

Isotopic composition of particulate organic nitrogen and its relationship to nitrate assimilation in the Mediterranean Sea

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SUMMARY: The shifts in the ^{15}N : ^{14}N ratio of particulate organic nitrogen (PON) suspended in the upper layer of the ocean have normally been interpreted in terms of changes in the isotopic composition of the source nitrogen used by the phytoplankton (nitrate, ammonium and dinitrogen gas) and/or the fractionation associated with the assimilation processes (^{14}N is preferentially taken up by phytoplankton). The objective of the present study was to test which process, isotopic fractionation during NO_3^- uptake or reduced N-form consumption by phytoplankton, was more important in explaining the variations in the ^{15}N signature of PON in the surface layer of a geostrophic front area (northwest Alboran Sea, Mediterranean Sea). Samples of PON were collected at 6 different stations during 4 seasonal surveys. The particulate organic carbon (POC) and PON concentrations averaged over the mixed layer (ML) ranged from 142 and 23 $\mu\text{g L}^{-1}$ in summer to 104 and 13 $\mu\text{g L}^{-1}$ in autumn respectively. The C:N molar ratio of organic matter also varied seasonally from 7.2 in summer, winter and spring to 9.3 in autumn. The range of variation of the $\delta^{15}\text{N}$ of PON in the ML was -0.1‰ to 6.5‰ . The lowest values of $\delta^{15}\text{N}$ (lower than 1‰) were obtained in summer and winter. However, the $\delta^{15}\text{N}$ of PON in autumn and spring was higher than 3.5‰ . Consequently, the particulate matter in the ML was enriched in ^{15}N by about 1.5‰ in autumn and spring with respect to summer and winter. The $\delta^{15}\text{N}$ of PON in the surface layer was negatively correlated with nitrate concentration ($r = -0.62$, $n = 22$, $p = 0.002$). The $\delta^{15}\text{N}$ was also negatively correlated with the proportion of nitrate to total inorganic nitrogen (i.e. the sum of nitrate, nitrite and ammonium). Furthermore, there was a negative correlation between nitrate relative to nitrate plus ammonium assimilation rates ($r\text{NO}_3^-$) and the $\delta^{15}\text{N}$ ($r = -0.71$, $n = 20$; $p < 0.001$) for the surface samples. $r\text{NO}_3^-$ was particularly low (lower than 0.2) for the surface samples collected during autumn, when primary productivity mainly depended on the consumption of ammonium coming from the recycling of organic matter. Therefore, $\delta^{15}\text{N}$ of seston was a sensitive indicator of the transition from productivity based on nitrate to productivity based on regenerated ammonium.

Keywords: Alboran Sea, mineralization, organic matter, phytoplankton, primary production, seasonal cycle.

RESUMEN: COMPOSICIÓN ISOTÓPICA DEL NITRÓGENO EN LA MATERIA ORGÁNICA EN SUSPENSIÓN Y SU RELACIÓN CON LA ASIMILACIÓN DE NITRATO EN EL MEDITERRÁNEO. – Los cambios en la relación ^{15}N : ^{14}N del nitrógeno orgánico particulado (NOP) se han interpretado en términos de cambios en la composición isotópica del nitrógeno consumido por el fitoplancton (nitrato, amonio y N_2) o del fraccionamiento isotópico durante la asimilación de nitrato (el fitoplancton consume preferentemente ^{14}N). El objetivo del presente estudio fue determinar cual de los dos procesos, fraccionamiento isotópico o consumo de formas reducidas de nitrógeno, pudo tener más importancia en la determinación del contenido relativo de ^{15}N en el NOP de la capa superficial en el frente geostrofico del sector noroeste de Alborán (Mar Mediterráneo). Para ello, se recogieron muestras de NOP en 6 estaciones durante 4 campañas oceanográficas estacionales. Las concentraciones de NOP y carbono orgánico particulado (COP) promedio en la capa de mezcla (CM) variaron entre 142 y 23 $\mu\text{g L}^{-1}$ en verano y 104 y 13 $\mu\text{g L}^{-1}$ en otoño, respectivamente. La razón molar C:N de la materia orgánica también varió estacionalmente desde 7.2 en verano, invierno y primavera hasta 9.3 en otoño. El rango de variación del $\delta^{15}\text{N}$ del NOP en la capa de mezcla fue de -0.1‰ hasta 6.5‰ . Los menores valores de $\delta^{15}\text{N}$ del NOP (menores de 1‰) se obtuvieron en verano e invierno. Por el contrario, $\delta^{15}\text{N}$ del NOP en otoño y primavera fue mayor que 3.5‰ . En consecuencia, la materia orgánica en la CM se enriqueció un 1.5‰ en ^{15}N en otoño y primavera respecto a verano e invierno. $\delta^{15}\text{N}$ del NOP en la capa de superficie se correlacionó negativamente con la concentración de nitrato ($r = -0.62$, $n = 22$, $p = 0.002$) y con la relación entre nitrato y nitrógeno inorgánico total (esto

es, la suma de nitrato, nitrito y amonio). Además se observó una correlación negativa entre la tasa de asimilación de nitrato relativa a nitrógeno inorgánico total ($r\text{NO}_3^-$) y $\delta^{15}\text{N}$ del NOP y ($r = -0.71$, $n = 20$, $p < 0.001$). $r\text{NO}_3^-$ fue particularmente bajo (menor de 0.2) en las muestras de superficie recogidas en otoño, cuando la productividad primaria dependió del consumo de amonio procedente del reciclado de la materia orgánica. Por lo tanto, el $\delta^{15}\text{N}$ del seston fue un indicador de la transición de una productividad basada en nitrato a otra basada en amonio regenerado.

