indicator value if the large variability in the isotopic composition of prey and predators, as occurs in upwelling ecosystems, is not adequately measured (O'Reilly et al., 2002). We overcome the temporal variability of $\delta^{15}N$ in plankton (Bode & Alvarez-Ossorio, 2004) by sampling at large spatial scales that integrate successional stages occurring at seasonal scales (Bode et al., 2004a). Seasonal averages of $\delta^{15}N$ were reported to reduce the incertidude in trophic position estimates caused by the variability of plankton signatures (Matthews & Mazumder, 2005). Significant differences in the isotopic composition of plankton between shelf areas, as those found in our study, can be interpreted as indicators of different food webs (Perry et al., 1999). In our case most planktivores are widely distributed and feed in both shelf and shelf-break areas (Carrera & Porteiro, 2003), thus differences between consumers collected in across-shelf transects are not expected. In the generally narrow shelf studied, along-shore differences due to differential upwelling fertilisation would be more relevant for food web characterisation. In this way, we found significant ^{15}N enrichment in plankton from shelf zone IIa, where upwelling is more active and new nitrate inputs are frequent during spring (Alvarez-Salgado et al., 2002), while plankton in zone III (Southern Bay of Biscay) was less enriched, likely due to the use of regenerated nitrogen.

5.3. Consumers in mid trophic levels: S. pilchardus

Taking sardine as a representative planktivore, the results of our feeding experiment, along with the comparison of field values in spring and summer, indicate that the isotopic signature in the muscle was relatively constant for periods between weeks and months, even in the absence of food. Thus, $\delta^{15}N$ in the muscle effectively integrates the diet over such periods, as reported for other fish species (Tieszen et al., 1983), and we can assume that the isotopic composition of consumers measured in our study match that of plankton sampled concurrently over a large area. The variability in the trophic position of sardine found in this study can be typical of mid trophic levels in pelagic food webs, which may experience both top-down and bottom-up controls (Cury et al., 2000). Their ability to consume a large variety of phyto- and zooplanktonic prey would allow overcoming the lack of a particular type and the patchy distribution of plankton. A large variability in muscle $\delta^{15}N$ values depending on the relative amount of phytoplankton consumed would be expected, as illustrated with the dispersion of $\delta^{15}N$ values with fish size. However, we found that the isotopic composition of muscle varied in general in a narrow range, due not only to the small difference in $\delta^{15}N$ values between plankton classes but also to the time integration of diets. This characteristic allows for an estimation of the relative mobility of sardines along the shelf, providing that there were different isotopic signatures of plankton in the different shelf areas, as demonstrated for zones IIa and III. This approach has been successfully employed to show mobility in other fish species (e.g. Hanson et al., 1997). Sardines feeding preferentially in zone IIa during relatively long periods would have higher $\delta^{15}N$ values than those feeding in other shelf zones. Our results revealed that sardines collected in zone II were generally more enriched than those collected in other zones and therefore that there was a low mobility between zones.

Mobility, however, may vary between years. Both absolute $\delta^{15}N$ and relative enrichment between plankton and sardines varied significantly between years, suggesting that the mobility of sardines was different. Relatively low values of plankton $\delta^{15}N$ before 2000 may be related to the strength of the winter poleward current during 1998 and 1999 (Cabanas et al., 2003), as this currents transports warm and low nitrate surface water (Alvarez-Salgado et al., 2003). The predominant use of ^{14}N relative to ^{15}N from regenerated nitrogen by phytoplankton in these years, would be transmitted up to zooplankton in the food web. The continuous decrease in upwelling intensity and the irregular pattern of the poleward index, however, suggest that the increase in plankton $\delta^{15}N$ after 2000 may have been due to the interaction between upwelling and downwelling events through changes in the nutrient sources. Unfortunately there are no time-series data of the isotopic signatures of nitrogen sources to verify this interpretation. Interestingly, sardine and plankton $\delta^{15}N$ followed an inverse interannual pattern. Both variables should be positively correlated, however, if nutrient inputs were dependent only of upwelling intensity, thus supporting the hypothesis of interaction between upwelling and downwelling. Large differences in $\delta^{15}N$ between consumer and prey are expected when there is nitrogen limitation and there is internal recycling in the former, whereas lower differences are expected when nitrogen is sufficient (Adams & Sterner, 2000). In this way, the negative relationship between $\delta^{15}N$ of plankton and sardines is consistent with a large isotopic fractionation and nitrogen recycling when plankton is mainly consuming regenerated nitrogen, as in 1999. Changes in the type of prey consumed may also alter the isotopic signature of sardines. In addition, sardines feeding in low nutrient areas may experience starvation and consequently higher isotopic fractionation than those in high nutrient areas. Due to the lack of a simple relationship between plankton $\delta^{15}N$ and upwelling-downwelling forcing the interpretation of the differential signatures between plankton and sardines would need further studies taking into account nutrient levels, plankton composition and sardine growth in the different areas wich are beyond the scope of the present study. These differences, however, can also be interpreted as the result of the large mobility of sardines in years when muscle $\delta^{15}N$ values are close to those of plankton, as in 2001 and 2003. Sardines feeding in all shelf zones would have $\delta^{15}N$ values lower than those feeding mainly in zone II, as plankton of zones I and III was less enriched. Moreover, the distribution of sardine $\delta^{15}N$ in shelf zones in years 1998 and 1999 was more heterogeneous than in 2001, thus indicating the adaptation to local plankton. Such changes in mobility are consistent with the interpretation of the sardine population as separate subpopulations that are restricted to different shelf areas in years of low abundance but merge when the population increase (Carrera & Porteiro, 2003).

