Available online at www.sciencedirect.com

SCIENCE @ DIRECT°

Taxonomic versus trophic structure of mesozooplankton: a seasonal study of species succession and stable carbon and nitrogen isotopes in a coastal upwelling ecosystem

Antonio Bode and Maria Teresa Alvarez-Ossorio

Bode, A., and Alvarez-Ossorio, M. T. 2004. Taxonomic versus trophic structure of mesozooplankton: a seasonal study of species succession and stable carbon and nitrogen isotopes in a coastal upwelling ecosystem. − ICES Journal of Marine Science, 61: 563−571.

Seasonal variations in mesozooplankton ($>200\ \mu m$) stable carbon and nitrogen isotope abundances were compared with the seasonal species succession pattern off A Coruña (Galicia, NW Spain). Mesozooplankton biomass, numerical abundance, species composition, and natural abundance of stable isotopes, along with water column temperature, salinity, chlorophyll a and dissolved nitrate concentrations, were studied in monthly samples during the years 2000 and 2001 at a coastal station (80-m depth). Biomass and abundance of mesozooplankton followed the annual cycle with maxima in spring and summer (year 2000) and occasionally also in autumn (year 2001). The succession pattern shown by the taxonomic assemblages was recognized in both years of study, despite differences in the timing of upwelling and the large input of continental water during most of 2000. Variations in neither carbon nor nitrogen isotopes were correlated with either abundance or biomass at annual time scales, but heavy isotopes significantly accumulated during periods of zooplankton increase (spring and early summer). The consumption of nitrate during the spring can explain in part the progressive accumulation of heavy nitrogen isotopes during this period. In addition, the abundance of carnivorous zooplankton (mainly carnivorous copepods, siphonophores, and chaetognaths) was linearly related to the increase in heavy isotopes during the spring and, in the case of nitrogen, also during the whole study period. The computed linear relationships between isotope abundances and plankton species composition provided a way to further analyse the pelagic foodweb through the estimation of isotope reference values for primary consumers.

A. Bode and M. T. Alvarez-Ossorio: Instituto Español de Oceanografía, Centro Oceanográfico de A Coruña, Apdo. 130, 15080 A Coruña, Spain; e-mail: maite.alvarez@co.ieo.es (M. T. Alvarez-Ossorio). Correspondence to A. Bode: tel: +34 981 205362; fax: +34 981 229077; e-mail: antonio.bode@co.ieo.es.

Introduction

The study of the trophic relationships in pelagic ecosystems is complicated by the large variability in diet of most species, which leads to unstructured foodwebs (Isaacs, 1973). Omnivory is widespread among all organisms, but a particularly large number of zooplankton species may feed on similar diets composed of a mixture of phytoplankton, detritus, and microplankton (e.g. Stoecker and Capuzzo, 1990; Irigoien et al., 1998; Batten et al., 2001). Experimental determinations of the contribution of non-autotrophic prey to the diet of zooplankton (mostly copepods) suggest estimates ranging from purely carnivorous diets to a variety of mixtures of algae and animal prey (Gifford and Dagg, 1991; Fessenden and Cowles, 1994;

Irigoien et al., 1998; Batten et al., 2001; Halvorsen et al., 2001). Such variety in the diet makes the quantification of flows between compartments or trophic levels difficult.

As heavy isotopes accumulate in consumers relative to their prey (Minagawa and Wada, 1984; Peterson and Fry, 1987), the value of natural abundance of stable isotopes in plankton has been used to analyse the trophic structure of various foodwebs (Mullin et al., 1984; Fry and Quiñones, 1994; Rolff, 2000). The relative enrichment in heavy isotopes between species or size classes can be interpreted as a measure of the complexity of a particular foodweb as it reflects the number of trophic steps from the primary producers. This is supported by the relative constancy of the isotopic fractionation between successive consumer levels found in several studies (Minagawa and Wada, 1984;

Vander Zanden and Rasmussen, 2001; Post, 2002), partic-ularly in the case of nitrogen isotopes ($+3.4\permil$). In this way, the trophic position of a particular compartment in the foodweb may be computed if we have an estimate of the isotopic value of the food at the base of the foodweb. How-ever, the choice of an appropriate reference value is complicated because the isotopic abundance of the phyto-plankton depends on the nutrient source (O'Reilly *et al.*, 2002) and on the species composition (Fry and Wainright, 1991), particularly in upwelling ecosystems. In addition, the isotopic fractionation between primary producers and her-bivores is highly variable (Vander Zanden and Rasmussen, 2001).

Recent studies with stable isotopes have demonstrated that the use of the natural abundance value of a primary consumer as the isotopic reference greatly reduces the uncertainty of estimations of trophic level in foodwebs of freshwater ecosystems (Vander Zanden and Rasmussen, 2001; Post, 2002; O'Reilly *et al.*, 2002). In marine pelagic ecosystems of temperate latitudes these primary consumers can be found among the zooplankton, but because species composition and abundance vary seasonally (e.g. Cole-brook, 1979) the selection of a single species as a reference may not be appropriate. Furthermore, several studies have shown that the isotopic composition of zooplankton size classes varies during the year ultimately due to changes in the sources of nutrients available for the primary producers (Goering *et al.*, 1990; Montoya *et al.*, 1990; Rolff, 2000).

In this study, we determined seasonal variations in stable carbon and nitrogen isotope abundances in zooplankton, which were compared with the seasonal species succession pattern off A Coruña (Galicia, NW Spain). The objective was to demonstrate the existence of trends in the isotopic composition of zooplankton over the annual species succes-sion and productivity cycles. The results of this study may provide clues for the estimation of natural abundance

values that can be used as the isotopic reference in the determination of trophic positions in marine pelagic ecosystems.