Palabras clave: ciclo estacional, fitoplancton, mar de Alborán, materia orgánica, mineralización, producción primaria.

INTRODUCTION

The $^{15}\text{N}:$ ^{14}N ratio (usually expressed in per thousand notation, $\delta^{15}\text{N}$) of the algal material is determined by the isotopic composition of the source nitrogen used by phytoplankton (NO_3^- , NH_4^+ and N_2) and the fractionation associated with the assimilation processes. Isotopic fractionation during NO_3^- uptake results in ^{15}N depletion in algal-N with respect to the source- NO_3^- since ^{14}N is preferentially taken up by phytoplankton (Waser *et al.*, 1998). $\delta^{15}\text{N}$ is the same as for subsurface consumed NO_3^- in the extreme event of NO_3^- being totally exhausted (Altabet and McCarthy, 1985; Wada and Hattori, 1991). Cyanobacterial nitrogen fixation also lowers the $\delta^{15}\text{N}$ of the algal material because of the lower $\delta^{15}\text{N}$ of atmospheric N_2 (0 ‰; Emerson *et al.*, 1991). Similarly, the consumption of reduced forms of N resulting from organic matter recycling (i.e. urea and NH_4^+ , which are normally depleted in ^{15}N ; Altabet, 1996) produces a decrease in the $\delta^{15}\text{N}$ of phytoplankton, although in areas where the primary productivity is supported exclusively by regenerated NH_4^+ and nitrogen recycling is intensive in the euphotic zone, the ^{15}N content of NH_4^+ , algal material and PON could be close to that of the source NO_3^- (Mino *et al.*, 2002; Tanaka and Saino, 2002).

Altabet *et al.* (1991) showed that the shifts in the ^{15}N content of PON during mineralization are very small and only detectable when the process is well developed. Consequently, variability of the $\delta^{15}\text{N}$ of PON in the euphotic layer has been mostly interpreted in terms of changes in the N-source used by phytoplankton (Saino and Hattori, 1980; Rau *et al.*, 1998; Mino *et al.*, 2002; Kumar *et al.*, 2004; Bode *et al.*, 2006). The objective of the present study is to test which process, isotopic fractionation during NO_3^- uptake or reduced-N consumption by the phytoplankton is more important in determining the ^{15}N signature of PON in a geostrophic front area located in the northwest Alboran Sea (Mediterranean Sea). The study area is characterised by the presence of a surface layer of nutrient-poor Atlantic water (SAW) entering through the Strait of Gibraltar and a layer of Mediterranean water (MW), which flows out below the SAW (Minas *et al.*, 1991; Ramírez *et al.*, 2005). Episodically, MW reaches the surface layer and promotes phytoplankton growth and the accumulation of chlorophyll *a* (Minas *et al.*, 1991; Rodríguez *et al.*, 1997; García-Górriz and Carr, 2001). The intensity of the upwelling varies seasonally due to the thermal stratification cycle (Mercado *et al.*, 2005, 2007) and/or the changes in the inflow and outflow

through the Strait of Gibraltar (García-Lafuente *et al.*, 2000; Gómez *et al.*, 2000). The variability of the local wind regime also produces short-term variability in upwelling intensity (Cano and García, 1991). A coupling between the new- NO_3^- supply towards the euphotic layer and the NO_3^- relative to NH_4^+ uptake has been described by Mercado *et al.* (2008a) based on the results of four seasonal cruises performed during an annual cycle. In that study, significant seasonal differences in the activity of the respiratory electron transport system were also described for the plankton fraction lower than 200 μm (Ramírez *et al.*, 2006). We hypothesise that these changes will be reflected in the $\delta^{15}\text{N}$ of PON suspended in the water column. In the present article, the vertical profiles of the $\delta^{15}\text{N}$ of organic matter collected during the same surveys are analysed in order to test this hypothesis.

MATERIALS AND METHODS

Hydrological data and seawater samples were collected at 6 stations located in the Alboran Sea during four cruises performed in July-2003, October-2003, February-2004 and May-2004 (Fig. 1). Sampling at stations 5 and 6 was not carried out during the spring cruise due to adverse weather conditions. A vertical profile of temperature, salinity and chlorophyll *a* (chl *a*) fluorescence was obtained in each station with a Seabird 25 CTD equipped with a fluorescence probe (Seapoint 6000). The mixed-layer (ML) depth was estimated according to the method proposed by Kara *et al.* (2000) based on using a finite difference criterion, where the ML depth is the depth at which the potential density has changed by a fixed amount from the surface reference value. Specifically, we calculated the ML as the depth at which the density difference from the surface was 0.5. This threshold was used as it gave the best fit for finding the pycnocline as determined from visual inspection of the density profiles. Samples of seawater were collected with Niskin bottles at 5 or 6 fixed depths (0, 10, 20, 50, 75 and 100 m) depending on the depth of the water column at each station (about 85 m at stations 1, 3 and 5 and more than 120 m at stations 2, 4 and 6). In addition, a sample was collected at the depth where the maximum fluorescence signal was detected (usually positioned between 20 and 50 m). Aliquots were collected to determine nutrients and chl *a* concentrations following the methods described in Ramírez *et al.* (2005) and Mercado *et al.* (2008a) respectively.