6. Conclusions

The relationship between trophic position and body size in the pelagic food web studied is less constrained than those reported for other ecosystems. As a result, the ratio between the sizes of predators and prey is largely variable. Widespread omnivory and plankton consumption by relatively large predators are the likely causes. Size-structuring among the groups considered, however, was stronger than within them, as found in other studies. Trophic positions of plankton consumers vary in a narrow range of values, suggesting potential competition for food. Low nitrogen isotope enrichment between prey and consumers indicate nutrient limitation and recycling at the base of the food web. This suggests a bottom-up control of planktivorous fish and agrees with the described seasonal variability of nitrogen fluxes and heterogeneous distribution of upwelling zones within the studied shelf. The composition of nitrogen isotopes in the muscle of sardine integrates fish diet over seasonal periods and reflects the composition of plankton over large shelf areas. Differential isotopic signatures in high and low upwelling zones are consistent with low mobility of sardines during periods of low population size, thus suggesting the use of isotopic abundance as a measure of the ability of sardines to exploit different areas. The lack of a simple relationship between the main oceanographic events at a regional scale and the isotopic signature of plankton and sardine, however, calls for more studies to understand the role of mobility and nutrient enrichment in the isotopic composition.

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Table 1. Procedence of samples. Zones are marked in Fig. 1.

Date	Cruise	Zone	Type of samples
March - April 1998	PELACUS 0398	II, III	sardine
March 1999	PELACUS 0399	II, III	sardine, plankton
March – April 2000	PELACUS 0300	II, III	plankton
April 2001	PELACUS 0401	II, III	sardine, plankton
March 2002	PELACUS 0302	II, III	sardine, plankton, other fish species
June 2002	Commercial landings	II	sardine
March – April 2003	PELACUS 0303	II, III	sardine, plankton, other fish species
March – April 2004	PELACUS 0304	II, III	sardine, plankton
June 2004	Capricornio 03050504	I, II	sardine, plankton

Table 2. Mean ± sd trophic position of pelagic consumers computed from mean (±sd) $\delta^{15}N$ values (‰) of primary plankton consumers. G: fish species groups with non significantly different means (ANOVA and Dunnett-C test, P<0.05). Trophic position = 2 + ($\delta^{15}N_{sardine}$ – $\delta^{15}N_{plankton}$) / 3.4. n: number of data.

	$\delta^{15}N$			
Component	Mean ± sd	n	G	Trophic position
Plankton 20-200 µm	5.6 ± 1.7	159	-	2.0
Sardina pilchardus larvae	9.3 ± 0.4	10	-	3.1
Sardina pilchardus	10.7 ± 0.8	447	b	3.5
Trachurus trachurus	11.3 ± 0.4	44	a	3.7
Scomber scombrus	10.6 ± 1.0	45	b	3.5
Macroramphosus scolopax	10.7 ± 0.3	10	b	3.5
Engraulis encrasicolus	10.5 ± 0.5	15	b	3.4
Capros aper	10.7 ± 0.2	10	b	3.5
Boops boops	10.3 ± 0.4	20	b	3.4
Allotheuthis spp.	10.1 ± 0.5	6	-	3.3
Delphinus delphis	13.1 ± 0.8	5	-	4.2

Table 3. Mean and sd values of $\delta^{15}N$ (‰) in plankton size-classes collected in shelf (< 200 m depth) and shelf-break waters (> 200 µm). P: Significance between means (ANOVA), n: number of data.

Size-class	Shelf			Shelf-break			
	mean	sd	n	mean	sd	n	P
<20 µm	4.9	1.5	122	5.3	1.3	53	0.088
20-40 µm	5.7	1.5	85	5.4	1.3	34	0.295
40-80 µm	5.5	1.6	98	5.4	1.2	36	0.700
80-200 µm	5.8	1.7	95	5.2	1.6	36	0.049
200-500 µm	5.9	1.6	113	4.8	1.8	46	0.000
500-1000 µm	6.7	1.5	114	5.7	1.6	48	0.001
1000-2000 µm	7.1	1.2	116	6.1	1.4	50	0.000

Table 4. Results of ANOVA on $\delta^{15}N$ (‰) of 200-500 μm plankton variability across cruise years (1999 to 2004) and three shelf zones (zones IIa, IIb and III in Fig. 1). SS: sum of squares, DF: degrees of freedom, MS: mean squares, F: variance ratio, P: significance.

Factor	SS	DF	MS	F	P
year	97.714	5	19.543	13.950	0.000
zone	30.053	2	15.027	10.727	0.000
year x zone	16.049	10	1.605	1.146	0.338
error	124.677	89	1.401		
total	3554.322	107			

Table 5. Results of the feeding experiment with *S. pilchardus* in captivity. Mean ± sd (n = 12). Differences between treatment and control values were analysed with Mann-Whitney tests.

