

The pelagic foodweb in the upwelling ecosystem of Galicia (NW Spain) during spring: natural abundance of stable carbon and nitrogen isotopes

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The structure of the pelagic foodweb in the upwelling ecosystem of Galicia (NW Spain) was studied by means of the enrichment in ^{13}C and ^{15}N isotopes in plankton (four size classes from 20 to $>1000\ \mu\text{m}$), sardine (primary consumer) and dolphin (top consumer). Samples were collected in March 1998, during the spawning period of sardines at the beginning of the upwelling season. Isotopic enrichment across plankton size classes was lower than either enrichment between plankton and sardine or enrichment between sardine and dolphin. This difference may be attributed to the presence of chain-forming phytoplankton in some plankton size classes but also to a large degree of omnivory among zooplankton. Also, there was a size-dependent effect on ^{15}N enrichment of sardine. Sardines $<18\ \text{cm}$ had a variable isotopic enrichment but generally higher than those of larger sardines, revealing omnivorous but mostly zoophagous diets. In contrast, sardines $\geq 18\ \text{cm}$ showed a linear decrease of ^{15}N enrichment with length, as a consequence of the increase in the efficiency of filter-feeding and in the amount of phytoplankton ingested. According to ^{15}N enrichment, we concluded that probably there are no more than four trophic levels in the studied ecosystem. Nevertheless, our results revealed that the pelagic food web in this upwelling area is more complex than expected due to the generalized omnivory in all organisms. Furthermore, the obtained relationships between $\delta^{15}\text{N}$ and the size of organisms provide the basis for a quantitative analysis of changes in the trophic structure of this ecosystem.

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Introduction

Pelagic ecosystems in upwelling areas were traditionally considered as dominated by relatively simple and short foodwebs. One of the most known examples is the direct transfer of organic matter from phytoplankton to some clupeid fishes (like sardines and anchovies) that form large populations and support intensive fisheries in these areas (Ryther, 1969; Blaxter and Hunter, 1982). However, as new information accumulated, it appeared that complex foodwebs can also be found in these ecosystems. The revision of this complexity came in part from the recent consideration of the microbial system as a

mechanism enlarging foodwebs (Moloney, 1992). Furthermore, simple foodwebs often result from studies of gut-content analysis of a limited number of individuals, which only can provide information on the most recent diet and are not able to identify all consumed items (see James, 1988 for a review). In contrast, studies measuring the natural abundance of stable isotopes in different body tissues reflect a time-integrated diet, and tend to support the view of more complex foodwebs (Sholto-Douglas *et al.*, 1991; Lindsay *et al.*, 1998). The latter approach has been employed in the study of trophic interactions between organisms in different pelagic ecosystems (e.g. Wada *et al.*, 1987; Fry, 1988;

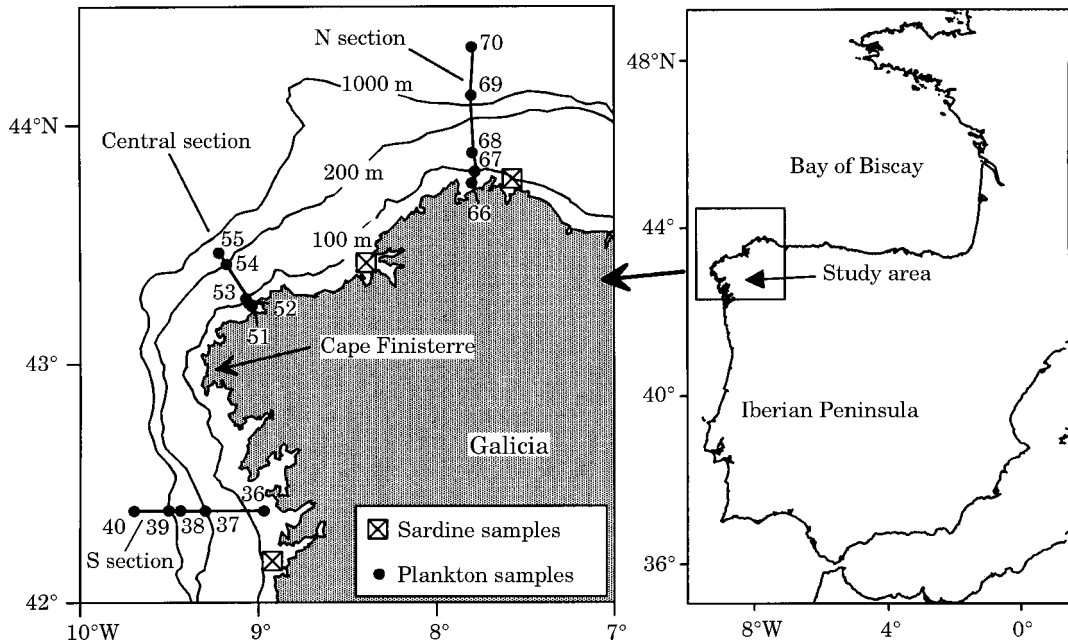


Figure 1. Map of study area with location of plankton (numbered dots) and sardine stations.

Hanson *et al.*, 1997; Rolff, 2000). Thus, Monteiro *et al.* (1991) demonstrated that large fish populations in the southern Benguela upwelling ecosystem were not mainly supported by direct feeding on phytoplankton, as Ryther's (1969) hypothesis suggested (Ryther, 1969).