Materials and methods

Zooplankton ($>200\ \mu m$) was collected monthly during the years 2000 and 2001 at Station 2 off A Coruña (Figure 1). Samples were obtained by double oblique tows of a Juday-Bogorov net (200-μm mesh size) between the surface and the bottom (maximum depth 80 m). Aliquots were taken for the determination of biomass (dry weight), numerical abun-dance and species composition, and natural abundance of stable carbon and nitrogen isotopes. Temperature and salin-ity profiles were measured with a Seabird 25 CTD and water column chlorophyll *a* (Chl *a*) and dissolved nitrate concentrations were analysed. Details of the sampling and analytical methods for these variables can be found in Casas *et al.* (1997). Zooplankton were identified under the microscope to the species level where possible. Particular attention was paid to identify species or groups with potentially carnivorous diets (Greene, 1985). Succession of zooplankton taxa was summarized by means of non-metric multidimensional scaling (Clarke and Warwick, 2001). The analysis was run on the Bray−Curtiss similarity matrix obtained from log-transformed abundance values of taxa present in $>20\%$ of samples. Successional phases were further analysed by means of the SIMPER procedure (contribution of individual taxa to sample group separation by the similarity matrix) and ANOSIM (significance of differences in similarity between sample groups) of the PRIMER statistical package (Clarke and Warwick, 2001).

For stable isotope determinations, dried (60$\degree$C, 48 h) and finely ground aliquots of zooplankton were packed in tin capsules for conversion into CO_2 and N_2 in an elemental
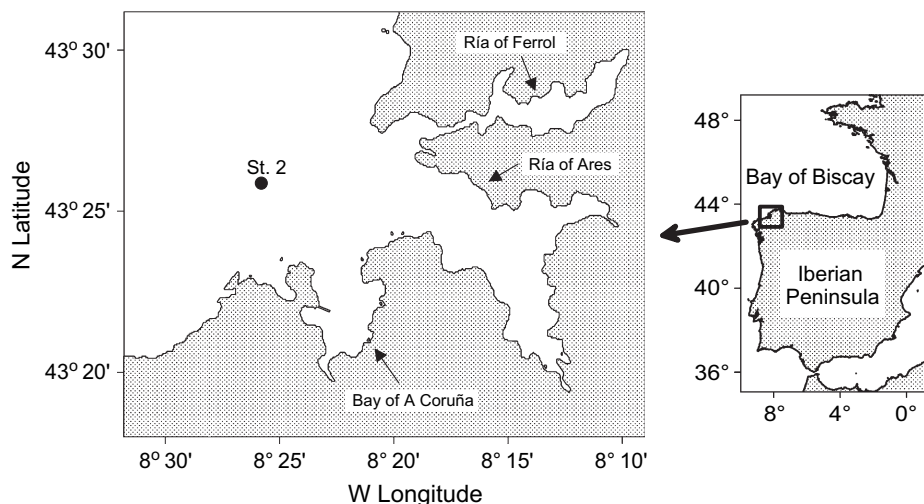

Figure 1. Map of the study site off NW Spain.

analyser (Carlo Erba CHNSO 1108) coupled to an isotope-ratio mass spectrometer (Finnigan Mat Delta Plus). Samples were not acidified to remove carbonates because other studies showed that the acidification may not cause substantial modification in carbon isotope results, but it may affect nitrogen determinations (Bunn *et al.*, 1995; Bode *et al.*, 2003). Carbon and nitrogen stable isotope abundance was expressed as $\delta^{13}C$ and $\delta^{15}N$ relative to VPDB (Vienna PeeDee Belemnite carbonate) and atmospheric N_2 isotope standards. Precision (± 1 standard error) of replicate determinations of both C and N stable isotopes was $< 0.03‰$.

The relationships between environmental, plankton, and stable isotope variables were analysed by non-parametric rank correlation coefficients (Spearman's ρ) and by robust regression (Least Trimmed Squares regression, see Venables and Ripley, 1997), the latter aimed at reducing the uncertainty in the estimations due to outliers, particularly when there are few observations. For these procedures, the statistical packages SPSS and S-Plus were employed.