Variable	Time interval	Control	Treatment	Significance
Length (cm)	0 - 30 d	19.8 ± 0.6	19.9 ± 1.0	$P > 0.05$
	31- 80 d	19.8 ± 1.4	20.3 ± 1.0	$P > 0.05$
Weight (g)	0 - 30 d	77.1 ± 15.6	79.2 ± 15.8	$P > 0.05$
	31 - 80 d	76.1 ± 24.9	86.0 ±16.0	$P < 0.05$
% N	0 - 30 d	10.6 ± 3.0	11.0 ± 4.1	$P > 0.05$
	31 - 80 d	11.5 ± 3.2	11.7 ± 2.6	$P > 0.05$
$\delta^{15}N$ (‰)	0 - 30 d	11.2 ± 0.6	11.2 ± 0.3	$P > 0.05$
	31 - 80 d	11.4 ± 0.7	11.3 ± 0.3	$P > 0.05$

Table 6. Results of ANOVA on $\delta^{15}N$ (‰) of sardine variability across cruise years (1998, 1999 and 2001) and three shelf zones (zones IIa, IIb and III in Fig. 1). SS: sum of squares, DF: degrees of freedom, MS: mean squares, F: variance ratio, P: significance.

Factor	SS	DF	MS	F	P
year	47.929	2	23.965	117.643	0.000
zone	3.615	2	1.807	8.872	0.000
year x zone	3.463	4	0.866	4.250	0.002
error	56.834	279	0.204		
total	33964.329	288			

Figure captions:

Fig. 1. Location of plankton and fish samples along the Atlantic shelf of the Iberian Peninsula. The 200 m isobath, taken as the limit for the shelf-break, is shown. The shelf is divided in several zones for spatial analysis of $\delta^{15}N$.

Fig. 2. Variability of $\delta^{15}N$ (‰) in plankton size-classes (a) and selected copepod genera (b). The upper size limit of each class is marked in the x-axis of panel a. Each box encompasses the 25% and 75% percentiles, the line indicates the median value and the vertical lines the range. The numbers below each box indicate the number of data. Significant differences between means (ANOVA and Dunnett-C tests, $P<0.05$) are indicated by different letters.

Fig. 3. Variability of $\delta^{15}N$ (‰) with individual length in *Sardina pilchardus*. Two size-classes (< 18 and ≥ 18 cm) were considered (see text).

Fig. 4. Relationship between mean $\delta^{15}N$ (‰) and individual weight (w, g). $\delta^{15}N$ standard errors are indicated by bars. The regression line is $\delta^{15}N = 9.386 + 0.194 \log_2(w)$ ($r = 0.970$, $P < 0.0001$, $n = 20$).

Fig. 5. Mean (+se) $\delta^{15}N$ values (‰) of phytoplankton (a) and zooplankton (b) in three shelf zones (see Fig. 1). Differences between zone means are not significant in both cases (ANOVA, $P>0.05$). The values of the smallest size-class sampled in each area (< 20 µm) are assumed to be representative of phytoplankton, while those of the 200-500 µm size-class (> 200 µm in zones I and IIa in Fig. 1) are assumed to represent zooplankton. The numbers within each bar indicate the number of data averaged.

Fig. 6. Mean (+se) δ^{15}N (‰) of plankton 200-500 μm sampled in different shelf zones marked in Fig. 1 (a) and years (b). Significant differences between means (ANOVA and Dunnett-C tests, $P < 0.05$, Table 4) are indicated by different letters. The numbers within each bar indicate the number of data averaged.

Fig. 7. Mean (+ se) δ^{15}N (‰) of sardine in zones I, II and III during 2004 (a) and in zones IIa, IIb and III in 1998, 1999 and 2001 (b). Significant differences between means (ANOVA and Dunnett-C tests, $P < 0.05$) are indicated by different letters.

Fig. 8. Annual mean (+se) δ^{15}N (‰) of 200-500 μm plankton (a) and sardine (b) from zone II (see Fig. 1). Significant differences between means (ANOVA and Dunnett-C tests, $P < 0.05$) are indicated by different letters. The numbers within each bar indicate the number of data averaged.

Fig. 9. Relationship between mean δ^{15}N (‰) of sardine and 200-500 μm plankton from zone II (see Fig. 1). Error bars represent standard errors. The regression line (δ^{15}N sardine = 12.7 – 0.4 δ^{15}N plankton, $r = 0.925$, $n = 5$, $P < 0.05$) is indicated by the dashed line.

Fig. 10. Annual variations of (a) mean difference between δ^{15}N (‰) of sardine and 200-500 mm plankton ($\Delta\delta^{15}$N) from zone II (continuous line) compared to sardine spawning stock biomass (SSB, Gg = 10^3 tonnes, dashed line) and (b) mean upwelling (continuous line) and poleward (dashed line) index values of the preceding year ($m^3 s^{-1} km^{-1}$). SSB was obtained from ICES fishery areas XIa and VIIIc (ICES, 2005). Missing $\Delta\delta^{15}$N values for 1998 and 2000 (open circles) were estimated from the regression line in Fig. 9.

