Dissolved and particulate organic nitrogen in shelf waters of northern Spain during spring

A. Bode*, M. Varela, M. Canle**, N. González

Instituto Español de Oceanografía, Apdo. 130, 15080 A Coruña, Spain

ABSTRACT: An extensive survey of nitrogen forms and phytoplankton of the euphotic zone on the northern Spanish shelf was made in March 1992. Dissolved inorganic nitrogen (DIN) included nitrate, nitrite and ammonium, which were determined by colorimetric analysis. Dissolved organic nitrogen (DON) was determined by persulfate oxidation of filtered seawater. In addition, particulate nitrogen, chlorophyll a, sestonic proteins and primary production measurements were carried out at selected stations. Surface waters in the western region (Galicia) were characterized by the presence of Eastern North Atlantic Central Water of subtropical origin (ENACWt). This water mass extended over the southern Bay of Biscay (Mar Cantábrico) where large phytoplankton accumulations occurred. Chlorophyll a concentration and primary production rates measured in the Mar Cantábrico were significantly higher than in Galicia. DON reached concentrations of up to 10 mmol m⁻³ in phytoplankton rich areas, particularly in the eastern part of the southern Bay of Biscay, where primary production was also high. Positive correlations were found between DON and particulate nitrogen, and between chlorophyll and primary production, while a negative correlation was found between DON and DIN. On average, dissolved nitrogen was ca 95% of total nitrogen, and DON formed from 52% (Galicia) to 61% of total nitrogen (Mar Cantábrico). The average concentration of DON in the Mar Cantábrico (7.82 mmol N m⁻³) was almost double that of Galicia. Using salinity as a conservative tracer, DON was partitioned and the excess DON (DONexcess) compared to the expected concentration resulting from mixing between continental water and ENACWt at each depth was computed. We found lower correlation values between DONexcess and phytoplankton biomass and production than between total DON and these variables, which suggests an uncoupling between phytoplankton and DON production and the participation of microplanktonic grazers in the release of DON in surface waters. However, considering nitrogen stocks integrated in the euphotic zone in each region, DONexcess accounted on average for only 7% of total nitrogen in Galicia, while in the phytoplankton-rich Mar Cantábrico it accounted for 26%. This suggests that phytoplankton spring blooms are one of the main sources of organic matter in this pelagic ecosystem.

KEY WORDS: Nitrogen speciation · Dissolved organic matter · Phytoplankton · Primary production · Northern Spain

INTRODUCTION

Plankton systems are in a dynamic balance controlled by the availability of nutrients and the loss of biomass by biological and physical processes. Tradition ally, most studies consider only 1 main type of control, but present evidence indicates that there are important interactions between bottom-up and top-down controls (Glibert 1998). In marine environments, dissolved organic nitrogen (DON) is a key component of these interactions because nitrogen is considered one of the main limiting elements (Dugdale & Goering 1967), and organic molecules can be readily assimilated by most planktonic organisms to complement the
pool of elements required for growth (Antia et al. 1991). Nitrogen cycling in the pelagic environment involves the mineralization of DON by microheterotrophs, but also the excretion of organic and inorganic nitrogen by meso- and macrozooplankton. At the same time zooplankton grazing contributes to the liberation of DON, mainly because of sloppy feeding (Roman et al. 1988), further reducing the dependence of external inputs of inorganic nitrogen to the pelagic system. Most DON in the ocean is considered biologically inert, as it often represents a major portion of the total dissolved nitrogen (TDN) and large pools are known to persist in deep waters (Sharp 1983). In the classic view, phytoplankton cells take up dissolved inorganic nitrogen (DIN), either advected from other areas or regenerated in situ (Dugdale & Goering 1967), and constitute the primary step in the transfer through the food web of particulate organic nitrogen (PON), which is eventually exported from the surface to deep waters. Therefore the fate of organic matter produced at the surface of the oceans has mainly been studied considering only particulate material (Berger et al. 1989). It was not until recently that the importance of DON in the cycling and export of organic matter was recognized (Jackson & Williams 1985, Bronk et al. 1994, Doval et al. 1997a, Slawyk et al. 1998, Álvarez-Salgado et al. 1999, Doval et al. 1999); it participates in key biogeochemical processes influencing carbon and nitrogen budgets.