Results

Environmental dynamics

The ecosystem studied is characterized by a typical seasonal cycle with minimum temperatures in winter throughout the water column and maximum values in summer, when maximum stratification arises as a consequence of solar heating of the surface (Figure 2a). Salinity changes are generally low, as there are no large rivers nearby, but in the period between March 2000 and April 2001 there was a large influence of low salinity water in the surface layer due to run-off; it was an unusually rainy year (Figure 2b). However, coastal upwelling superimposes on the seasonal cycle by keeping the water near the bottom at $< 14°C$ for most of the year and by introducing nitrate to the surface during the spring and summer (Figure 2c), which produces a series of phytoplankton blooms as a result of temporary breakdown of stratification (Figure 2d). Also, in this study, the run-off water in the winter of 2001 left an unusual amount of nitrate in the surface, exceeding the values found in bottom layers. The changes in surface waters during most of 2001 were restricted to a narrow layer near the surface and apparently did not affect bulk biological variables, as the water column averaged chlorophyll followed the pattern found in previous studies in the same area (Valdés *et al.*, 1991; Casas *et al.*, 1997). Primary productivity increased during spring–early summer (from March to June), following the depletion of nitrate in the surface waters. This is illustrated by the significant negative correlation between surface nitrate and the Julian day for the spring–early summer period, whereas there was no significant correlation
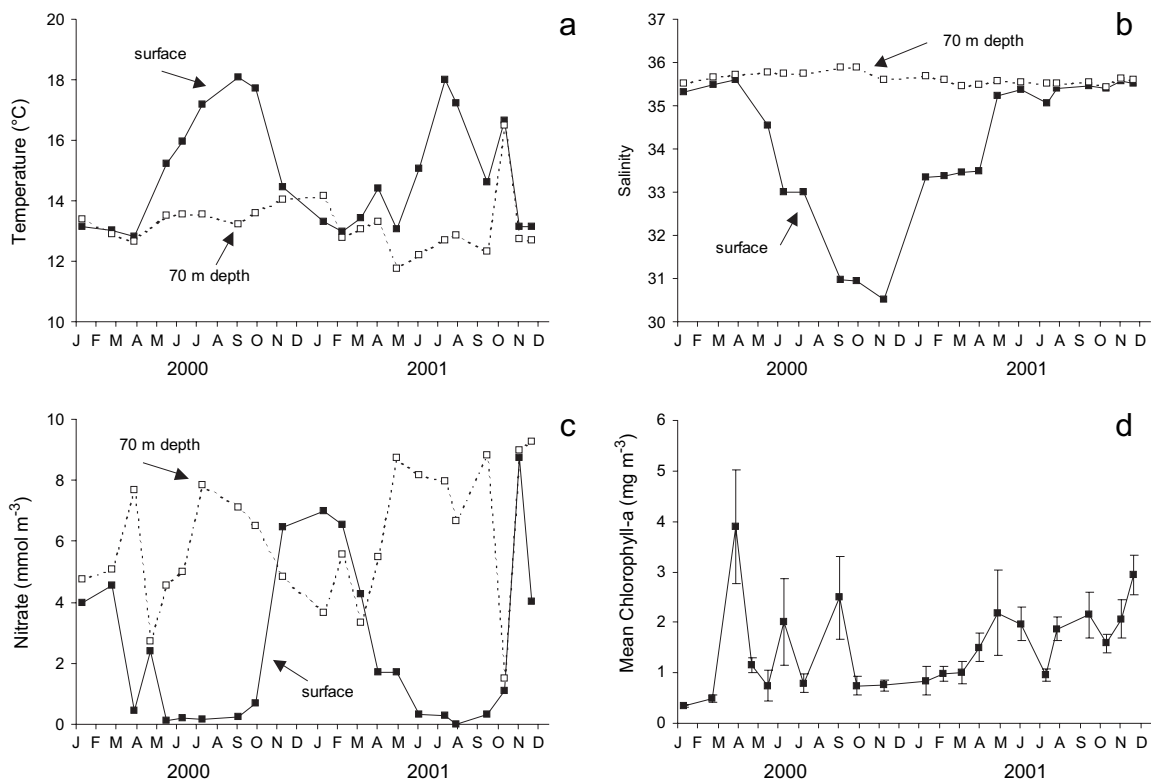

Figure 2. Variations of temperature (a), salinity (b), dissolved nitrate (c), and mean ($\pm$ s.e.) water column chlorophyll *a* concentration (d) during the study.

using all data (Table 1). Chlorophyll concentrations generally increased during spring, but because of the high variability found in the year 2000 we did not find significant correlations between mean chlorophyll and the Julian day (Figure 2d).

Zooplankton species succession

Mesozooplankton abundance (Figure 3a) followed an annual cycle with maximum values in spring and summer (year 2000) and occasionally also in autumn (year 2001). These maxima generally followed those of the phytoplankton and were caused mainly by the reproduction of holoplanktonic species such as Cladocera (Figure 3b) and copepods (Figure 3c), as well as the influx of meroplanktonic larvae into the community (Figure 3e). Taxa with potentially carnivorous diets included copepods (*Aetideus armatus*, *Paraeuchaeta hebes*, *Metridia lucens*, *Pleuromamma gracilis*, and *Candacia armata*), siphonophores, and ctenophores. Carnivores also displayed two annual abundance peaks, but in this case maximum numbers were generally reached by early summer (Figure 3d). Most of the variation in the abundance of carnivores was due to carnivorous copepods, significantly correlated with total carnivore abundance (Spearman's $\rho = 0.598$, $p < 0.01$). Similarly, variations in zooplankton biomass (not shown) were significantly correlated with those in total abundance (Spearman's $\rho = 0.851$, $p < 0.01$). The seasonal pattern coincides with previous studies of zooplankton succession and biomass seasonality at the same station (Valdés *et al.*, 1990, 1991;

Bode *et al.*, 1998) and in nearby areas of the NW Iberian Peninsula (Colebrook, 1979; Cabal, 1993).

Using multidimensional scaling, the succession pattern of the taxonomic assemblages was arranged in two main phases of high and low plankton abundance, respectively (Figure 4). Such phases were recognized in both years of study despite the differences in the input of continental water. The separation of samples into these groups was confirmed by the analysis of the similarity matrix with the ANOSIM procedure (global R statistic = 0.592, $p < 0.001$). Small-sized (e.g. *Acartia clausii*) and juvenile stages of copepods contributed to $> 50\%$ of similarity of samples within each of the phases of high and low total zooplankton abundance. In contrast, the contribution of the abundance of carnivorous taxa to average similarity in these groups was $< 1\%$ (SIMPER analysis). The high zooplankton abundance phase occurred from February to October, when surface temperature generally increased and surface nitrate decreased, and coincided with the period of high chlorophyll concentration in the water column (Figure 2).