Studies of pelagic foodwebs have shown that a large fraction of total variance in the natural abundance of stable isotopes in organisms ranking from plankton to fishes was due to individual organism size. On the one hand, heavy isotopes accumulate in consumer organisms of individual sizes larger than those of their preys in plankton (Montoya *et al.*, 1990; Fry and Quiñones, 1994) and planktivorous fishes (Sholto-Douglas *et al.*, 1991; Hanson *et al.*, 1997). The enrichment in heavy isotopes of a predator compared to its prey was approximately constant across all trophic levels in aquatic ecosystems, which allows for the determination of the trophic position of the analysed organisms (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001; Post, 2002). On the other hand, organisms accumulate heavy isotopes during growth because of the preferential mobilization of light isotopes in metabolic reactions (Wada and Hattori, 1991). If there are no substantial changes in diet during the growth of a given species, the bodies of young individuals would display lower abundances of heavy isotopes than those of old individuals (Rau *et al.*, 1981; Lindsay *et al.*, 1998; Jennings *et al.*, 2002). In contrast, variations in the amount of preys from different trophic positions consumed or metabolic changes, as those induced by starvation (Doucet *et al.*, 1999), would cause changes in the abundance of heavy

isotopes in the consumer tissues. Therefore, the measurement of the natural abundance of stable isotopes in key organisms of different individual size would allow not only for the determination of foodweb complexity in a given ecosystem (Jennings *et al.*, 2001, 2002) but also for the study of changes in diet during the growth of some organisms.

An important fishery of sardine (*Sardina pilchardus*, Walbaum) occurs in Galicia (NW Iberian Peninsula, Figure 1), a region at the northern limit of one of the major upwelling areas in the NE Atlantic (Wooster *et al.*, 1976; Fraga, 1981). In recent years, the stock of sardine dramatically decreased to historically minimum values (ICES, 1999), but no direct relationships were found between recruitment and variability in upwelling (Robles *et al.*, 1992; Guisande *et al.*, 2001) or general hydrographic conditions (López-Jamar *et al.*, 1995). However, survival of sardine eggs was clearly related to their biochemical condition and to the organic content of available food particles for spawning sardines in the region, suggesting a major role of food web interactions in the success of local sardine populations (Riveiro *et al.*, 2000). Previous studies of gut contents indicated that larval sardines (4–24 mm of total length) feed primarily on zooplankton (Conway *et al.*, 1994), while adults consumed variable proportions of phyto- and zooplankton, depending on availability at the time of sampling (Varela *et al.*, 1988, 1990).

The objective of this study was to ascertain the degree of complexity in food web interactions in the pelagic ecosystem of Galicia by measuring the natural

abundance of stable carbon and nitrogen isotopes in selected organisms of different size. For this purpose, we first determined the variability of stable isotope abundance with organism size and sampling zones within the study area. Later, we used stable isotope abundance to determine the trophic position of each selected organisms taking into account their individual size. Plankton was selected as the base of the food web and sardine was selected as representative of the planktivorous consumers. Also, the common dolphin was taken as an example of a top pelagic predator in this ecosystem (González *et al.*, 1994; Santos *et al.*, 1996).

Material and methods

Sampling and oceanographic observations

Samples were collected from 14 to 23 Mar 1999 on RV “Thalassa”, during the season of peak spawning of sardine (López-Jamar *et al.*, 1995) and phytoplankton spring blooms in Galicia (Bode *et al.*, 1994). Upwelling intensity was estimated from daily upwelling index values computed from geostrophic winds at a location off the Galician coast by Lavin *et al.* (2000). Surface chlorophyll distributions during the sampling period were obtained from SeaWiFS satellite images provided by the Plymouth Marine Laboratory (UK). Stations for plankton sampling were distributed in across-shelf transects from the coast to the shelf-break (Figure 1). Water samples from 3 m depth were collected with a submersible pump to determine chlorophyll-*a* concentration in acetonic extracts of phytoplankton collected on glass-fibre Millipore Type-F filters by vacuum filtration and analysed by the fluorimetric method (Parsons *et al.*, 1984). Plankton was collected during the night by means of vertical hauls of a WP2-type net of 20 µm mesh-size from 100 m depth to the surface. Such sampling extended well below the euphotic zone (Bode *et al.*, 1994) thus likely collecting phytoplankton as well as most of zooplankton species migrating to the surface during the night (Fernández de Puelles *et al.*, 1996). Samples were subsequently fractionated through sieves of 200, 500 and 1000 µm mesh-size and each fraction was first carefully washed with filtered seawater, transferred to glass fibre filters, and stored frozen until further processing in the laboratory. Sardines (11 to 24 cm total length) were collected at three locations (Figure 1) using a pelagic trawl with vertical aperture of 24 m for 15–30 min of effective sampling. Each specimen was first measured (± 5 mm), weighed (± 0.2 g) and then dissected to obtain portions of white muscle that were stored frozen for isotopic analysis. In addition, we collected portions of muscle of the common dolphin (*Delphinus delphis* Linnaeus) from individuals stranded on the coast (n=5, 3 males and 2 females).

Stable isotope determinations

To measure the ^{13}C and ^{15}N content, plankton samples were first dried (50°C, 24 h), and then ground and packed in tin capsules for conversion to CO_2 and N_2 in an elemental analyser (Carlo Erba CHNSO 1108) coupled to an isotope-ratio mass spectrometer (Thermo Finnigan Mat Delta Plus). Plankton samples were not acidified to remove carbonates as in other studies (e.g. Fry, 1988) because the acidification of some spare replicates showed no significant changes in the relative abundance of ^{13}C or ^{15}N (Kruskal–Wallis test, $p>0.05$, $n=21$). Muscle samples from sardine and dolphin were freeze-dried in the laboratory, and then treated with a mixture of chloroform, methanol and water to remove lipids (Bligh and Dyer, 1959) in order to minimize the differences in ^{13}C caused by the variable content of fatty tissue of individual fish (Sholto-Douglas *et al.*, 1991). After lipid removal, muscle samples were dried (50°C, 24 h), ground and packed in tin capsules, and fed into the mass spectrometer as described for plankton samples. The natural abundance of ^{13}C and ^{15}N was expressed as isotope ratios (‰) according to the formula:

$$\delta X = [(R_{\text{sample}} - R_{\text{std}}) / R_{\text{std}}] 1000$$

where $X = ^{13}\text{C}$ or ^{15}N , $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, and $\text{std} = \text{Peedee Belemnite carbonate for } \delta^{13}\text{C}$, and atmospheric nitrogen for $\delta^{15}\text{N}$. Three aliquots of each sample were analysed separately to obtain a mean δX value. Precision (± 1 standard error) for both C and N triplicate isotopic determinations was better than 0.03‰.

Variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values across organisms was expressed as isotope size-spectra following the procedure used by Fry and Quiñones (1994), which consisted in plots of natural abundance values versus the \log_2 of nominal organism carbon biomass for each size-fraction. Nominal biomass of individuals for plankton size classes was computed from the geometric mean of lower and upper mesh sizes for each class and the equations in Rodriguez and Mullin (1986) relating size and carbon biomass of zooplankton. Sardine samples were grouped in four \log_2 individual biomass size classes computed from individual fresh weights and a conversion factor of $0.04 \text{ g C [g fresh weight]}^{-1}$ (Peters, 1983). Dolphin individual weights were estimated as the geometric mean of fresh weight values from the literature (Carwardine, 1995).

To obtain a preliminary estimation of the trophic structure of the pelagic ecosystem, we considered a simplified food web based on phytoplankton as the first trophic level and composed of consumer organisms (grouped in size classes) that preyed on several food sources. Instead of being classed in a single trophic level, each consumer would have a trophic position defined by

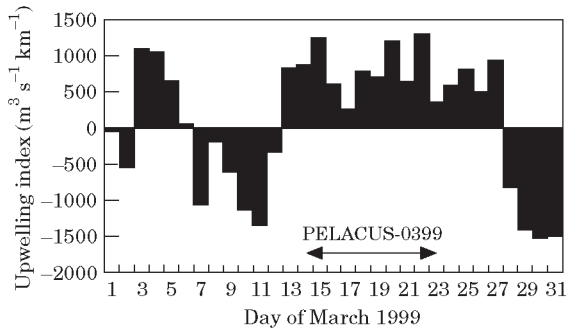


Figure 2. Daily upwelling index values during March 1999 computed from geostrophic winds at 43°N, 11°W (Lavin *et al.*, 2000).

the relative proportions of the different preys in its diet. The trophic position was computed according to the formula:

$$\text{Trophic position}_{\text{consumer}} = 1 + \frac{(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{phytoplankton}})}{\Delta\delta^{15}\text{N}}$$

where $\delta^{15}\text{N}_{\text{consumer}}$ is the mean $\delta^{15}\text{N}$ value for a given size class. The $\delta^{15}\text{N}$ value of 20–200 μm plankton was attributed to primary producers ($\delta^{15}\text{N}_{\text{phytoplankton}}$) and thus represents the reference value at the base of the food web (trophic position=1). Also a mean trophic fractionation ($\Delta\delta^{15}\text{N}$) of 3‰ between adjacent trophic levels was assumed to take into account the lower fractionation reported for primary consumers (Vander Zanden and Rasmussen, 2001; Post, 2002).

Results

Oceanographic conditions

The sampling cruise was at the beginning of the upwelling season under the influence of predominantly northeastern winds which caused positive upwelling index values (Figure 2). A large phytoplankton bloom developed over the shelf (Figure 3). The bloom was initially restricted to coastal waters but later extended to outer shelf and oceanic regions, particularly in the southern part of the study area where the displacement of surface water by the upwelling exported chlorophyll-rich surface waters offshore. Measured chlorophyll concentrations from surface samples collected during the cruise were higher than 2 mg Chl *a* m^{-3} at shelf stations of the southern section, under the influence of the nearby rias, whereas values generally decreased at stations in central and northern sections (Figure 4).

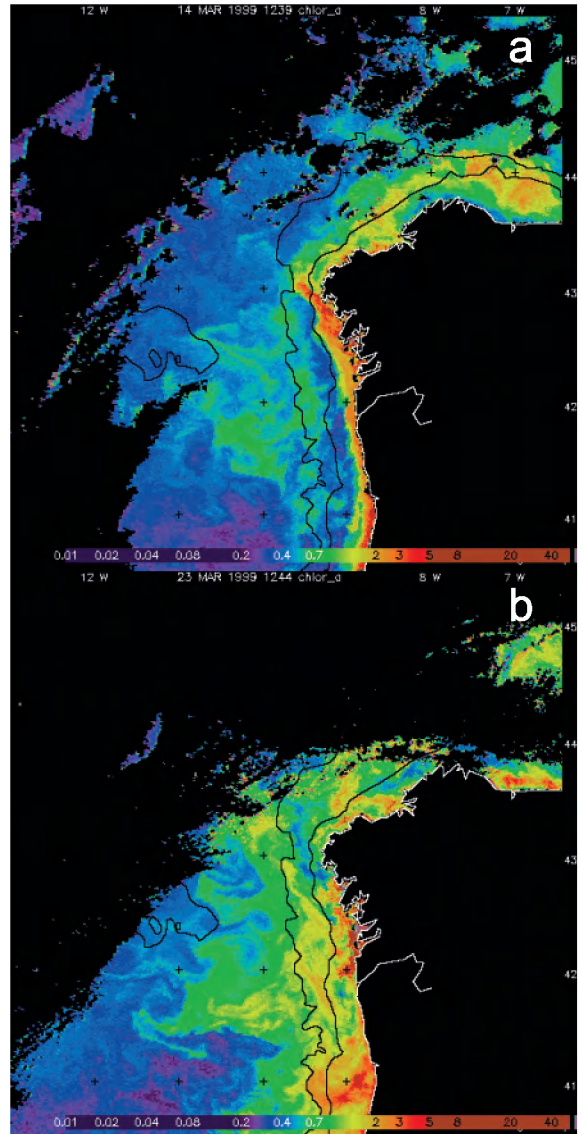


Figure 3. Surface chlorophyll concentration (mg m^{-3}) for the first (a) and last day (b) of the sampling cruise estimated from SeaWiFS images processed at CCMS–Plymouth Marine Laboratory.