The waters on the continental shelf of northern Spain form one of the most productive European coastal seas. The northwestern region (Galicia) is seasonally under the influence of a wind-driven upwelling that fertilizes surface waters, generally from March to October (Fraga 1981). The upwelling also affects the northern coasts (Mar Cantábrico, southern Bay of Biscay), but generally with lower intensity (Botas et al. 1990). In addition to upwelling events, spring blooms in open shelf waters were the main contributors to annual phytoplankton production in both Galicia (Bode et al. 1994a, 1996, Casas et al. 1997) and Mar Cantábrico (Fernández & Bode 1991, 1994). The predominantly westerly winds during winter and early spring in the northwestern Iberian shelf favour the progress of a poleward current flowing parallel to the shelf break from the Portuguese coast (Frouin et al. 1990). This current transports Eastern North Atlantic Central Water (ENACW) into the Bay of Biscay and significant amounts of the subtropical type of ENACW, named ENACWt (Rios et al. 1992), were reported in the Mar Cantábrico during the spring (Botas et al. 1988, Lavín et al. 1992a,b). There is evidence that spring blooms in this region are greatly influenced by the intrusion of oceanic waters over the shelf, causing frontal zones in which plankton accumulates (Bode et al. 1990, Fernández et al. 1991, 1993, Bode et al. 1994b, Barquero et al. 1998). Biological and chemical studies in these waters have never considered DON and primary production at a regional scale, although there are data from the Ria de Vigo in Galicia (Fraga 1960, 1967, Fraga & Vives 1961, Doval et al. 1997a, Álvarez-Salgado et al. 1999).

The aim of the present study was to analyse the distribution of DIN, DON and PON in the euphotic zone of the northwestern Spanish shelf during the period of occurrence of spring blooms. First, the distribution of particulate and dissolved nitrogen forms in the 2 main subregions of the northwestern Iberian shelf (Galicia and Mar Cantábrico), characterized by different amounts of ENACW and by a distinct phytoplankton species composition, biomass and production, is introduced. Second, DON is partitioned into conservative (DONconserv) and non-conservative (DONexcess) fractions using a 2-end-member mixing model and the salinity of water samples as a conservative tracer (Álvarez-Salgado et al. 1999), with the aim of obtaining a rough estimation of the production of DON in the euphotic zone. Finally, the relationships between DON and other variables, particularly phytoplankton biomass and production, are examined.

**MATERIALS AND METHODS**

Samples were collected during PROSARP-0392 cruise on RV ‘Cornide de Saavedra’ from 6 to 20 March 1992 (Fig. 1). Water-column temperature and salinity were measured with a CTD Seabird SBE-25. Water samples were collected at 3 to 6 depths within the euphotic zone at all stations using 5 l Niskin bottles. Photosynthetically active irradiance (PAR) was measured at each station using a submersible quantum sensor (Li-Cor). Stations in selected transects were sampled at 6 depths to determine primary production (Fig. 1). DIN forms included nitrate, nitrite and ammo-

![Fig. 1. Map of sampling stations on the shelf of northern Spain and limits for the Galicia and Mar Cantábrico regions. Arrows and numbers indicate transects where primary production was measured.](image-url)
nium, and were determined in samples stored frozen on board by the autoanalyzer methods described in Grasshoff et al. (1983). TDN was determined as nitrate by persulphate oxidation of seawater (Grasshoff et al. 1983). Twenty ml of seawater for TDN determination were filtered on board through Whatman GF/F filters and collected in 50 ml Pyrex bottles containing 3 ml of a mixture of K2S2O8 (185 mmol l–1), H3BO3 (485 mmol l–1) and NaOH (350 mmol l–1). The Pyrex bottles were tight-capped and placed in an autoclave at 115°C for 30 min. Oxidized samples were kept stored in the Pyrex bottles until nitrate determination in the laboratory. DON concentrations were computed as the difference between TDN and DIN. The average coefficient of variation of TDN in duplicate samples was 8%, while that of DIN was 3%. Using these values and the procedure described in Hansell (1993), we estimated that the coefficient of variation of TDN at the average DIN and TDN concentrations measured during this study was 13%.