Two litres of seawater pre-filtered through a 200 μm mesh, were filtered onto precombusted (450°C for

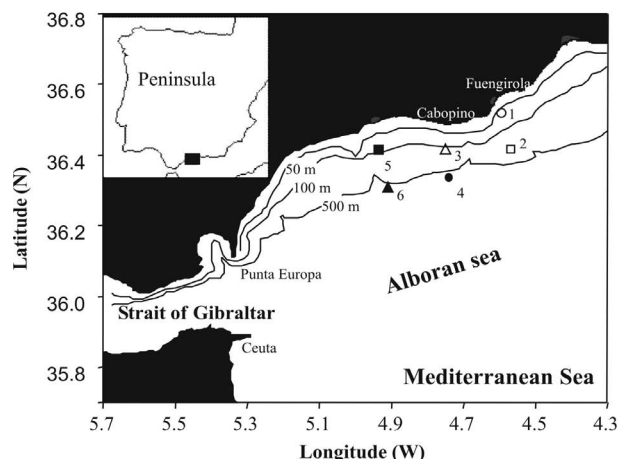


FIG. 1. – Map of the study area showing the sampling stations (station 1: open circles; station 2: open squares; station 3: open triangles; station 4: closed circles; station 5: closed squares; station 6: closed triangles). The contour lines indicate the bathymetry.

2 h) Whatman GF/F filters. The filters were dried at 60°C and stored frozen. In the lab, they were exposed to HCl fumes overnight (to remove the inorganic carbon) and then dried and pelleted for isotopic analysis. Particulate organic nitrogen (PON) and carbon (POC) concentrations and natural abundance stable nitrogen isotope composition in the filters were measured with a FlashEA1112 (ThermoFinnigan) elemental analyser connected to a Deltaplus (ThermoFinnigan) continuous flow isotope-ratio mass spectrometer. The isotopic abundances in the samples were normalised to the isotopic abundance in the standard (atmospheric N_2) and expressed in per mil notation ($\delta^{15}\text{N}$). The precision (repeatability) of duplicate analyses for POC, PON and $\delta^{15}\text{N}$ were 0.3%, 0.9% and 0.2‰ respectively.

The estimated variables were expressed as the mean value \pm standard deviation (SD). Significant differences among the four seasonal surveys were tested at $p < 0.05$ with a Kruskal-Wallis H -test (Zar, 1984). The relationship between variables was studied by Pearson's correlation analysis (Statistica'99 Edition, Statsoft, Inc.).

RESULTS

Particulate organic matter

Most of the profiles of POC (Fig. 2) and PON (Fig. 3) concentrations showed a maximum located within the upper 20-30 m (the maximum was located at about 40-50 m depth at only three stations sampled in summer). The POC and PON maxima were located at the surface during spring. During each survey the spatial variability of POC and PON within the upper 20-30 m was remarkable, particularly in summer, when concentrations of POC and PON reached 300 and 50 $\mu\text{g L}^{-1}$ respectively at station 5, while concentrations lower than 50 and 10 $\mu\text{g L}^{-1}$ were obtained at station 2. POC and PON concentrations tended to decrease below the ML.

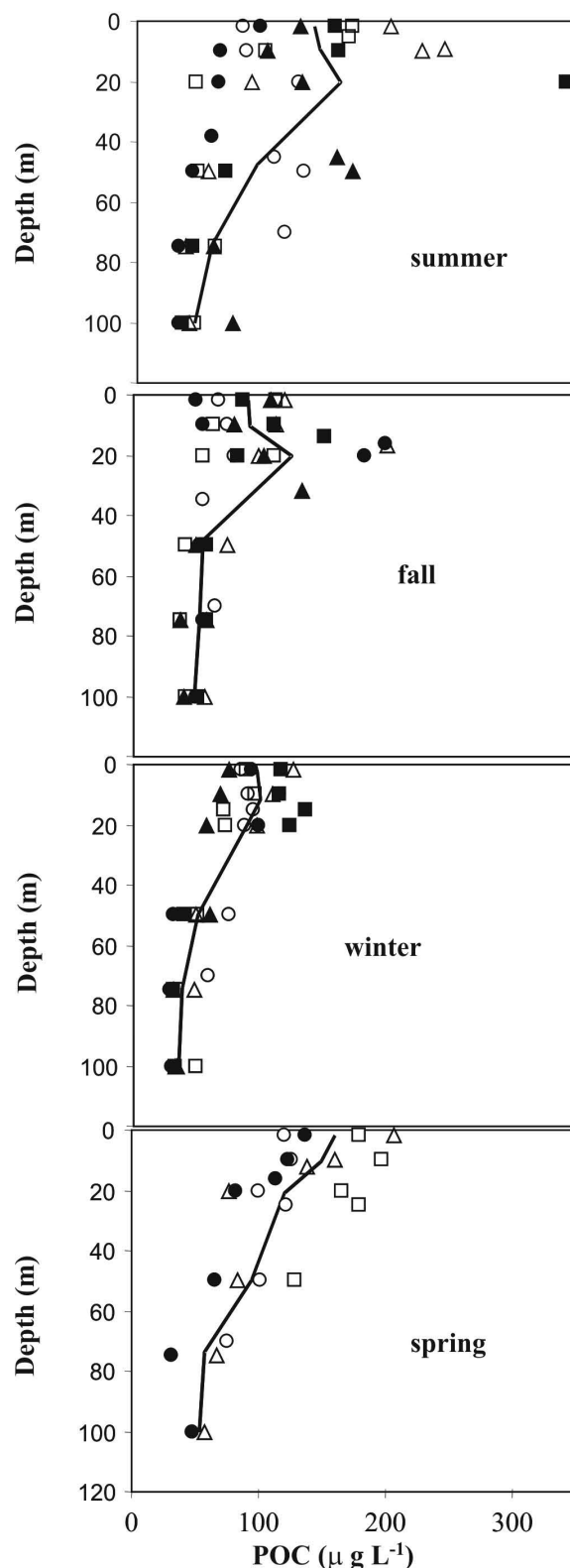


FIG. 2. – Vertical distribution of the particulate organic carbon concentration (POC, $\mu\text{g L}^{-1}$) obtained at the sampling stations during the four seasonal surveys (station 1: open circles; station 2: open squares; station 3: open triangles; station 4: closed circles; station 5: closed squares; station 6: closed triangles). The solid lines represent the averaged seasonal profiles.