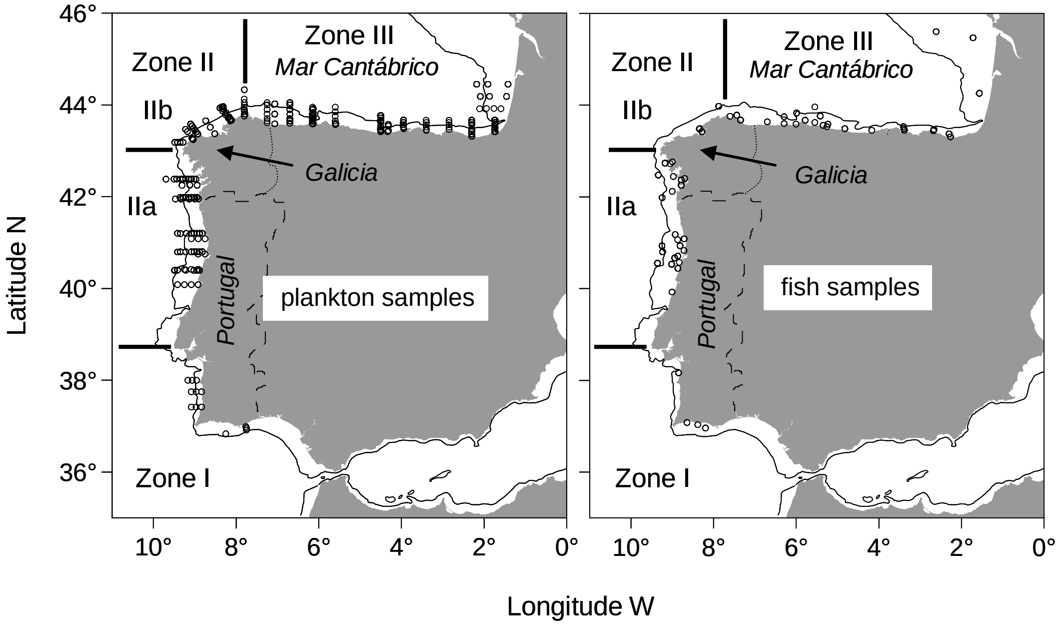

46°
44°
42°
40°
38°
36°
Latitude N
Zone II
Zone III
Mar Cantábrico
IIb
IIa
Galicia
Portugal
plankton samples
fish samples
Zone I
10°
8°
6°
4°
2°
0°
Longitude W

Fig. 1

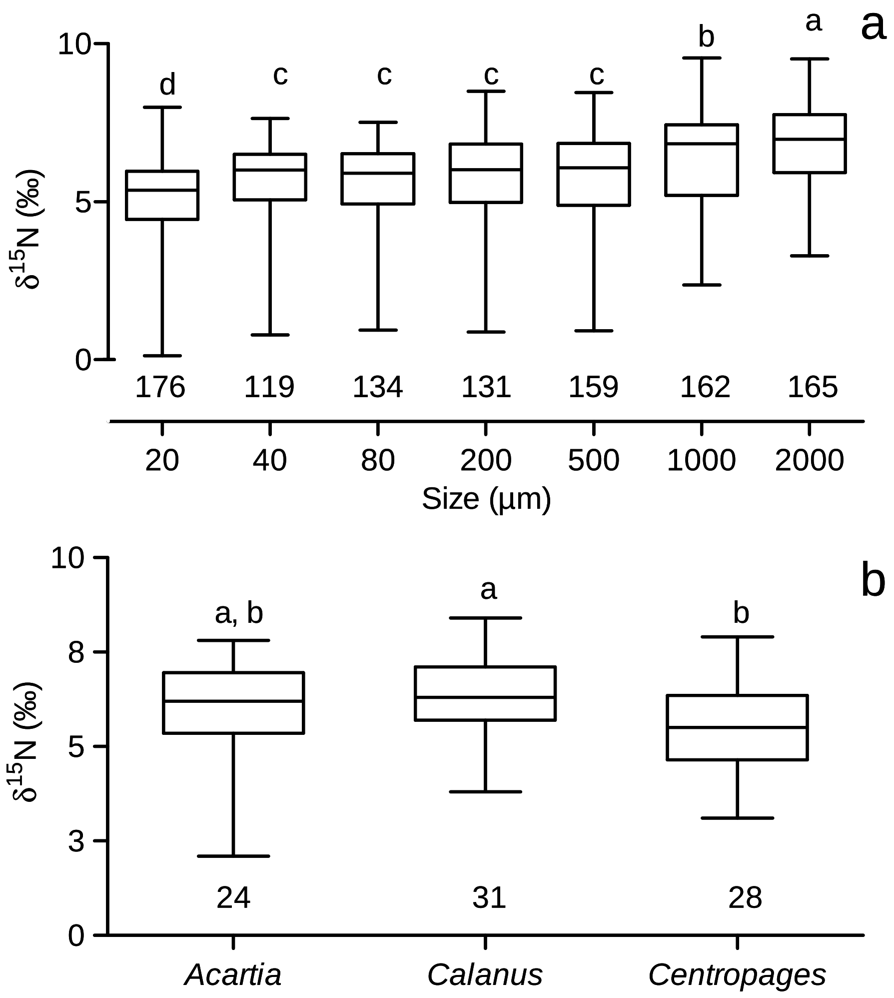

a
δ15N (‰)
10
5
0
d
c
c
c
c
b
a
176
119
134
131
159
162
165
20
40
80
200
500
1000
2000
Size (µm)
b
δ15N (‰)
10
8
5
3
0
a, b
a
b
24
31
28
Acartia
Calanus
Centropages