PON was determined in samples obtained from up to 1 l of seawater filtered through Whatman GF/F filters on board and stored frozen until further processing in the laboratory. There samples were dried at 60°C and fed into a CHN analyzer with combustion and reduction temperatures set at 740 and 640°C respectively, to prevent combustion of carbonates (Fraga 1976, Grupo SARP 1993). As a measure of the phytoplankton contribution to PON (Dortch & Packard 1989), chlorophyll-to-protein ratios were computed for some samples. Particulate proteins were determined in some samples obtained by filtering up to 1 l of seawater through Whatman GF/F filters that were stored frozen on board. Protein concentrations (NProt) were measured by the method of Dortch et al. (1984) using bovine serum albumin (SIGMA 905-10) as the standard. Protein to nitrogen conversion was made using the equivalence 0.153 g N (g protein)–1 obtained from measurements of the standard in the CHN analyzer.

Chlorophyll a (chl a) concentrations were measured on 250 ml samples filtered on board through Nucleopore membrane filters (0.8 µm pore size) and stored frozen. Chl a was extracted in 90% acetone overnight and its fluorescence measured in a fluorometer (Parsons et al. 1984). Primary production was measured on board using samples from 6 depths within the euphotic zone. Duplicate 250 ml seawater aliquots from each depth level were inoculated with 4 µCi (148 kBq) of NaH14CO3 and incubated on board in simulated 'in situ' conditions, using neutral density screens to simulate 5 irradiance levels corresponding to 100, 50, 25, 10 and 1% of photosynthetic irradiance received at the surface. Samples were incubated at the irradiance level closest to irradiance of the sampling depth. Incubations were performed around noon and lasted for up to 3 h. Additional aliquots were incubated in the dark for dark carbon uptake correction. After incubation, all samples were filtered through Whatman GF/F filters that were placed in vials with water-soluble scintillation cocktail. Radiation of 14C taken up by phytoplankton was determined with a liquid scintillation counter. All results reported are listed in Grupo SARP (1993). Samples for phytoplankton species determination, preserved with Lugol’s solution and observed with an inverted microscope, were collected at the same depths as the samples for primary production. In this paper, only species or groups with mean abundances higher than 0.2 cells ml–1 are reported. Unidentified forms of dinoflagellates and flagellates were classified by size-groups.

DON was partitioned using a 2-end-member mixing model, considering that the conservative characteristics of the water-column sampled were the result of mixing of ENACW with surface water of continental influence (Álvarez-Salgado et al. 1999). The expected concentration of DON from mixing between ENACW and continental water (DONconserv) was estimated as:

\[
DON_{\text{conserv}} = \frac{S}{S_{\text{ENACW}}} \cdot DON_{\text{ENACW}} + \frac{S_{\text{ENACW}} - S}{S_{\text{ENACW}}} \cdot DON_{\text{C}}
\]

where S is the salinity measured at each depth sampled for DON, S_{\text{ENACW}} is the salinity maximum of ENACW found during the study (35.926 psu, Stn 72), DON_{\text{ENACW}} is the average concentration of DON measured in samples from below 70 m depth from Galicia (4.03 mmol N m–3, ±1.06 SE, n = 6) and DON_{\text{C}} is the average concentration of DON reported for continental waters in Galicia (25 mmol N m–3, Doval et al. 1997a).