Overall stable isotope variability

There was a large overlap in isotopic content of the selected size classes particularly among plankton classes (Figure 5), however the mean values of $\delta^{15}\text{N}$ of plankton, sardine and dolphin were significantly different (Kruskal–Wallis test, $p < 0.01$, $n = 142$). The mean enrichment in ^{15}N with doubling organism size, estimated from the slope of the regression line between $\delta^{15}\text{N}$ and $\log_2(w)$ was 0.28‰. Values of $\delta^{13}\text{C}$ were more variable than those of $\delta^{15}\text{N}$ and the corresponding mean increase with doubling size was only 0.10‰.

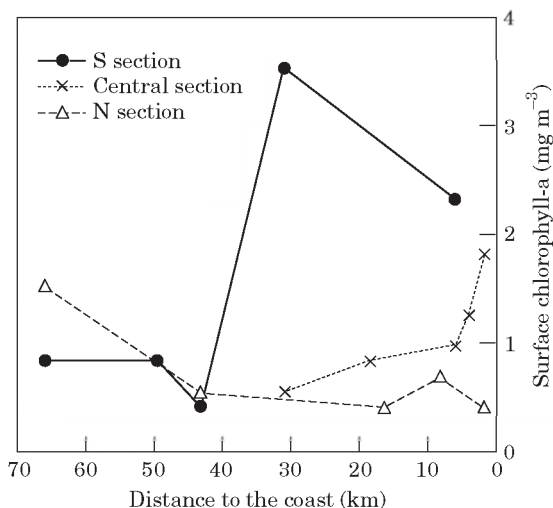


Figure 4. Surface chlorophyll concentration (mg m^{-3}) measured at the plankton sampling stations during the cruise.

Stable isotope variability across plankton size classes

No significant differences were found between mean values of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in the different transects for each plankton size class (Kruskal–Wallis test, $p > 0.05$), suggesting that plankton composition and trophic position within each size class was similar in the whole study area at the time of sampling. However, most samples from coastal stations in the northern and southern transects (e.g. stations 36 and 37) displayed higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than those from off-shore stations (e.g. stations 39 and 40). In contrast, isotopic values from stations in the central transect were less variable (Figure 6).

There was no clear trend in $\delta^{13}\text{C}$ with the size of plankton (Figure 6), since low values were found at the largest sizes at some stations (e.g. stations 69 and 37) but the converse occurred at other stations (e.g. stations 51 and 39). In contrast, $\delta^{15}\text{N}$ generally increased with size. A mean enrichment of $\delta^{15}\text{N} = 0.15\text{‰}$ with doubling organism size can be computed if the values from the smallest size class are included, but the mean enrichment computed using values from the three larger classes increased up to 0.39‰ (Figure 7). Such differences in enrichment when the $20\text{--}200\ \mu\text{m}$ size class is included agree with the low enrichment between primary producers and herbivores reported in most aquatic systems (Vander Zanden and Rasmussen, 2001).

Stable isotope variability in sardine

The regression line between $\delta^{13}\text{C}$ and the mean value of $\log_2(w)$ for each size class using all data points was non significant, but a mean decrease of 0.21‰ with doubling organism size was computed for $\delta^{15}\text{N}$ (Figure 8). When

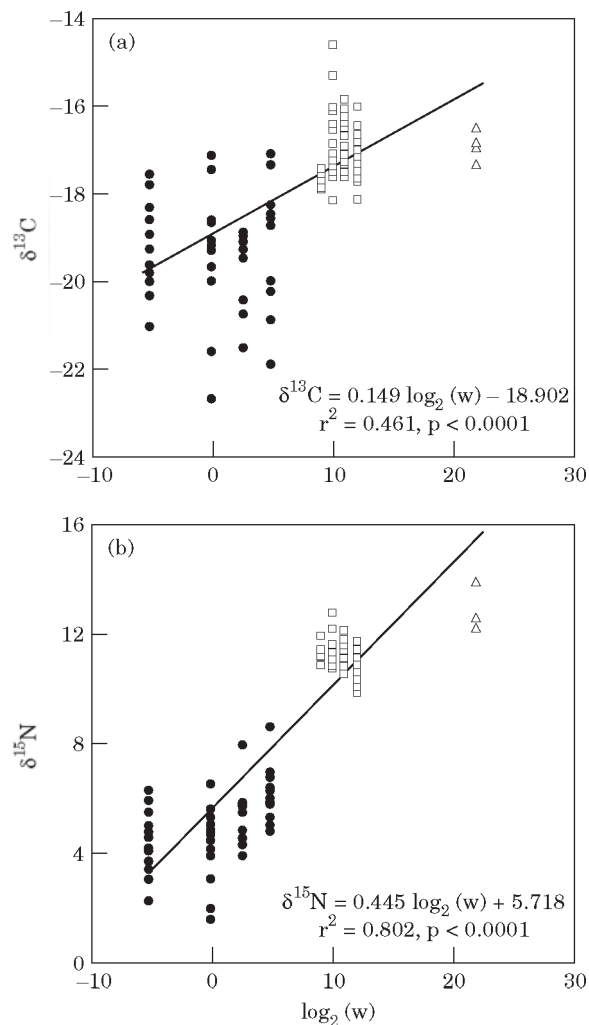


Figure 5. Values of $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) for plankton (dots), sardine (open squares) and dolphin samples (open triangles) grouped in size classes. Size was expressed as \log_2 of individual weight (w) in mg C . Regression lines for $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ versus $\log_2(w)$ and their significance were shown.