Fig. 2

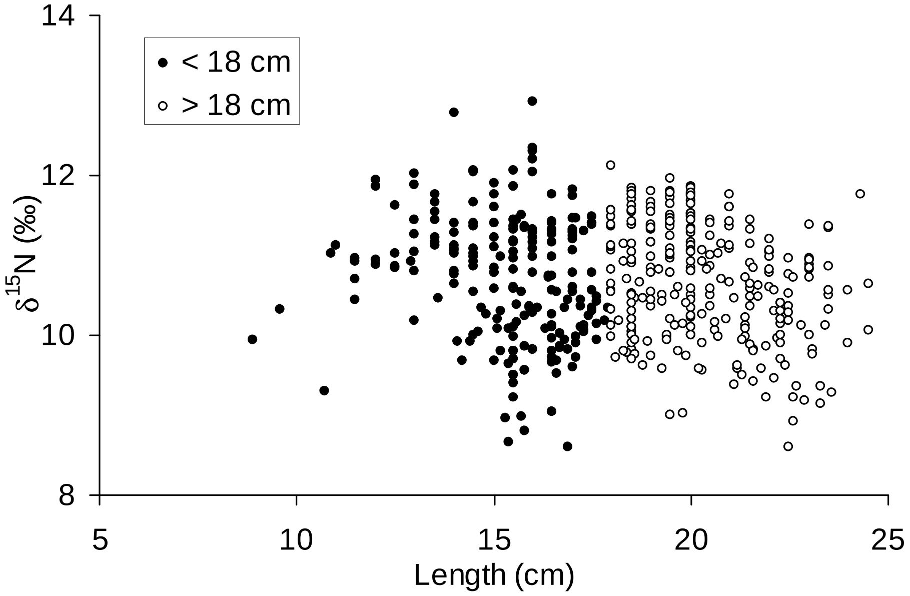
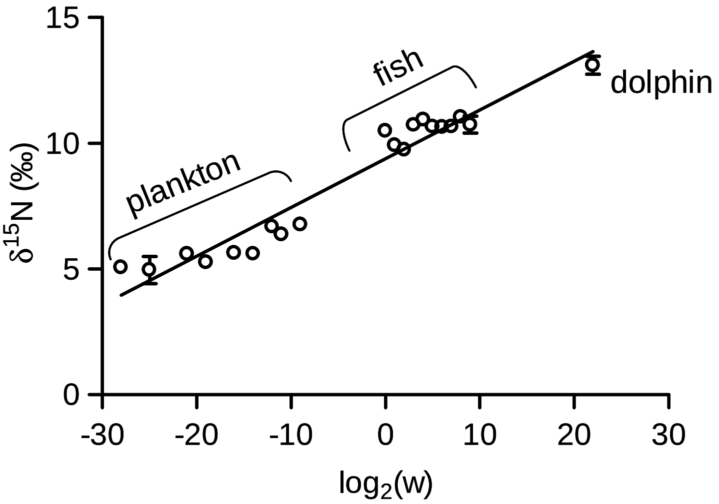


Fig. 3



fish
dolphin
plankton
$\delta^{15}N$ (‰)
15
10
5
0
-30
-20
-10
0
10
20
30
$\log_2(w)$