DON_{\text{conserv}} concentrations were subtracted from measured DON (DON_{\text{total}}) to estimate the excess DON at each depth (DON_{\text{excess}}).

Linear regression equations between nitrogen concentrations, biomass and primary production values were computed to describe the observed relationships. All regression equations were computed using Model-II regression (Sokal & Rohlf 1981). Values given as mean ± SE throughout.

RESULTS

Oceanographic conditions and distribution of DIN and PON

The study area displayed surface waters in Galicia warmer and saltier than waters in the Mar Cantábrico (Fig. 2). Salinity reached values higher than 36.0 psu in the southwestern part of the region and remained generally higher than 35.8 psu up to the western limit of the Mar Cantábrico. Low salinity values were found...
near the coast, particularly in the vicinity of the Galician rias and in the southeastern part of the Bay of Biscay. Nitrate concentrations at the surface were high, particularly off the shelf and in some coastal locations; the latter were under the influence of runoff, as evidenced by low salinity values (Fig. 2). Concentrations lower than 1 mmol N-NO$_3$– m$^{-3}$ were found over the shelf in Galicia and in the eastern and western parts of the Mar Cantábrico, while the central region of the study area had surface nitrate concentrations exceeding 4 mmol m$^{-3}$. Surface chl a concentrations were generally high in areas with less than 2 mmol N-NO$_3$– m$^{-3}$. Values higher than 2 mg chl a m$^{-3}$ were found in localized areas in the Galician shelf and in most of the eastern part of the Mar Cantábrico, with maximum concentrations higher than 6 mg chl a m$^{-3}$. Chl a concentration was generally lower than 0.5 mg m$^{-3}$ off the Galician shelf and in the central region of the Mar Cantábrico, its distribution following a pattern opposite to nitrate (Fig. 2).

In general, water between 40 to 200 m depth was quite homogeneous, displaying characteristics of ENACW in both Galicia and Mar Cantábrico regions (Fig. 3). Surface water at some stations, notably in the Mar Cantábrico, contained noticeable amounts of freshwater. According to Fig. 3, all stations were influenced by ENACW, since most observations lie in a line parallel to those describing the main subtypes of ENACW, and particularly by the presence of variable amounts of the subtropical component (ENACW$_t$, Rios et al. 1992). Stations from Galicia showed higher temperature and salinity values, while stations from the Mar Cantábrico had lower values (Figs. 3 & 4). These characteristics are indicative of the southern origin of the water, which would progressively cool and mix with surface and continental water as the poleward current flowed along the northwestern Iberian coast.

The average depth of the mixing zone at stations sampled for primary production measurements was 68.3 m (±10.1 m, $n = 24$), and there were no significant differences between stations in Galicia and those in the Mar Cantábrico (Mann-Whitney test, $p > 0.05$). However, the euphotic zone was shallower in the Mar
Cantábrico stations (47.5 ± 3.6 m, n = 12) than in Galicia (67.1 ± 4.0, n = 12, Mann-Whitney test, p < 0.01).

DIN in the water column sampled was comprised mainly of nitrate (mean value 78% of DIN), while nitrite and ammonium accounted for small fractions (mean values 6 and 16% of DIN respectively). Average vertical profiles of DIN and PON were also different in both regions (Fig. 4). DIN increased from ca 3 mmol N m–3 in the surface to ca 5 mmol N m –3 near the bottom of the euphotic zone in Galicia, where values were in general less variable than at Mar Cantábrico stations. PON profiles did not display noticeable maxima and showed similar average values at the surface and near the bottom of the euphotic zone in Galicia. In contrast, stations from the Mar Cantábrico had on average nearly twice as much PON at the surface as those from Galicia, and values at the various depths sampled were quite variable.