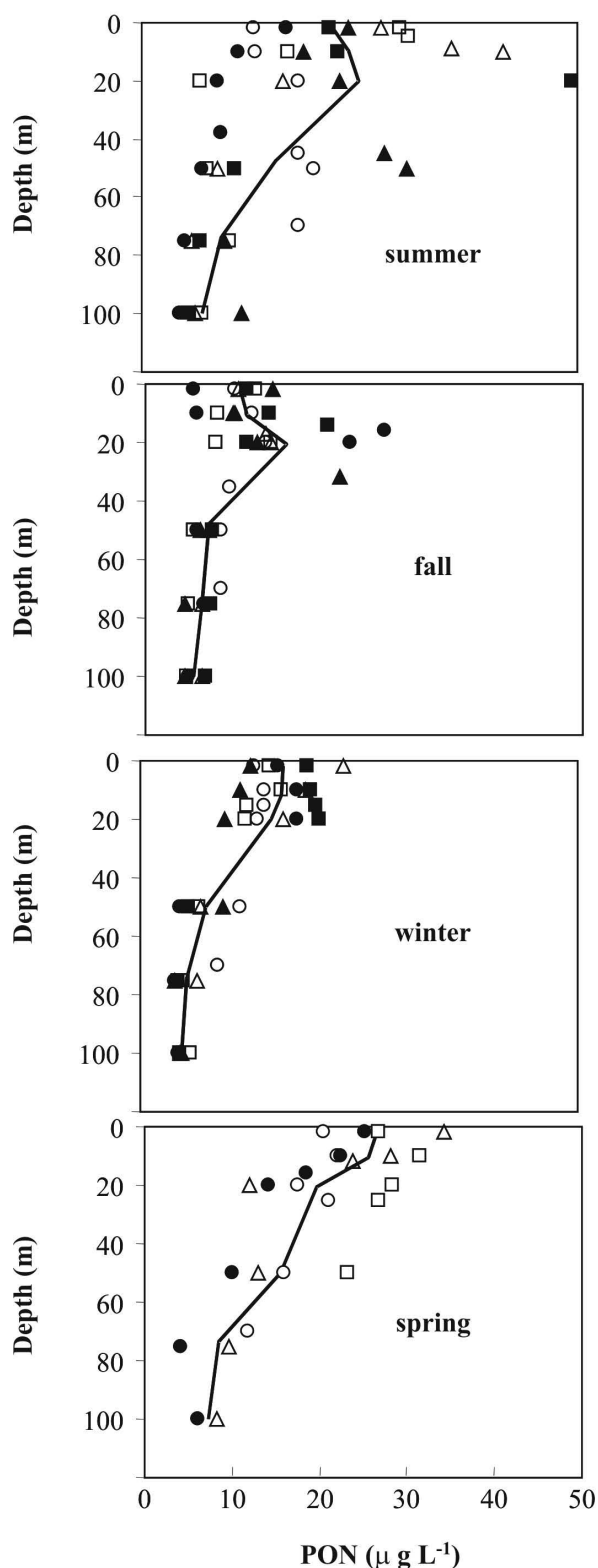


FIG. 3. – Vertical distribution of the particulate organic nitrogen concentration (PON, $\mu\text{g L}^{-1}$) at the sampling stations during the four seasonal surveys (station 1: open circles; station 2: open squares; station 3: open triangles; station 4: closed circles; station 5: closed squares; station 6: closed triangles). The solid lines represent the averaged seasonal profiles.

TABLE 1. – Seasonal means (\pm SD) of concentrations of particulate organic carbon (POC, mg L^{-1}) and nitrogen (PON, mg L^{-1}), carbon to nitrogen molar ratio of the organic matter (C:N) and $\delta^{15}\text{N}$ (‰) seasonally averaged for the mixed layer and for the layer between the lower limit of ML to the bottom (deep layer). For each row, significant differences among medians were tested at $p < 0.05$ with a Kruskal-Wallis H-test. Means with different superscripts were significantly different at $p = 0.05$.

	Summer	Autumn	Winter	Spring
mixed layer				
POC	142 ± 77^a	104 ± 77^b	96 ± 21^b	139 ± 39^a
PON	23 ± 12^a	13 ± 5^b	16 ± 4^b	23 ± 6^a
C:N	7 ± 1^a	9 ± 2^b	7 ± 0.4^a	7 ± 0.4^a
$\delta^{15}\text{N}$	2.7^a	4.1^b	2.3^b	3.7^a
deep layer				
POC	65 ± 39^a	$51 \pm 10^{a,b}$	42 ± 13^b	73 ± 29^a
PON	10 ± 7^a	6 ± 1^b	$6 \pm 2^{a,b}$	11 ± 6^a
C:N	8 ± 0.4^a	10 ± 1^b	$8 \pm 1^{a,b}$	8 ± 1^a
$\delta^{15}\text{N}$	5.8	5.4	4.9	5.5

The POC and PON concentrations were significantly higher in summer and spring than in autumn and winter (Table 1). The vertical patterns of POC and PON were similar to those obtained for chl *a* (a complete description of the vertical profiles of chl *a* can be found in Mercado *et al.*, 2008a). In fact, chl *a* and PON concentrations in the ML were strongly correlated in summer ($r = -0.92$, $n = 18$, $p < 0.001$) and winter ($r = -0.85$, $n = 27$, $p < 0.001$). The correlation was also significant in autumn ($r = -0.62$, $n = 21$, $p < 0.001$) and spring ($r = -0.62$, $n = 15$, $p = 0.007$). These correlations indicate that most of the organic matter suspended in the upper layer corresponds with algal material.