Fig. 4

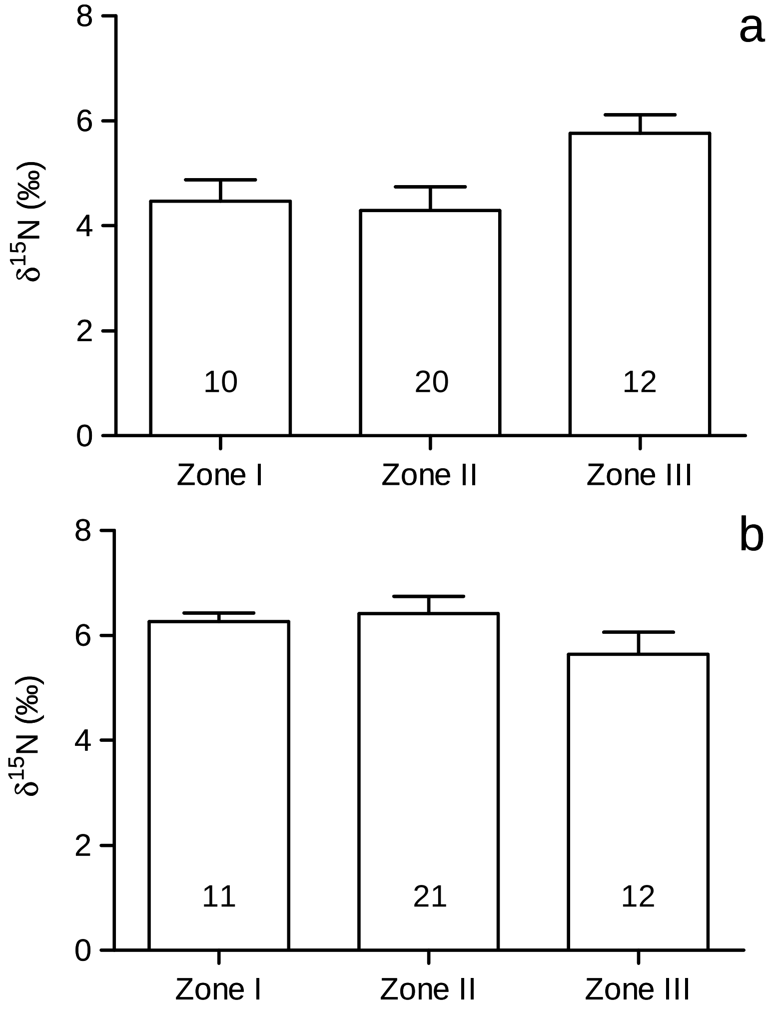

a
8
6
4
2
0
$\delta^{15}N$ (‰)
10
20
12
Zone I
Zone II
Zone III
b
8
6
4
2
0
$\delta^{15}N$ (‰)
11
21
12
Zone I
Zone II
Zone III

Fig. 5

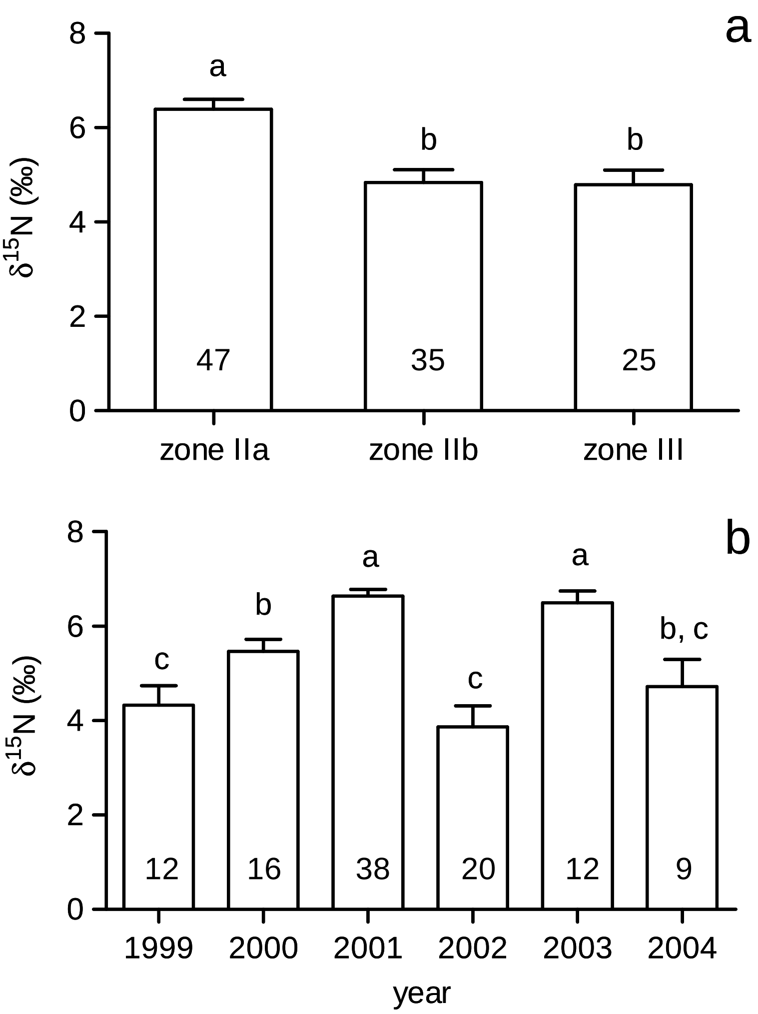

a
8
6
4
2
0
$\delta^{15}N$ (‰)
a
b
b
47
35
25
zone IIa
zone IIb
zone III
b
8
6
4
2
0
$\delta^{15}N$ (‰)
c
b
a
c
a
b, c
12
16
38
20
12
9
1999
2000
2001
2002
2003
2004
year