Distribution of phytoplankton, chl a and primary production

Although most phytoplankton species and groups identified were well distributed in the study area, each region was characterized by different abundance values (Table 1). In addition to the dominant flagellates, stations in the Mar Cantábrico displayed significantly higher abundances of small dinoflagellates, Cryptomonas and chain-forming diatoms such as Chaetoceros spp., Leptocylindrus danicus and Pseudonitzschia cf. pungens than stations in Galicia. The predominance of diatom species in the Mar Cantábrico was consistent with a higher development of phytoplankton blooms in this region compared to Galicia.

Chl a and primary production (PP) profiles from Galicia were relatively homogeneous; maximum values did not reach 5 mg chl a m–3 and 50 mg C m–3 h–1, respectively, and were distributed above 20 m depth (Fig. 5). Vertical profiles for the Mar Cantábrico exhibited high variability, with maxima located between the surface and approximately 30 m depth. Both mean chl a concentrations and primary production rates measured in the Mar Cantábrico (chl a = 2.13 ± 0.30 mg m–3, PP = 20.84 ± 5.84 mg C m–3 h–1, n = 61) were significantly higher than those measured in Galicia (chl a = 0.82 ± 0.09 mg m–3, PP = 6.23 ± 1.41 mg C m–3 h–1, n = 76, Mann-Whitney test, p < 0.01). However, chlorophyll-specific production rates (CSPR) were similar in both
Table 1. Mean abundance (±SE, n = 54, cells ml⁻¹) of the main phytoplankton species in Galicia and Mar Cantábrico during the study. Mann-Whitney test: *p < 0.05, **p < 0.01, ***p < 0.001, ns: not significant

<table>
<thead>
<tr>
<th>Phytoplankton</th>
<th>Galicia Mean ± SE</th>
<th>Mar Cantábrico Mean ± SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinoflagellates &lt;30 µm</td>
<td>0.54 ± 0.05</td>
<td>0.87 ± 0.06</td>
<td>***</td>
</tr>
<tr>
<td>Chaetoceros socialis Lauder</td>
<td>0.58 ± 0.11</td>
<td>0.70 ± 0.13</td>
<td>ns</td>
</tr>
<tr>
<td>Chaetoceros spp.</td>
<td>0.11 ± 0.03</td>
<td>0.40 ± 0.07</td>
<td>**</td>
</tr>
<tr>
<td>Guinardia flaccida (Castracane) Peragallo</td>
<td>0.41 ± 0.07</td>
<td>0.00 ± 0.00</td>
<td>***</td>
</tr>
<tr>
<td>Lauderia borealis Gran</td>
<td>0.47 ± 0.08</td>
<td>0.62 ± 0.11</td>
<td>ns</td>
</tr>
<tr>
<td>Leptocylindrus danicus Cleve</td>
<td>0.01 ± 0.01</td>
<td>0.55 ± 0.09</td>
<td>***</td>
</tr>
<tr>
<td>Nitzschia longissima (Brébisson) Grunow</td>
<td>0.13 ± 0.03</td>
<td>0.26 ± 0.03</td>
<td>**</td>
</tr>
<tr>
<td>Pseudonitzschia cf. pungens Grunow</td>
<td>0.47 ± 0.06</td>
<td>1.02 ± 0.09</td>
<td>***</td>
</tr>
<tr>
<td>Pseudonitzschia delicatissima (Cleve) Heiden</td>
<td>0.12 ± 0.02</td>
<td>0.32 ± 0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Rhizosolenia delicatula Cleve</td>
<td>0.47 ± 0.06</td>
<td>0.67 ± 0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Rhizosolenia fragilissima Bergon</td>
<td>0.37 ± 0.08</td>
<td>0.08 ± 0.02</td>
<td>*</td>
</tr>
<tr>
<td>Rhizosolenia stolterfothi Peragallo</td>
<td>0.23 ± 0.04</td>
<td>0.12 ± 0.03</td>
<td>ns</td>
</tr>
<tr>
<td>Schroederella delicatula (Peragallo) Pavillard</td>
<td>0.01 ± 0.01</td>
<td>0.23 ± 0.06</td>
<td>**</td>
</tr>
<tr>
<td>Flagellates &gt;10 µm</td>
<td>1.20 ± 0.04</td>
<td>1.49 ± 0.05</td>
<td>***</td>
</tr>
<tr>
<td>Flagellates 5–10 µm</td>
<td>2.35 ± 0.04</td>
<td>2.55 ± 0.04</td>
<td>**</td>
</tr>
<tr>
<td>Flagellates 2–5 µm</td>
<td>2.92 ± 0.03</td>
<td>3.14 ± 0.04</td>
<td>***</td>
</tr>
<tr>
<td>Phaeocystis pzechti (Hariot) Lagerheim</td>
<td>0.11 ± 0.04</td>
<td>0.24 ± 0.09</td>
<td>ns</td>
</tr>
<tr>
<td>Cryptomonas spp.</td>
<td>0.44 ± 0.06</td>
<td>1.07 ± 0.10</td>
<td>***</td>
</tr>
</tbody>
</table>