considering individual weights, sardine samples exhibited a characteristic variation of heavy isotope content with size, with values of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ first increasing with size and later decreasing for larger size classes). The abundance of ^{13}C increased with size up to the point when the sardine reached 18 cm in length, with a mean enrichment of 0.78‰ with doubling individual weight, which was equivalent to an enrichment of 0.25‰ per cm of length (Figure 8). Sardines of length ≥ 18 cm displayed a significant mean decrease of 0.56‰ in $\delta^{13}\text{C}$ with doubling individual weight (or 0.10‰ per cm of length). The depletion in heavier isotopes in sardines ≥ 18 cm was more marked in the case of nitrogen, having a mean decrease of 1.16‰ with doubling individual weight (or 0.22‰ per cm of length). However,

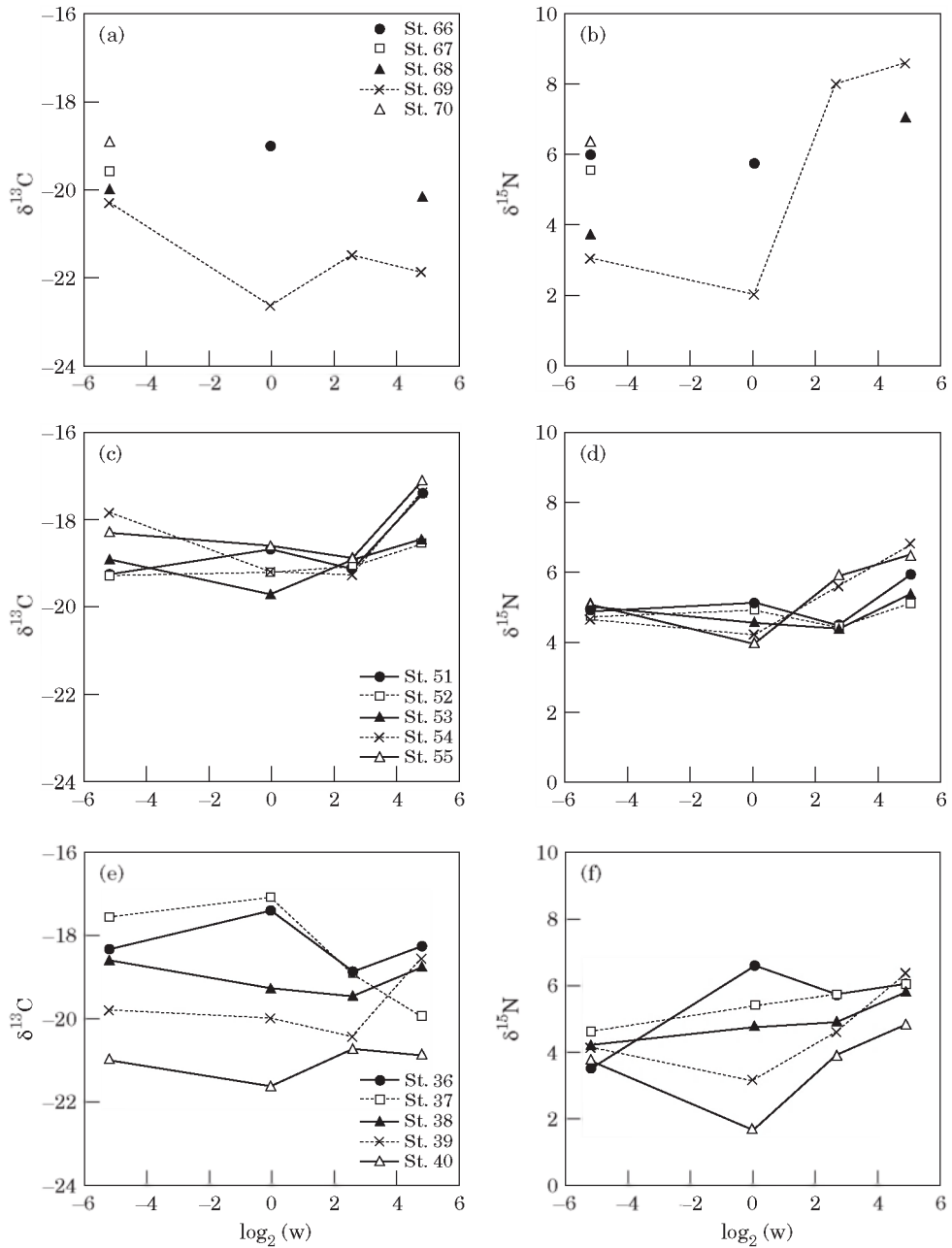


Figure 6. Values of $\delta^{13}\text{C}$ (a, c, e) and $\delta^{15}\text{N}$ (b, d, f) for plankton size classes at each station of northern (a, b), central (c, d) and southern sections (e, f). Size was expressed as \log_2 of individual weight in mg.

there was no significant correlation between $\delta^{15}\text{N}$ and size for sardines <18 cm. These results suggest a marked shift in the diet of sardines when they reach 18 cm, and the depletion in the heavier isotopes would be the consequence of an increase in the consumption of plankton organisms with a low position in the food web.

Food web structure

Small differences were found in mean $\delta^{15}\text{N}$ values between the 20–200 μm plankton and other classes (Table 1). Such low isotope fractionation values are expected if each size class contained a mixture of organisms from various trophic levels (Fry and Quiñones,