Fig. 6

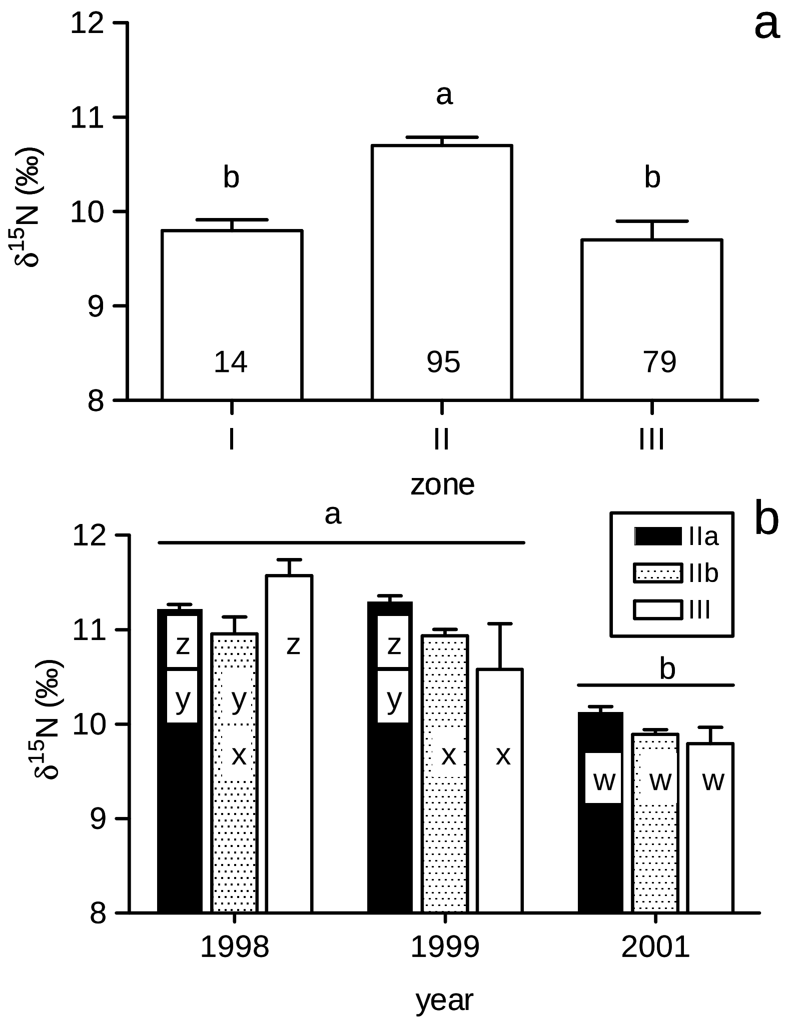

a
12
11
10
9
8
δ15N (‰)
a
b
b
14
95
79
I
II
III
zone
b
12
11
10
9
8
δ15N (‰)
a
b
IIa
IIb
III
z
y
z
y
x
y
z
z
y
x
x
w
w
w
1998
1999
2001
year

Fig. 7

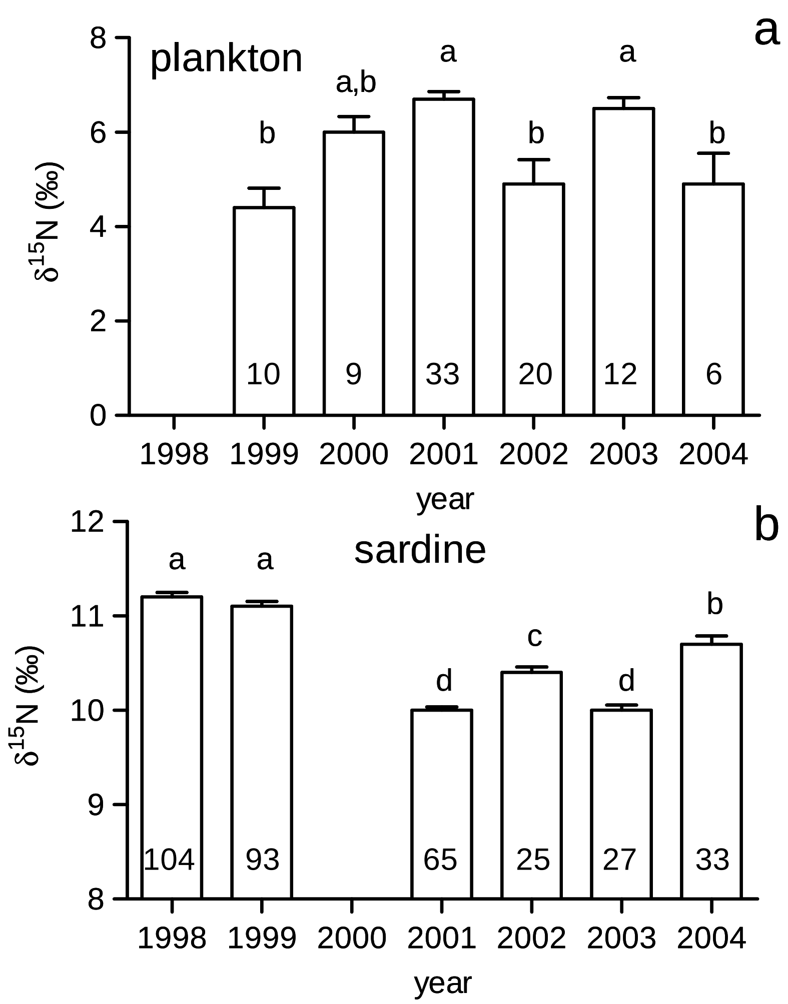

a
plankton
$\delta^{15}N$ (‰)
8
6
4
2
0
b
a,b
a
b
a
b
10
9
33
20
12
6
1998
1999
2000
2001
2002
2003
2004
year
b
sardine
$\delta^{15}N$ (‰)
12
11
10
9
8
a
a
d
c
d
b
104
93
65
25
27
33
1998
1999
2000
2001
2002
2003
2004
year