Stable isotope variability

Both $\delta^{13}C$ and $\delta^{15}N$ displayed maximum values in late spring (May 2000) or early summer (June 2001), but high values were found also in autumn of 2001 (Figure 5). Natural abundance values of both isotopes were significantly correlated either when considering all samples or samples from the spring–early summer period (Table 1). Values of $\delta^{13}C$ (but not of $\delta^{15}N$) showed a significant

Table 1. Spearman rank correlation coefficients and significance (lower panel), and number of available paired data for selected variables (upper panel). Significance is indicated by asterisks: *$p < 0.05$; **$p < 0.01$; n.s.: non-significant. Correlations were computed using all available data either for the whole study or only data for the spring–early summer period (March–June).

|	Julian day	Surface t	Surface NO_3	Mean Chl a	Carnivores	$\delta^{13}C$	$\delta^{15}N$
				All data			
Julian day	—	20	21	21	20	20	21
Surface t	n.s.	—	20	20	19	20	20
Surface NO_3	n.s.	$-0.681**$	—	21	20	21	21
Mean Chl a	n.s.	n.s.	n.s.	—	20	21	21
Carnivores	n.s.	n.s.	n.s.	0.538*	—	20	20
$\delta^{13}C$	n.s.	n.s.	n.s.	0.466*	0.501*	—	21
$\delta^{15}N$	n.s.	n.s.	n.s.	n.s.	0.623**	0.464*	—
			Spring–early summer data (March–June)				
Julian day	—	7	8	8	8	8	8
Surface t	n.s.	—	8	7	8	8	8
Surface NO_3	$-0.898**$	n.s.	—	8	8	8	8
Mean Chl a	n.s.	n.s.	n.s.	—	8	8	8
Carnivores	n.s.	n.s.	n.s.	n.s.	—	8	8
$\delta^{13}C$	n.s.	n.s.	n.s.	0.738*	0.905**	—	8
$\delta^{15}N$	0.874**	n.s.	$-0.762*$	n.s.	0.833*	0.881**	—
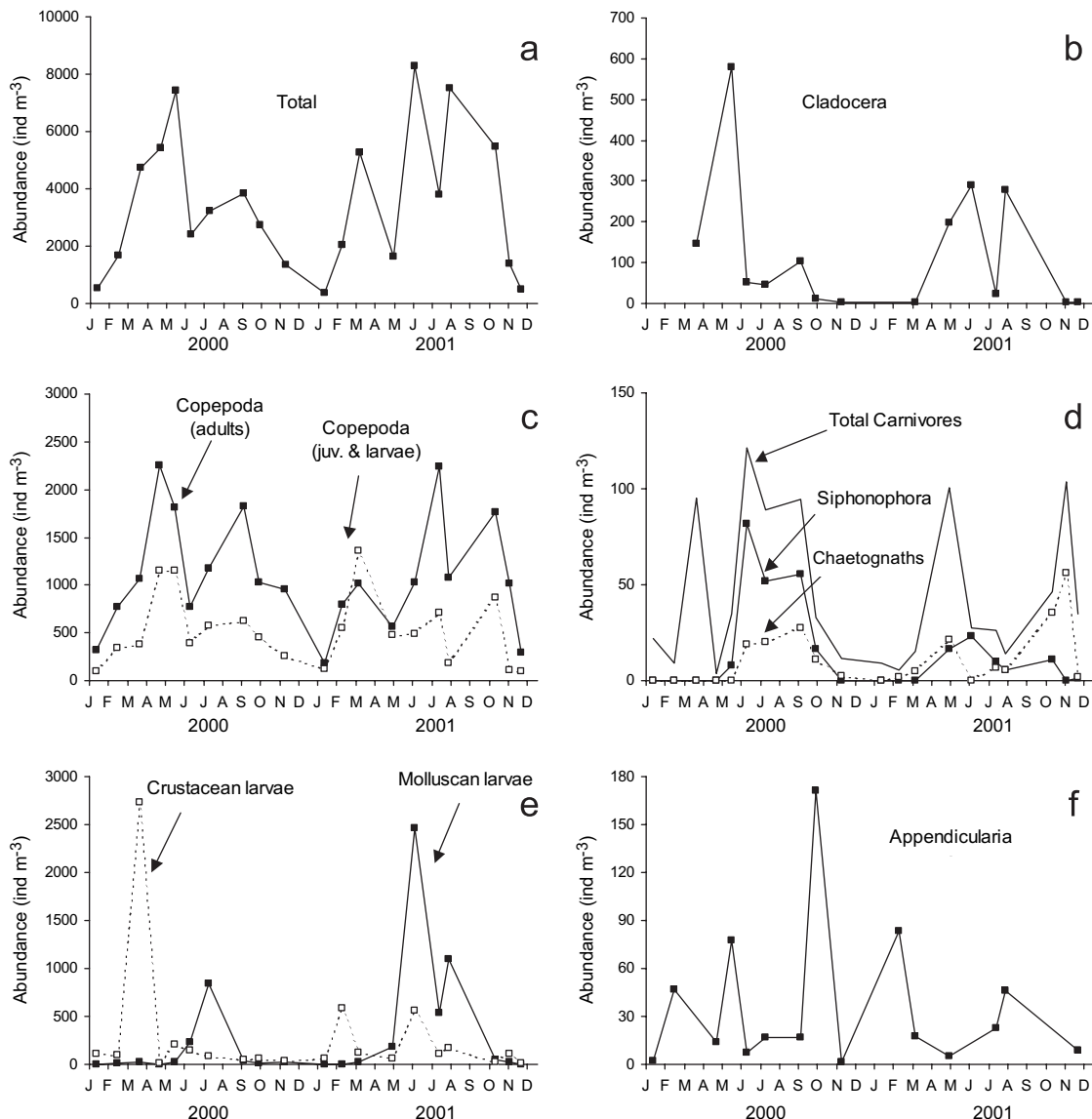
Figure 3. Variations in zooplankton abundance during the study. Total abundance (a) and abundance of Cladocera (b), Copepoda (c), Siphonophora, Chaetognaths and other carnivorous taxa (d), crustacean and molluscan larvae (e), and Appendicularia (f) are shown.

increase with mean Chl *a* (Figure 6; Table 2). In contrast, a significant increase of δ^{15}N values (but not of δ^{13}C) was found during the spring, coinciding with a decrease of surface nitrate concentrations. No significant correlations were found between either δ^{13}C or δ^{15}N and temperature or nitrate considering all sampling dates (Table 1).