The C:N molar ratio of particulate organic matter ranged from 6.3 to 13.02. The variability was more prominent in autumn (Table 1), when noticeably high values of C:N (higher than 10) were obtained in several samples. In spite of this variability, most of the profiles showed an increase in the C:N ratio with increasing depth (although the gradient was less pronounced in summer and autumn).

The $\delta^{15}\text{N}$ of organic matter

The relative abundance of ^{15}N in the particulate matter collected throughout the study period ranged from -0.1‰ to 13‰ . In most of the profiles, $\delta^{15}\text{N}$ decreased just below the surface to a minimum roughly coincident with the lower limit of the ML. Below this minimum, the $\delta^{15}\text{N}$ increased and usually reached the highest values at 75 to 100 m depth (Fig. 4 and Table 1). The range of variation of the $\delta^{15}\text{N}$ in the ML was -0.1‰ to 6.5‰ , and the lowest values were obtained at station 3 in summer and station 6 in winter (Fig. 4). On average, the particulate matter in this layer was enriched in ^{15}N by about 1.5‰ in autumn and spring with respect to summer and winter (Table 1). However, there was no clear seasonal variation pattern of $\delta^{15}\text{N}$ for the deeper layer, although $\delta^{15}\text{N}$ averaged for the water column was significantly lower in winter.

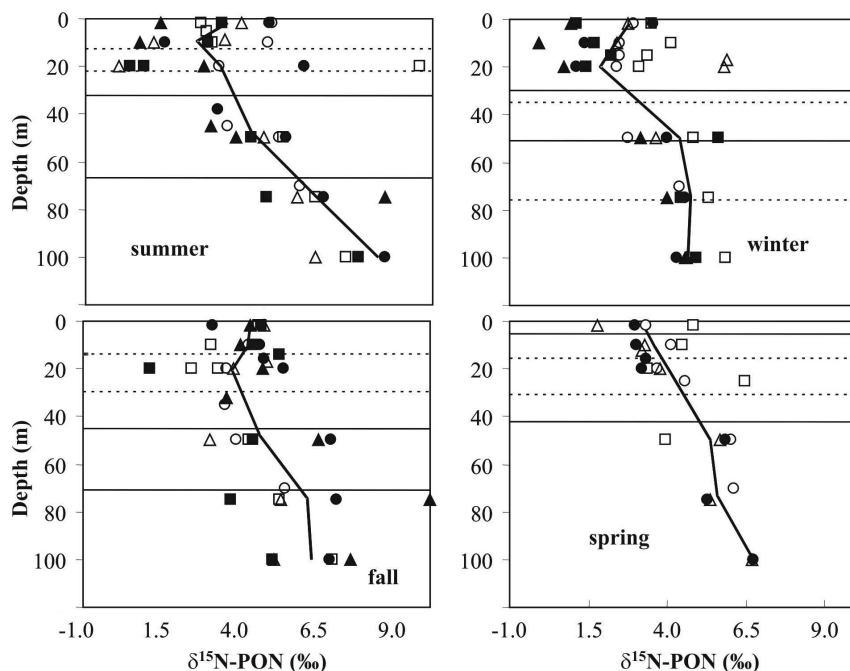


FIG. 4. – The $\delta^{15}\text{N}$ (‰) of particulate organic nitrogen at the sampling stations during the four seasonal surveys (station 1: open circles; station 2: open squares; station 3: open triangles; station 4: closed circles; station 5: closed squares; station 6: closed triangles). The solid lines represent the averaged seasonal profiles. The horizontal solid and dotted lines indicate the ranges of variation of the 37.5 isohaline position and the lower limit of the mixed layer respectively.