Fig. 8

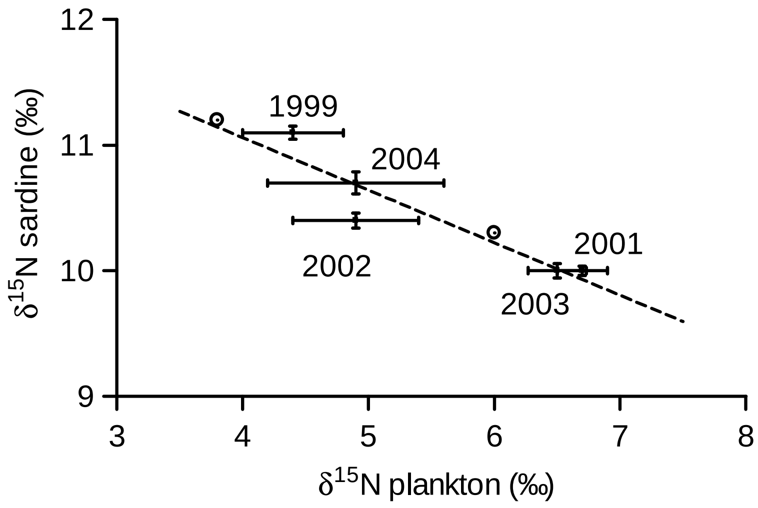

12
11
10
9
$\delta^{15}N$ sardine (‰)
3
4
5
6
7
8
$\delta^{15}N$ plankton (‰)
1999
2004
2002
2001
2003

Fig. 9

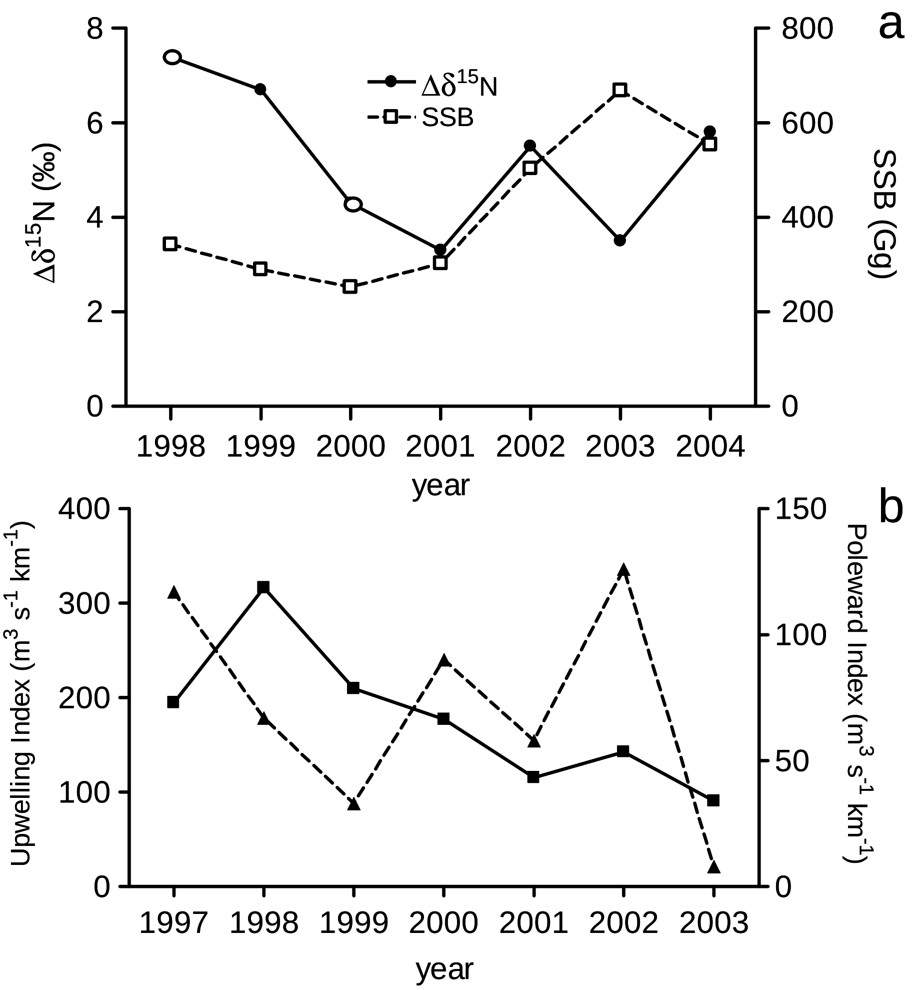

a
8
6
4
2
0
800
600
400
200
0
Δδ15N (‰)
SSB (Gg)
Δδ15N
SSB
1998
1999
2000
2001
2002
2003
2004
year
b
400
300
200
100
0
150
100
50
0
Upwelling Index (m3 s-1 km-1)
Poleward Index (m3 s-1 km-1)
1997
1998
1999
2000
2001
2002
2003
year

Fig. 10