Similarly, variations in carbon and nitrogen isotopes in zooplankton were not correlated with either total abundance or abundance of the dominant taxa, copepods ($p > 0.05$). No significant correlations were found either when considering only samples from spring and early summer. However,

abundance of carnivorous zooplankton was linearly related to the variations in δ^{13}C and δ^{15}N (Figure 6). Even when the determination coefficients were low, the regressions improved notably when using only spring–early summer data (Table 2). Furthermore, the regressions between δ^{13}C or δ^{15}N and carnivore abundance allow for estimation of the natural abundance values expected in a zooplankton community without predators (i.e. composed only from primary consumers). These values can be estimated from the intercepts of the robust regression lines relating isotope and carnivore abundances (Table 2).
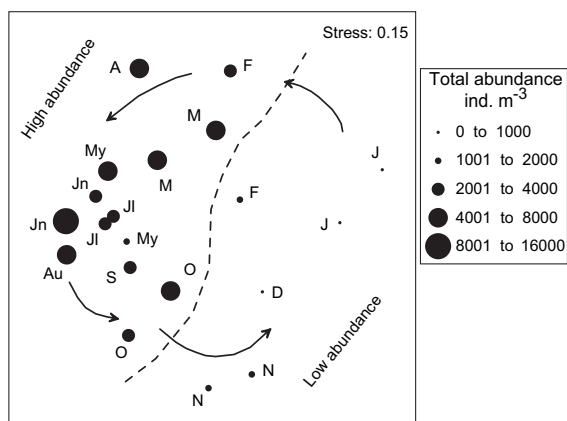
Figure 4. Non-metric multidimensional scaling plot of samples obtained from abundance data for zooplankton taxa. The stress value indicates the adequacy of the scaling in representing the seasonal ordination (Clarke and Warwick, 2001). Total mesozooplankton abundance is superimposed as circles with diameter proportional to their value in each sample scaled to the variability range of each variable. Sampling dates are indicated by letters and the succession patterns by arrows. Sampling dates are grouped in two periods of high and low plankton abundance.

Discussion

This study has shown that zooplankton annual succession patterns in NW Spain based on the taxonomic composition of the community are different compared with those taking into account trophic aspects. On the one hand, species succession is characterized by the rapid increase in numbers of almost all taxa from the late autumn and winter period to spring, mostly dominated by copepods (Valdés *et al.*, 1990, 1991; Cabal, 1993; Bode *et al.*, 1998). For most of the year (spring to early autumn) a series of abundance peaks of various taxa, including meroplanktonic larvae, contributes to maintaining high abundance and biomass values in the water column, with maximum values in spring and autumn. On the other hand, heavy carbon and nitrogen stable isotopes accumulate in zooplankton during the spring–early summer period, but decrease thereafter. Such a trend is shown particularly by nitrogen, while the abundance of carbon isotopes was more variable. These results contrast with those from ecosystems where the annual primary production is characterized by a single spring bloom that supports the subsequent growth of zooplankton (e.g. Goering *et al.*, 1990), but also with the pattern displayed by ecosystems with phytoplankton blooms in autumn (Montoya *et al.*, 1990) and in summer (Rolff, 2000). The growth of phytoplankton during spring is fuelled mostly by dissolved nitrate, which is expected to become progressively enriched in ^{15}N as it is consumed by phytoplankton because of isotopic fractionation (Wada and Hattori, 1991). The decrease in dissolved nitrate concentration is generally accompanied by an increase in $\delta^{15}N$ values (Montoya *et al.*,
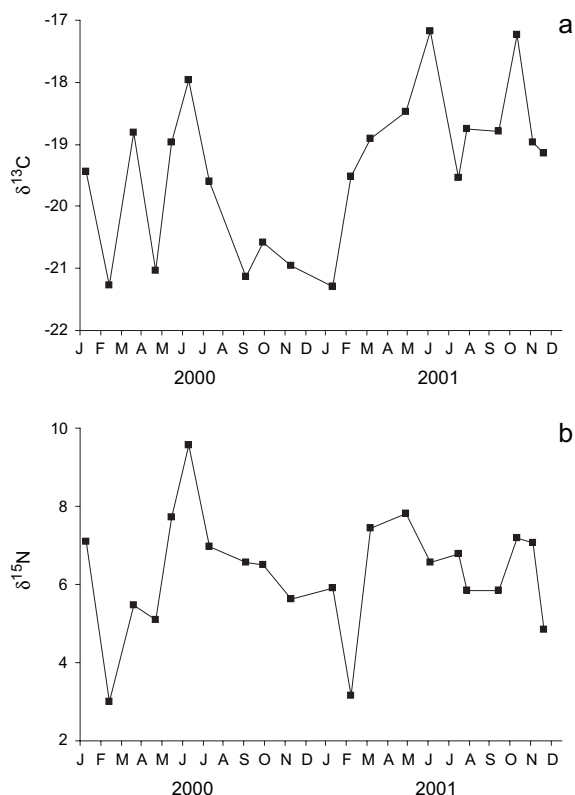

Figure 5. Variations in mesozooplankton $\delta^{13}C$ (a) and $\delta^{15}N$ (b) during the study.