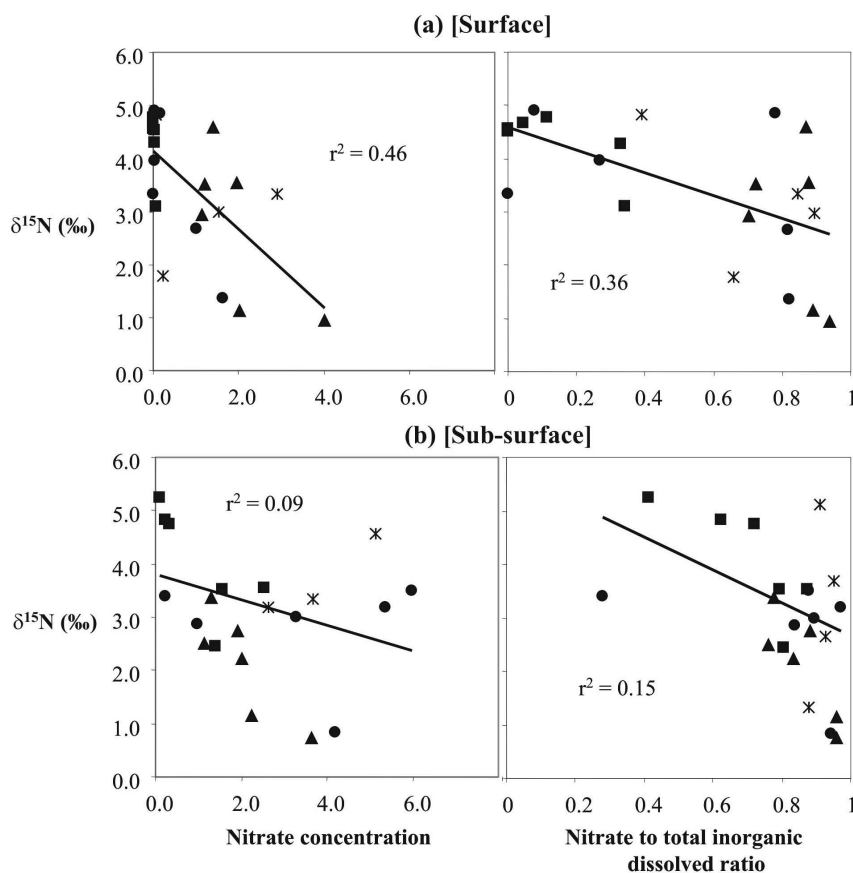


FIG. 5. – Relationship between nitrate concentration (NO_3^- , μM) and $\delta^{15}\text{N}$ of PON ($\delta^{15}\text{N}$ -PON) and ratio of nitrate to dissolved inorganic nitrogen ($\text{NO}_3^-:\text{DIN}$) and $\delta^{15}\text{N}$ of PON for surface (a) and sub-surface chl *a* fluorescence maximum (b) samples in summer (circles), autumn (squares), winter (triangles) and spring (asterisks).

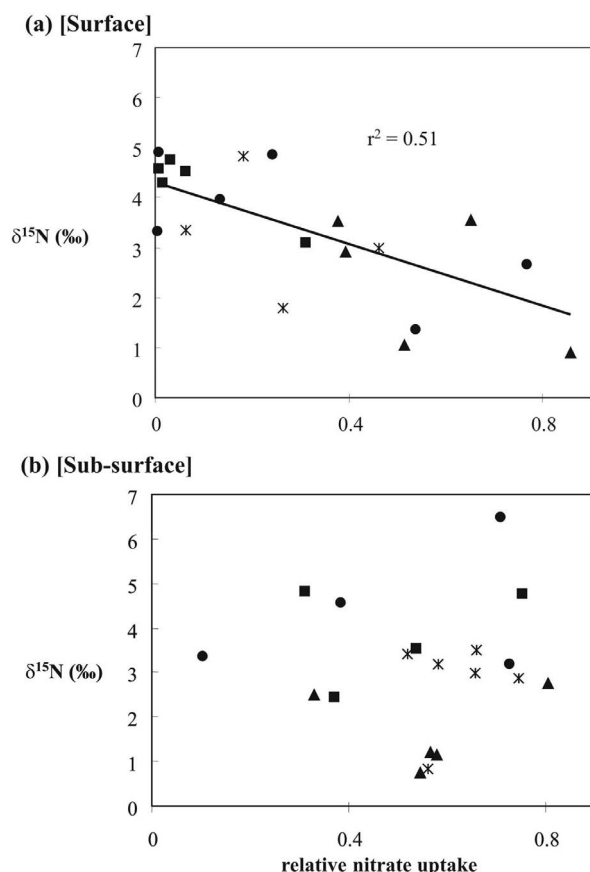


FIG. 6. – Relationship between the nitrate relative to nitrate plus ammonium uptake rate and the $\delta^{15}\text{N}$ of particulate organic nitrogen ($\delta^{15}\text{N}$ -PON) for surface (a) and sub-surface chl *a* fluorescence maximum (b) samples in summer (circles), autumn (squares), winter (triangles) and spring (asterisks).

The variability of $\delta^{15}\text{N}$ in the ML was substantial during each survey, which was probably linked to hydrological variability. Fig. 4 shows the range of variation of the depths of the ML and the 37.5 isohaline (which is an indicator of the intensity of the MW upwelling in our study area; Ramírez *et al.*, 2005; Mercado *et al.*, 2008a) during each survey to showcase this hydrological variability. In summer and autumn the ML depth varied from 13 to 30 m. The position of the 37.5 isohaline was quite variable (from 32 to 74 m) although it was located below the ML at all stations. Water column stratification was disrupted in winter when the ML spread below 50 m depth. During this season, the 37.5 isohaline was located within the ML. The ML depth ranged from 16 to 31 m in spring although the most conspicuous hydrological feature during this survey was the shallow position of the 37.5 isohaline at some stations. In spite of this hydrological variability, the correlation between $\delta^{15}\text{N}$ and salinity or temperature in the ML was not statistically significant.

The relative nitrate uptake ($r\text{NO}_3^-$) at each station was calculated from the nitrogen uptake rates published in Mercado *et al.* (2008a), where nitrate uptake