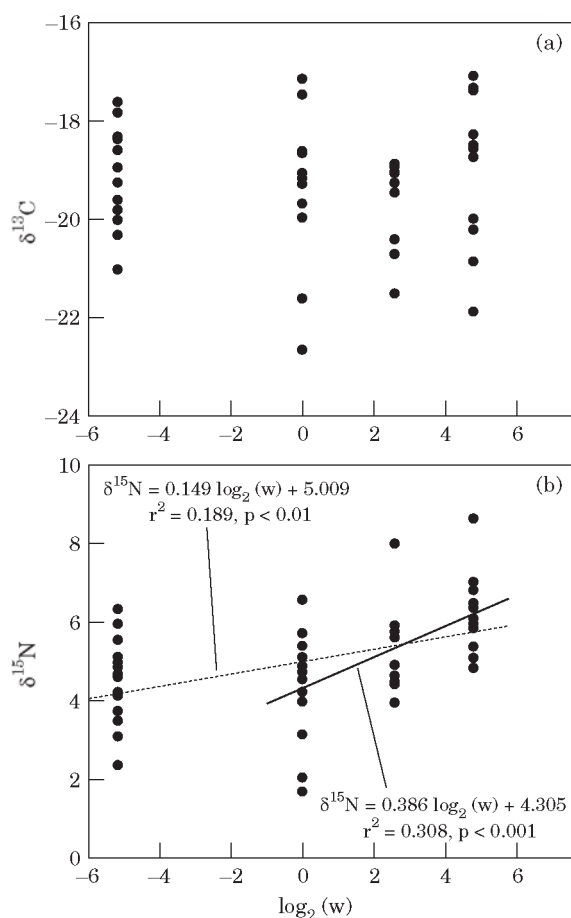


Figure 7. Values of $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) for plankton size classes. Size was expressed as \log_2 of individual weight (w) in mg C. Regression lines for $\delta^{15}\text{N}$ versus $\log_2(w)$ considering all size classes (discontinuous line) or only the three larger size classes (continuous line), along with their significance, were shown. No significant regression lines could be computed for $\delta^{13}\text{C}$.

1994). Visual inspection of fresh samples revealed the presence of phytoplankton-derived particles in nearly all size classes, particularly in those $<500\ \mu\text{m}$, as expected by the dominance of chain-forming diatoms in spring phytoplankton blooms in the region (Bode *et al.*, 1994; Casas *et al.*, 1997; Teira *et al.*, 2001). Furthermore, the decrease in mean $\delta^{15}\text{N}$ value in the 200–500 μm size class, can be interpreted as the consequence of the omnivorous trophic habits of most copepods present in this size-fraction during spring (Barquero *et al.*, 1998). Given that most of phytoplankton biomass is likely to be contained in the 20–200 μm size class, and the uncertainty in the mean trophic position of the organisms collected in the 200–500 μm size class, we used the mean value of the former size class as the isotopic baseline for the computation of trophic positions along the pelagic food web. However, these calculations must be taken as

a first approach, since the errors associated to trophic position estimates using primary producers almost doubled those using primary consumers (Vander Zanden and Rasmussen, 2001).

According to these calculations, sardines appeared mostly as secondary consumers, having a tendency towards herbivory in larger fishes that led to a slightly lower mean content in ^{15}N in samples from specimens $\geq 18\ \text{cm}$. Such changes in trophic position of sardines are more apparent if we employ the negative relationship between $\delta^{15}\text{N}$ and size in Figure 8 to compare the change in mean trophic position between sardines of different size. For instance, an individual of 18 cm in length would have a trophic position of 3.4 trophic levels, whereas at 24 cm its trophic position would be 3.0 trophic levels. However, zooplankton (both herbivores and carnivores) must have been a large fraction of their diet to produce the observed $\delta^{15}\text{N}$ value in the muscle of sardines, as trophic positions of both large and small sardines are about 3 trophic levels, which indicates the relative importance of the consumption of carnivorous preys. Finally, although dolphins were situated near the fourth trophic level in this pelagic ecosystem, preying mostly on secondary consumers like sardines, their mean $\delta^{15}\text{N}$ value was only 0.7 trophic levels above the trophic position of sardines. This suggests the existence of a relatively large degree of omnivory in these top predators as in the other organisms considered in this study.

Discussion

Our preliminary analysis using stable isotopes suggests a relatively complex food web off Galicia in which omnivory prevails in the various compartments considered. In the case of the sardine we obtained a mean trophic position of ca. 3 trophic levels, which corresponds to a secondary consumer. Such result confirms the conclusions obtained in previous studies (Cushing, 1978; Monteiro *et al.*, 1991) revealing that upwelling ecosystems are more complex than expected if the large populations of planktivorous fishes would feed directly on phytoplankton (Ryther, 1969). However, the computation of trophic positions from the natural abundance of stable isotopes is based on some assumptions that require further examination.

First, the samples must be representative of the main trophic levels of the ecosystem studied and integrate the diets at time scales at which nutrient sources vary (Fry, 1988; Minagawa and Wada, 1984; Wada and Hattori, 1991; Hanson *et al.*, 1997; O'Reilly *et al.*, 2002). Plankton and planktivorous fishes are at the base of the pelagic food web in upwelling ecosystems, as shown by similar studies with stable isotopes in the Benguela upwelling (Sholto-Douglas *et al.*, 1991; Monteiro *et al.*,