1990). Correspondingly, the phytoplankton becomes progressively enriched in ^{15}N as dissolved nitrate is removed from the water in the upper mixed layer (Montoya *et al.*, 1990) and this has been related to the enrichment of zooplankton (Goering *et al.*, 1990; Montoya *et al.*, 1990; Rolff, 2000). The linear increase in zooplankton $\delta^{15}N$ from spring to early summer, as surface temperature increases and nitrate decreases in our study, fits well with the nutrient depletion process described. In turn, the decrease in $\delta^{15}N$ values from the annual maximum in early summer can be explained by the progressive use of regenerated nitrogen by phytoplankton (mostly ammonium excreted by zooplankton), which is depleted in ^{15}N (Montoya *et al.*, 1990). However, the absence of a clear increase in $\delta^{15}N$ from zooplankton as a response to phytoplankton blooms during summer and autumn in our study suggests that the accumulation of ^{15}N during spring and early summer is due to a combination of trophic and species succession processes.

First, most of the nitrate used by phytoplankton during the spring is likely to be supplied by the winter mixing of Eastern North Atlantic Central Waters (ENACW) with shelf waters (e.g. Casas *et al.*, 1997). Upwelling events of ENACW replace some of the depleted nitrate in the subsurface layers, thus maintaining relatively high levels of phytoplankton biomass during the spring and summer. In
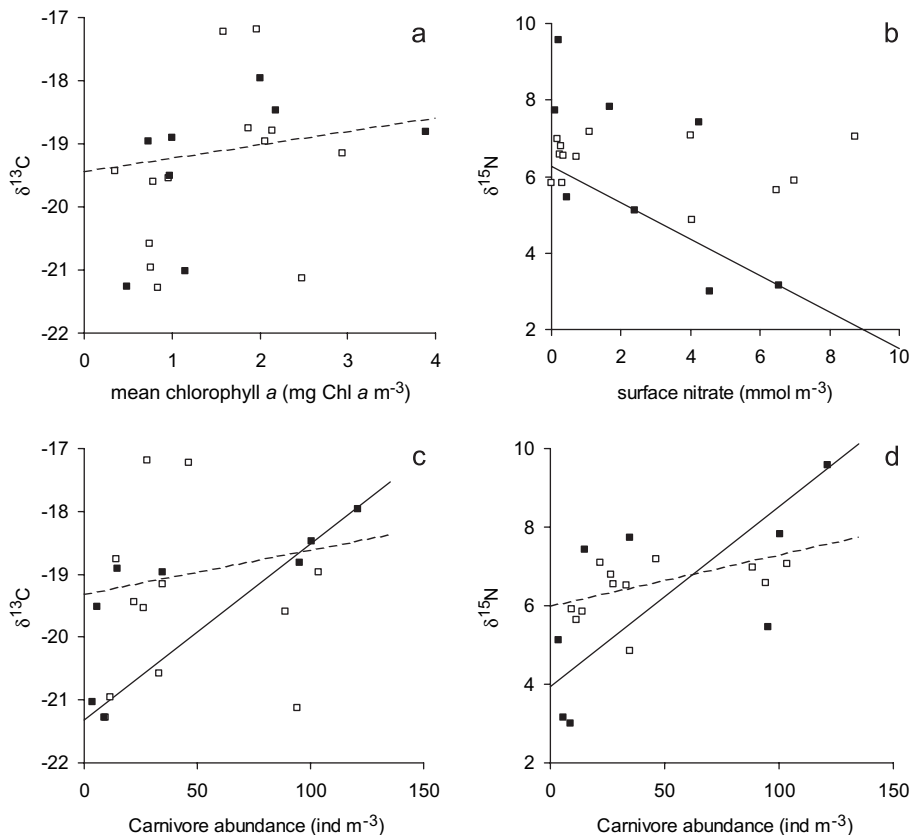
Figure 6. Relationships between $\delta^{13}C$ and mean chlorophyll concentration (a), $\delta^{15}N$ and surface nitrate concentration (b), and between $\delta^{13}C$ or $\delta^{15}N$ and total carnivore abundance (c and d, respectively). Separate regression lines (robust regression, see Table 2 and Methods) were computed using all samples (all dots, dashed line) or spring and early summer samples (filled symbols, continuous line). Only significant regression lines were shown.

contrast, nutrients provided by run-off seem of low importance in the study area, despite the clear signal of low salinity water found in the surface during the year 2000. The upwelled nitrate is expected to originate from regeneration in deep oceanic layers and thus to be relatively enriched in ^{15}N (e.g. Wada and Hattori, 1991). Previous measurements in the study area during the spring indicate that the isotopic signature of deep-water nitrate is consistently displayed by surface phytoplankton (Bode *et al.*, 2003) thus

favouring enrichment of the zooplankton consumers, as observed during the spring in this study. Despite the occurrence of upwelling events also in summer, phytoplankton growth during summer and autumn in this region depends mainly on regenerated nitrogen (Bode and Varela, 1994; Joint *et al.*, 2001), which would explain the general decrease in the values of zooplankton $\delta^{15}N$ observed.

Second, the rapid increase in the number of carnivorous groups during the spring, following the peak in omnivorous

Table 2. Parameters of the regression lines relating $\delta^{13}C$ or $\delta^{15}N$ and selected variables (see correlation values in Table 1). The adjusted lines were in the form: $Y = a + bX$. r^2: robust determination coefficient. p: significance indicated by asterisks (as in Table 1). Robust regression techniques were used (see Venables and Ripley, 1997). Separate lines were fitted using all data (n = 20) or data for the spring—early summer period (March—June, n = 8). Only significant lines displayed in Figure 6 were shown.