was measured from incubation experiments performed with samples obtained at the surface and sub-surface fluorescence maximum (SFM) depth, which coincided approximately with the lower limit of the ML and the euphotic layer. As shown in Mercado *et al.* (2008a), the lowest values of $r\text{NO}_3^-$ were obtained in surface samples during the stratification period (i.e. in summer, autumn and spring), while $r\text{NO}_3^-$ usually increased in the SFM samples. In winter, $r\text{NO}_3^-$ in the surface samples was not significantly different from that in the SFM samples. Coincidentally, the higher $\delta^{15}\text{N}$ -PON values (from 3.3 to 4.4 ‰) were obtained in those surface samples in which the nitrate concentration was below the detection limit. In fact, there was a significant correlation between $\delta^{15}\text{N}$ and nitrate concentration for the surface samples ($r = -0.62$, $n = 22$, $p = 0.002$; Fig. 5a). $\delta^{15}\text{N}$ was also negatively correlated with the proportion of NO_3^- to total inorganic nitrogen (i.e. the sum of nitrate, nitrite and ammonium). Similarly, there was a negative correlation between $r\text{NO}_3^-$ and $\delta^{15}\text{N}$ ($r = -0.71$, $n = 20$; $p < 0.001$, Fig. 6a). Note that there was substantial variability of $r\text{NO}_3^-$ in surface waters during each season, particularly during summer when $r\text{NO}_3^-$ ranged from less than 0.10 (stations 1 and 5) to 0.77 (station 2) and the $\delta^{15}\text{N}$ of PON varied by approx. 3.5‰. The highest $r\text{NO}_3^-$ (0.86) in surface samples was obtained in winter, coinciding with a relatively low $\delta^{15}\text{N}$ (0.9 ‰). The variability of both $r\text{NO}_3^-$ and the $\delta^{15}\text{N}$ of PON was reduced in spring, although it is noteworthy that quite different values of the $\delta^{15}\text{N}$ of PON were obtained in stations with similar values of $r\text{NO}_3^-$. In contrast to the surface data, the correlation between $\delta^{15}\text{N}$ and NO_3^- concentration (Fig. 5b) or $r\text{NO}_3^-$ (Fig. 6b) for the SFM samples was not statistically significant.

DISCUSSION

The range of variation in the $\delta^{15}\text{N}$ -PON obtained with our data for the surface layer (from -0.1 ‰ to 6.5‰) is relatively wide and comparable with the variability reported for larger ocean areas. For example, the $\delta^{15}\text{N}$ of PON in surface samples collected during a latitudinal transect along the Atlantic Ocean varied from -0.8 to 5.4‰ (Mino *et al.*, 2002), and Kumar *et al.* (2004) reported values for the $\delta^{15}\text{N}$ of PON ranging from 2 to 7.6‰ in the Bay of Bengal. The only published article for the Alboran Sea reporting values of the $\delta^{15}\text{N}$ of organic matter suspended in the surface layer shows a variation range of -0.2 to 2.7 ‰, which was obtained during a west-to-east transect along the entire basin (Pantoja *et al.*, 2002). This variation range is lower than that described in our study; however, the data published by Pantoja *et al.* (2002) were obtained in winter, and therefore they did not account for the possible seasonal variability (note that our $\delta^{15}\text{N}$ values for winter fit this range of variation well).

Nitrogen isotopic fractionation associated with N assimilation by phytoplankton controls to a large extent the $\delta^{15}\text{N}$ of PON in the surface layer in different ni-

trate and ammonium rich oceanic areas (Atalbet *et al.*, 1991, Benner *et al.*, 1997, Waser *et al.*, 1998, 2000). In our system, the $\delta^{15}\text{N}$ of PON in the surface samples was negatively correlated with the NO_3^- concentration, which suggests that this process also plays a major role in determining the $\delta^{15}\text{N}$ signature of PON. Similarly, Altabet and Francois (1994) and Altabet (2001) obtained a negative correlation between the $\delta^{15}\text{N}$ of PON and the NO_3^- concentration, which was attributed to partial NO_3^- utilisation in areas of the Southern Ocean and the Equatorial Pacific. The isotopic fractionation could also explain the vertical pattern with the lowest $\delta^{15}\text{N}$ of PON at the base of the euphotic layer where NO_3^- availability is higher than above, commonly observed in our study area. A similar vertical distribution of $\delta^{15}\text{N}$ of PON was described by Altabet and McCarthy (1986). Likewise, the lowest values of the $\delta^{15}\text{N}$ of PON obtained in winter, when the water column was homogenised and the removal of NO_3^- was low in comparison with its ambient concentration, could be due to isotopic fractionation during NO_3^- uptake.

Our analysis indicates that the $\delta^{15}\text{N}$ of PON was negatively correlated with the NO_3^- relative to NH_4^+ uptake rates (note that the urea uptake rates were negligible in most of the samples, Mercado *et al.*, 2008a). This relationship is the opposite to what is expected since the increased use of recycled N (as derived from low rNO_3^-) should result in ^{15}N depletion in the algal matter ($\delta^{15}\text{N}$ of NH_4^+ is normally smaller than the $\delta^{15}\text{N}$ of NO_3^- ; Altabet, 1988, Checkley and Miller, 1989). The highest values of the $\delta^{15}\text{N}$ of PON in the surface layer were obtained in samples collected during autumn and summer at stations where NO_3^- was almost completely depleted and NH_4^+ was the main N-source. According to Ramírez *et al.* (2006), the PON-normalised respiratory activity of the planktonic community lower than 200 μm in autumn was almost doubled in comparison to winter and spring, which indicates that intensive N recycling was produced. Furthermore, the water column was strongly stratified in autumn while the 37.5 isohaline was located well below the lower limit of the ML. Consequently, the re-supply of new NO_3^- by vertical diffusion towards the surface was reduced during this season. Under these hydrological constraints, the surface layer could be considered as a closed system (*sensu* Waser *et al.*, 2000). According to the equations that describe the evolution of the $\delta^{15}\text{N}$ of PON during the drawdown of NO_3^- in a closed system provided by Waser *et al.* (2000), a progressive increase in the $\delta^{15}\text{N}$ of PON should be expected. In the extreme event of NO_3^- being totally exhausted, the isotopic ^{15}N content in dissolved inorganic nitrogen, algal material and PON should be close to that of the source NO_3^- consumed provided the residence time of PON is long. Concordantly, the $\delta^{15}\text{N}$ of PON averaged for the stations in which NO_3^- was depleted in ML was $4.3 \pm 0.6\text{‰}$, i.e. close to the $\delta^{15}\text{N}$ of NO_3^- reported by Pantoja *et al.* (2002) for the Alboran Sea.