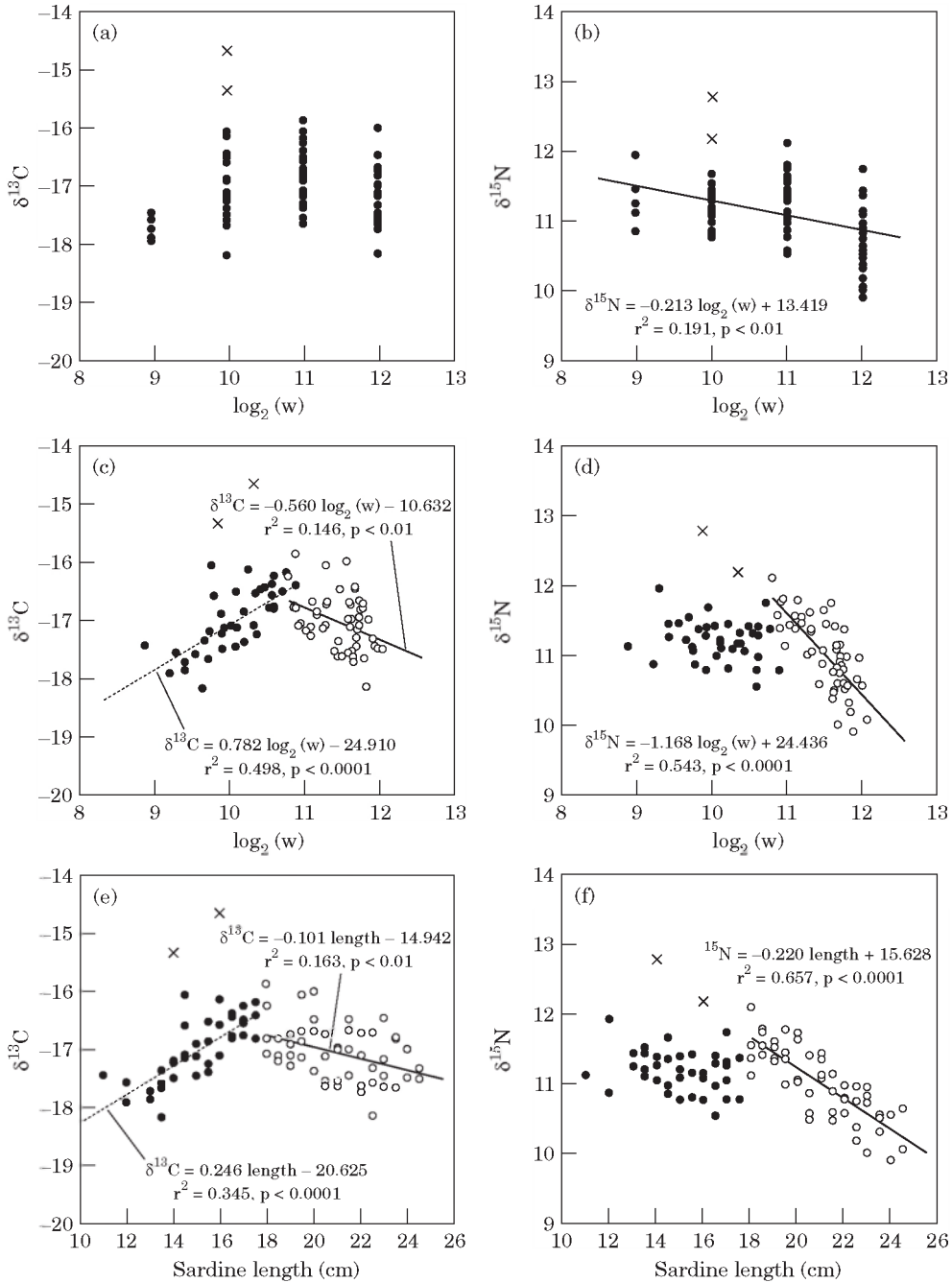


Figure 8. Plots of $\delta^{13}\text{C}$ (a, c, e) and $\delta^{15}\text{N}$ (b, d, f) versus size for sardine samples. Size was expressed as \log_2 of individual weight (w) in mg C, either grouped in 4 size classes (a, b) or using the individual weights (c, d), and also as individual length in cm (e, f). Regression lines for $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ versus size, along with their significance, were shown. Separate regression lines were computed for sardines < 18 cm (filled circles and discontinuous lines) and ≥ 18 cm (open circles continuous lines). Two outliers, not used in the computation of regression lines, were indicated as crosses.

1991). The addition in our study of the dolphin, a piscivorous predator, allowed for the characterization of the upper trophic levels. The obtained food web structure is representative of a time scale of several weeks, as

the isotopic composition of muscle proteins of fishes integrated dietary changes over this time period (Tieszen *et al.*, 1983; Hesslein *et al.*, 1993). In contrast, planktonic organisms are rapidly enriched in the new nutrients from

Table 1. Mean trophic positions of organisms in the pelagic ecosystem of Galicia computed from differences in average $\delta^{15}\text{N}$ values between plankton 20–200 μm and other plankton size classes, sardines and dolphin. A constant enrichment of 3‰ in $\delta^{15}\text{N}$ between adjacent trophic levels is assumed (see text).

Organism/size class	$\delta^{15}\text{N}$	$\Delta\delta^{15}\text{N}$	Trophic position
Plankton 20–200 μm	4.5	—	1.0
Plankton 200–500 μm	4.3	–0.2	1.0
Plankton 500–1000 μm	5.3	0.8	1.3
Plankton >1000 μm	6.2	1.7	1.6
Sardine <18 cm	11.3	6.8	3.3
Sardine 18 cm	11.1	6.6	3.2
Dolphin	13.1	8.6	3.9

the upwelling (O'Reilly *et al.*, 2002) and therefore the samples obtained in the present study would represent the source of food most likely enriched in nutrients directly derived from the upwelling. When interpreting food web structure at a seasonal scale, the variability in isotopic composition of the different plankton size classes according to the nutrient sources used and species succession, as reported in other systems (Rohlf, 2000), need to be taken into account due to the dependence of phytoplankton production on regenerated nutrients during some periods in the Galician upwelling (Alvarez-Salgado *et al.*, 1993; Bode and Varela, 1994; Castro *et al.*, 1994; Casas *et al.*, 1997; Teira *et al.*, 2001).