X	Y	Data	a	b	r^2	p
$\delta^{15}N$	Surface NO$_3$	Spring—early summer	6.252	−0.475	0.441	*
$\delta^{13}C$	Carnivore abundance	All data	−19.308	0.007	0.230	*
$\delta^{13}C$	Carnivore abundance	Spring—early summer	−21.316	0.028	0.825	**
$\delta^{15}N$	Carnivore abundance	All data	5.992	0.013	0.117	**
$\delta^{15}N$	Carnivore abundance	Spring—early summer	3.925	0.047	0.342	*

species (mostly copepods) is consistent with the progressive enrichment in ^{15}N in zooplankton, as consumer species are enriched in heavy isotopes relative to their prey (Minagawa and Wada, 1984; Peterson and Fry, 1987). In our study, the contribution of carnivores to δ^{15}N values appears to be higher than the contribution of regenerated nitrogen, because there is a positive correlation between carnivore abundance and δ^{15}N not only when considering samples for the spring period but also when considering samples from all seasons (Figure 6). It can be argued that the regression of δ^{15}N with zooplankton abundance values may lead to misleading interpretations of isotopic enrichment during zooplankton succession because of possible changes in the average size of individuals. Although the use of direct biomass estimates of the various zooplankton taxa would be desirable, we do not expect a large deviation from the actual patterns when using abundance values, because we found a highly significant correlation between total abundance and biomass but also because previous studies in the same sampling area showed that most of the variations in total zooplankton biomass and abundance were due to copepods with a mean individual size of 0.9 mm throughout the year (Valdés *et al.*, 1990, 1991). According to these studies, meroplanktonic larvae and juvenile stages of copepods have a modal size of 0.4 mm, while large zooplankton (such as siphonophores) may reach several millimetres in length. Neither the occasional high number of small zooplankton nor the generally low number of large specimens seem to affect the biomass—abundance relationship in the study area (Valdés *et al.*, 1990).

Given the large variability found in both carbon and nitrogen isotope abundances in mesozooplankton through the annual cycle, there is not a simple way to estimate a single natural abundance value to use as a reference value when computing trophic positions, as was proposed for freshwater ecosystems (Vander Zanden and Rasmussen, 2001; Post 2002; O'Reilly *et al.*, 2002). One approach is to use the mean value of natural abundance measured in the size fraction that likely contains primary producers (e.g. Bode *et al.*, 2003). Another approach would be to measure the natural abundance of isotopes in selected herbivore species, but it is difficult to identify exclusive herbivores among marine zooplankton, as most grazers identified in the study region are omnivores (Batten *et al.*, 2001; Halvorsen *et al.*, 2001). Furthermore, the separation under the microscope of zooplankton by species, and subsequent determination of natural abundance values is a labour-intensive procedure. In this study we propose the use of the intercept of the line relating isotope abundances and carnivore abundance in the estimation of isotope reference values for this ecosystem. Use of the regression model allows for elimination of the effect of true carnivores on δ^{15}N values, as they are always present in the samples from the whole zooplankton community. For instance, we can use the value of the intercept for δ^{15}N using data from the spring—early summer period (+3.9‰) as an estimate of the isotopic reference value for a zooplankton community composed only of primary consumers (i.e. excluding species that almost exclusively prey on other mesozooplankton). Such consumers may not be full-time herbivores (e.g. most copepods), but their isotopic signatures are expected to be close to those of phytoplankton, as the isotopic enrichment in microbial and protozoan foodwebs is generally small (e.g. Rau *et al.*, 1990). Using the reference value (which would indicate a trophic position value of 2 if we consider that the primary producers have a trophic position value equal to 1), the trophic position of other pelagic compartments in the foodweb could be estimated (e.g. Vander Zanden and Rasmussen, 2001). For instance, taking into account the mean values of δ^{15}N from sardines (*Sardina pilchardus*), a planktivorous fish, and from the common dolphin (*Delphinus delphis*), a piscivorous predator, measured near the study area by Bode *et al.* (2003), the reference value for zooplankton without carnivores determined in this study (+3.9‰), and a trophic fractionation factor between successive consumers of 3.4‰ (Vander Zanden and Rasmussen, 2001; Post, 2002), we can estimate that the sardines (δ^{15}N = +6.7‰) have a trophic position of 2.8, which is consistent with the omnivorous diet of this species. In turn, dolphins (δ^{15}N = +8.6‰) would have a mean trophic position of 3.4, equivalent to a primary carnivore. These figures are 0.5 trophic levels lower than those derived from previous estimations made by using the δ^{15}N value of the phytoplankton as the reference (Bode *et al.*, 2003), which were subject to large uncertainties (e.g. Vander Zanden and Rasmussen, 2001). In this way, the results of this study may facilitate the interpretation of trophic analysis of the pelagic ecosystem through the measurement of stable isotopes in various compartments of the foodweb. However, the relationships given, although significant, do not account for a large fraction of the observed variability in isotope composition. Future studies will demonstrate the validity of the proposed regression approach by examining in greater detail some aspects not considered here, as the biomass-specific values of natural abundance of C and N isotopes in selected zooplankton species.

References

Batten, S., Fileman, E. S., and Halvorsen, E. 2001. The contribution of microzooplankton to the mesozooplankton diet in an upwelling filament off the northwest coast of Spain. Progress in Oceanography, 51: 385−398.

Bode, A., Alvarez-Ossorio, M. T., and González, N. 1998. Estimations of mesozooplankton biomass in a coastal upwelling area off NW Spain. Journal of Plankton Research, 20: 1005−1014.

Bode, A., Carrera, P., and Lens, S. 2003. The pelagic foodweb in the upwelling ecosystem of Galicia (NW Spain) during spring: natural abundance of stable carbon and nitrogen isotopes. ICES Journal of Marine Science, 60: 11−22.