The correlations between the $\delta^{15}\text{N}$ of PON and NO_3^- and rNO_3^- were not significant for the SFM samples. The SFM was located close to the lower limit of the ML (which normally coincided with the nutricline) at most of the stations. At this depth level, NO_3^- should be supplied continuously by vertical diffusion. Therefore, the system cannot be considered closed with respect to the nutrients at this depth level. Consequently, in addition to the isotopic fractionation, other processes and/or factors potentially influence the $\delta^{15}\text{N}$ of PON in the SFM. (1) Mineralization theoretically contributes to increasing the ^{15}N content of organic matter. However, according to Altabet *et al.* (1991), this process acts slowly in comparison with the residence time of the particulate matter in the upper layer of the ocean. Moreover, it only produces small shifts in the $\delta^{15}\text{N}$ of PON. Therefore, it should be expected that the contribution of mineralization to the variability in the $\delta^{15}\text{N}$ obtained in our study is limited. (2) Changes in the $\delta^{15}\text{N}$ of NO_3^- have been proposed as the main source of variability of the $\delta^{15}\text{N}$ of PON in some areas of the Atlantic Ocean (Waser *et al.*, 2000, Mahaffey *et al.*, 2004). The ^{15}N signatures of NO_3^- in SAW and MW can be quite different, for example Pantoja *et al.* (2002) reported values of 3.5‰ for deep Mediterranean water NO_3^- in the Alboran Sea, while the $\delta^{15}\text{N}$ - NO_3^- of the Atlantic water varies from 5 to 7‰. Therefore, the recently upwelled NO_3^- should be depleted in ^{15}N in comparison with the NO_3^- present in SAW entering through the Strait of Gibraltar. However, the correlation between salinity in the ML (which is an indicator of the MW upwelling intensity) and the $\delta^{15}\text{N}$ of PON was not statistically significant. Moreover, the intensity of the MW upwelling was higher in spring according to the position of the 37.5 isohaline. However, the values of the $\delta^{15}\text{N}$ of PON in this season were higher than in winter or summer. (3) $\delta^{15}\text{N}$ values from -2‰ to 0‰ have been described for N_2 -fixer cyanobacteria (Minagawa and Wada, 1986). Consequently, N_2 fixation should decrease the ^{15}N signal of PON. However, our data precludes the possibility of significant N_2 fixation since most of the $\delta^{15}\text{N}$ values obtained in our study are above 2‰ and all the samples in which the $\delta^{15}\text{N}$ was below this threshold were collected at stations where most of the primary productivity was based on NO_3^- assimilation. (4) Part of the variation in the $\delta^{15}\text{N}$ of PON may have been produced by the presence of non-algal organisms (bacteria and microzooplankton) and/or detritus. According to Waser *et al.* (2000), the effect of changes in trophic representation in PON can be evaluated by examining the relationship between the $\delta^{15}\text{N}$ of PON and the ratio of PON to chl *a*. In our study, this relationship for the SFM samples was statistically significant ($r = 0.46$, $n = 22$; $p = 0.03$), which indicates that the mixture of trophic levels could contribute to the variations in the $\delta^{15}\text{N}$ of PON at this depth level. Similarly, the high value of the $\delta^{15}\text{N}$ of PON (higher than 9‰) registered at station 2 in summer coincided with a relatively high ratio of PON to chl *a* (17.6),

which indicates that this high value was probably a consequence of the presence of non-algal material.

In summary, the negative relationships between the $\delta^{15}\text{N}$ of PON and NO_3^- concentration and rNO_3^- obtained for the surface samples indicate that the variability of the $\delta^{15}\text{N}$ of PON in the study area can be roughly predicted from the expected evolution of the $\delta^{15}\text{N}$ of PON during the drawdown of NO_3^- in a closed system. Consequently, the isotopic fractionation associated with NO_3^- uptake is the main factor controlling the ^{15}N imprint of the organic matter at this depth level. Changes in the nitrate availability for primary production in the surface layer are mainly driven by the hydrological forcing that produces seasonal shifts in the vertical positions of the 37.5 isohaline and the lower limit of the ML. Consequently, our study demonstrates that the $\delta^{15}\text{N}$ of seston is a sensitive indicator of the seasonal transition from productivity based on regenerated ammonium to one based on new nitrate supplied by vertical diffusion. In spite of this seasonal variability, the spatial variability of the $\delta^{15}\text{N}$ of PON in the surface layer was large within seasons, which probably reflects relatively short-term and local hydrographical differences resulting in inhomogeneous NO_3^- supply into the upper layer. High hydrological variability in the northwestern area of the Alboran Sea has been attributed to local events, in a specific kind of westerlies which produce an intensification of the upwelling of MW (Ramírez *et al.*, 2005, Mercado *et al.*, 2008b). In addition, other events like cyclonic eddies due to filaments derived from the main Atlantic current which comes in thought the Strait of Gibraltar and internal wave breaking which is produced by Atlantic tide currents could also contribute to this local variability (Ramírez *et al.*, 2005). It should be expected that hydrological and climate changes at regional scale modify the intensity and frequency of the local episodic events of MW upwelling. Consequently, the short-scale variability should be taken into account in interpreting changes in the $\delta^{15}\text{N}$ of sinking particles and sediment cores in this area of the Mediterranean Sea given that these changes are normally attributed to shifts in N recycling on the ocean surface.

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