Second, the choice of appropriate values for mean trophic fractionation between trophic levels and for the reference baseline are two of the most critical aspects for trophic position computations. Recent reviews of published studies concluded that the use of a mean $\delta^{15}\text{N}$ value of 3.4‰ is a robust estimate of trophic fractionation between consumers, although values for plant-herbivore fractionation are more variable (Vander Zanden and Rasmussen, 2001; Post, 2002). In our study we have chosen a mean trophic fractionation of 3‰ per trophic level for a food web of up to three consumer levels and one plant-herbivore link with a mean fractionation of 2.5‰ (Vander Zanden and Rasmussen, 2001). Considering all other sources of error, the variability of trophic fractionation values has a small effect in the computation of trophic positions (Post, 2002). More important are the errors caused by the reference baseline, and the use of $\delta^{15}\text{N}$ values from primary consumers is preferred, but the separation of pure phytoplankton or herbivorous zooplankton is not always possible in pelagic samples. In this study we assumed that the smallest size class of plankton sampled contained mostly phytoplankton and used its mean $\delta^{15}\text{N}$ value as the reference baseline. Our $\delta^{15}\text{N}$ results for the 20–200 μm size class are consistent with the values given in the literature as typical for phytoplankton (Owens, 1987;

Goering *et al.*, 1990; McClelland and Valiela, 1998). Taking into account the mean value of $\delta^{15}\text{N}$ for that size class (4.5‰) and the value of 6.5‰ measured in nitrate below the upper mixing layer in the area (A. Bode, unpublished results), we can estimate the apparent fractionation factor for nitrate uptake by the 20–200 μm plankton as 1.0019, which is within the range published for marine phytoplankton species (Wada and Hattori, 1991; Horrigan *et al.*, 1990; Montoya *et al.*, 1990) and is consistent with the preference for nitrate of phytoplankton in upwelling systems (Dortch and Postel, 1989; Dugdale *et al.*, 1990). Using primary producers as the reference baseline and published mean $\delta^{15}\text{N}$ values for aquatic food webs, Vander Zanden and Rasmussen (2001) estimated that the error of trophic position estimates would be ca. 0.4 trophic levels for a typical food web. Such an error would imply that there were no significant differences between mean trophic positions within plankton or sardine size classes in our study, but at the same time would still allow for the characterization of trophic positions of large consumers, as the differences between plankton, sardine and dolphin trophic position exceeded the assumed error. Also, the differences found between mean $\delta^{15}\text{N}$ of sardines of 18 and 24 cm can be considered as indicators of true differences in trophic position.

In the size class approach chosen in our study there is no information about the relative contribution of planktonic species to a given size class, and therefore to the isotopic signature of that size class. However, it is well established that small sizes are dominated by primary producers while secondary and tertiary consumers are usually of large size (e.g. Peters, 1983; Rodriguez and Mullin, 1986; Fry and Quiñones, 1994). Also, studies of $\delta^{15}\text{N}$ enrichment across size classes of fishes demonstrated that trophic positions within a community were largely determined by the size of the organisms while inter-specific relationships between maximum body mass and $\delta^{15}\text{N}$ were of low relative importance (Jennings *et al.*, 2001, 2002). The overall increase in trophic position, was generally caused by the intra-specific accumulation of heavy isotopes with the growth in body mass (Rau *et al.*, 1981; Lindsay *et al.*, 1998; Jennings *et al.*, 2002). However, we found a significant negative relationship between size and $\delta^{15}\text{N}$ for sardines ≥ 18 cm, a size that is generally attained after the first year of life (ICES, 1999). We interpreted such relationship as the consequence of the increasing consumption of phytoplankton by adult sardines, as most clupeids are known to employ both particle-feeding and filter-feeding (James, 1988). Similarly, King and MacLeod (1976) showed the change from zoophagy to a more phytophagous diet with age in *E. capensis* using gut content studies and anatomical considerations of the filtering mechanism of this clupeid, while Sholto-Douglas *et al.* (1991), when noting the decrease in $\delta^{15}\text{N}$ in various

tissues of two clupeid species with length, did not discard completely possible changes in diet but suggested that the observed changes would be the result of protein metabolism.

The north Iberian sardine is well suited to use a filter feeding mode, compared to other clupeids (Blaxter and Hunter, 1982). On the one hand it has a mesh of relatively long gill-rakers in its branchial archs that are able to intercept plankton, and its epibranchial organ concentrating food items is one of the largest among clupeids. The length of gill-rakers and the number of denticles increase during growth (Andreu, 1953, 1960) reducing the size of particles that can be intercepted. On the other hand, *S. pilchardus* has a larger proportion of red muscle than other clupeids and its white muscle is highly vascularized (Blaxter and Hunter, 1982), which allows for continuous swimming and the exploitation of diverse habitats. The mean trophic position of sardine obtained in this study is consistent with the description of gut contents for juvenile and adults of this species (Cépède, 1907; Oliver, 1951; Oliver and Navarro, 1952; Varela *et al.*, 1988, 1990). Although a completely phytophagous diet is rare among clupeids, the abundant phytoplankton collected by the gill-rakers when the fish actively prey upon zooplankton would complement their diet (Cushing, 1978).

Furthermore, from the computed mean increase in $\delta^{15}\text{N}$ with doubling size from plankton to dolphins (0.28‰), and a mean trophic enrichment of 3.4‰ (Vander Zanden and Rasmussen, 2001; Post, 2002), we can estimate a mean value for the ratio of predator to prey body sizes as $2^{3.4/0.28}$ of ca. 4500:1. Such value is consistent with the expected importance of plankton organisms, of a size much smaller than those of their predators, in the pelagic ecosystem (Sheldon *et al.*, 1977). Particularly, filter-feeding is associated to large values of the predator-to-prey body size ratio. For instance, Cushing (1978) estimated that this ratio would be up to two orders of magnitude larger in case of filter-feeders compared to predators, which would have a value of ca. 100. In contrast, much lower values were reported in case of demersal and benthic communities, where the average predator-to-prey ratio was ca. 400:1 (Jennings *et al.*, 2002). Although the use of a mean fractionation value for the whole food web is subject to substantial errors (Vander Zanden and Rasmussen, 2001), and possibly small fractionation of nitrogen would occur in the organisms of the microbial food web (Fry and Quiñones, 1994), our results from stable isotope analysis suggest the dominance of omnivory through the pelagic food web in the Galician upwelling, in agreement with the results of gut content studies. Also, the relationships between $\delta^{15}\text{N}$ and the size of organisms obtained provide a quantitative tool to analyse changes in the trophic structure of this ecosystem related to upwelling dynamics.

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