Bode, A., and Varela, M. 1994. Planktonic carbon and nitrogen budgets for the N−NW Spanish shelf: the role of pelagic nutrient regeneration during upwelling events. Scientia Marina, 58: 221−231.

Bunn, S. E., Loneragan, N. R., and Kempster, M. A. 1995. Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: implications for food-web studies using multiple stable isotopes. Limnology and Oceanography, 40: 622−625.

Cabal, J. A. 1993. Estructura y dinámica de las poblaciones de copépodos planctónicos en la costa central de Asturias. PhD thesis, Universidad de Oviedo, Spain. 349 pp.

Casas, B., Varela, M., Canle, M., González, N., and Bode, A. 1997. Seasonal variations of nutrients, seston and phytoplankton, and upwelling intensity off La Coruña (NW Spain). Estuarine, Coastal and Shelf Science, 44: 767−778.

Clarke, K. R., and Warwick, R. M. 2001. Change in marine communities: an approach to statistical analysis and interpretation. PRIMER-E Ltd., Plymouth, UK. 171 pp.

Colebrook, J. M. 1979. Continuous plankton record: seasonal cycles of phytoplankton and copepods in the North Atlantic Ocean and North Sea. Marine Biology, 51: 23−32.

Fessenden, L., and Cowles, T. J. 1994. Copepod predation on phagotrophic ciliates in Oregon coastal waters. Marine Ecology Progress Series, 107: 103−111.

Fry, B., and Quiñones, R. B. 1994. Biomass spectra and stable isotope indicators of trophic level in zooplankton of the northwest Atlantic. Marine Ecology Progress Series, 112: 201−204.

Fry, B., and Wainright, S. C. 1991. Diatom sources of ^{13}C-rich carbon in marine food webs. Marine Ecology Progress Series, 76: 149−157.

Gifford, D. J., and Dagg, M. J. 1991. The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepod *Acartia tonsa* Dana and *Neocalanus plumchrus* Murukawa. Marine Microbial Food Webs, 5: 161−177.

Goering, J., Alexander, V., and Haubenstock, N. 1990. Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific bay. Estuarine, Coastal and Shelf Science, 30: 239−260.

Greene, C. H. 1985. Planktivore functional groups and patterns of prey selection in pelagic communities. Journal of Plankton Research, 7: 35−40.

Halvorsen, E., Hirst, A. G., Batten, S. D., Tande, K. S., and Lampitt, R. S. 2001. Diet and community grazing by copepods in an upwelled filament off the NW coast of Spain. Progress in Oceanography, 51: 399−421.

Irigoien, X., Head, R., Klenke, U., Meyer-Harms, B., Harbour, D., Niehoff, B., Hirche, H.-J., and Harris, R. 1998. A high frequency time series at weathership M, Norwegian Sea, during the 1997 spring bloom: feeding of adult female *Calanus finmarchicus*. Marine Ecology Progress Series, 172: 127−137.

Isaacs, J. D. 1973. Potential trophic biomasses and trace-substance concentration in unstructured marine food webs. Marine Biology, 22: 97−104.

Joint, I., Rees, A., and Woodward, M. 2001. Primary production and nutrient assimilation in the Iberian Upwelling in August 1998. Progress in Oceanography, 51: 303−320.

Minagawa, M., and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between δ^{15}N and animal age. Geochimica et Cosmochimica Acta, 48: 1135−1140.

Montoya, J. P., Horrigan, S. G., and McCarthy, J. J. 1990. Natural abundance of ^{15}N in particulate nitrogen and zooplankton in the Chesapeake Bay. Marine Ecology Progress Series, 65: 35−61.

Mullin, M. M., Rau, G. H., and Eppley, R. W. 1984. Stable nitrogen isotopes in zooplankton: some geographic and temporal variations in the North Pacific. Limnology and Oceanography, 29: 1267−1273.

O'Reilly, C. M., Hecky, R. E., Cohen, A. S., and Plisnier, P.-D. 2002. Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. Limnology and Oceanography, 47: 306−309.

Peterson, B. J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics, 18: 293−320.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology, 83: 703−718.

Rau, G. H., Teyssie, J.-L., Rassoulzadegan, F., and Fowler, S. W. 1990. ^{13}C/^{12}C and ^{15}N/^{14}N variations among size-fractionated marine particles: implications for their origin and trophic relationships. Marine Ecology Progress Series, 59: 33−38.

Rolff, C. 2000. Seasonal variation in δ^{13}C and δ^{15}N of size-fractionated plankton at a coastal station in the northern Baltic Proper. Marine Ecology Progress Series, 203: 47−65.

Stoecker, D. K., and Capuzzo, J. M. 1990. Predation on protozoa: its importance to zooplankton. Journal of Plankton Research, 12: 891−908.

Valdés, L., Alvarez-Ossorio, M. T., and Varela, M. 1990. Relación entre la estructura de tallas y la biomasa del zooplancton nerítico en la plataforma de La Coruña (NO de España). Boletín del Instituto Español de Oceanografía, 6: 97−106.

Valdés, L., Alvarez-Ossorio, M. T., Lavín, A., Varela, M., and Carballo, R. 1991. Ciclo anual de parámetros hidrográficos, nutrientes y plancton en la plataforma continental de La Coruña (NO, España). Boletín del Instituto Español de Oceanografía, 7: 91−138.

Vander Zanden, M. J., and Rasmussen, J. B. 2001. Variation in δ^{15}N and δ^{13}C trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography, 46: 2061−2066.

Venables, W. N., and Ripley, B. D. 1997. Modern and Applied Statistics with S-PLUS. Springer-Verlag, New York. 548 pp.

Wada, E., and Hattori, A. 1991. Nitrogen in the sea: forms, abundances, and rate processes. CRC Press, Boca Raton. 208 pp.