Fig. 5. Average (±SE) vertical profiles of (a,d) chl a, (b,e) primary production (PP), and (c,g) chlorophyll-specific production rate (CSPR) in Galicia and Mar Cantábrico regions. Numbers along chlorophyll profiles indicate the number of samples averaged for each depth.
areas (8.22 ± 1.18 mg C [mg chl a h]–1, n = 137, Mann-Whitney test, p > 0.05).

Average chl a-to-NProt ratios in the Mar Cantábrico (0.55 ± 0.06, n = 25) were nearly twice those measured in Galicia (0.28 ± 0.03, n = 28, Mann-Whitney test, p < 0.001), indicating that phytoplankton contributed more to the composition of seston in the former area compared to the latter.

**Distribution of DON and fractionation of total nitrogen**

The average concentration of DON measured in Galicia (4.58 ± 0.20 mmol N m–3, n = 65) was significantly lower than the value obtained in the Mar Cantábrico (7.78 ± 0.23 mmol N m–3, n = 72, Mann-Whitney test, p < 0.0001). However, the vertical distribution of the conservative fraction of DON was similar in both regions and its concentration value was always close to that of ENACW, while the non-conservative fraction was responsible for most of the variation in DON in the water-column sampled in both regions (Fig. 6). In Galicia, only samples close to the surface or to the bottom of the euphotic zone displayed significant amounts of DONexcess, and in some cases at intermediate depths there was an apparent deficit in DON compared to the expected concentration considering only conservative mixing. In the Mar Cantábrico, there was always a significant excess of DON not accounted for by conservative mixing, particularly between the surface and 30 m depth.

Using average values of the various fractions of depth-integrated nitrogen, from the surface to the bottom of the euphotic zone in each region (67 m in Galicia, 48 m in the Mar Cantábrico), we estimated that Galicia waters contained a higher fraction of nitrogen as DIN than the Mar Cantábrico, where the organic components were estimated to account for 67% of total nitrogen (Fig. 7). Moreover, the non-conservative fraction of DON in the Mar Cantábrico (26%) was nearly 4 times the average value of total nitrogen accounted for this fraction in Galicia (7%).

**Relationships between DON and other variables**

Most of the measured components of particulate and dissolved organic matter variables were significantly correlated (Table 2). PON was significantly correlated with NProt (r = 0.673, n = 50, p < 0.01). Also, both PON and DON were positively related to chl a, suggesting a significant contribution of phytoplankton to organic nitrogen. In the same way, DON was negatively correlated with DIN and positively with primary
production variables. However, the consideration of DONexcess (which could only be estimated in some samples due to the lack of valid salinity data) did not increase the significance of the correlations found with the former variables, and even produced non significant coefficients (Table 2). Similarly, no improvement in the correlations and regression lines was obtained when using separately Galicia and Mar Cantábrico samples (ANCOVA, p > 0.05).

**DISCUSSION**

The situation found during the cruise was characteristic of the transition between the winter period, with a mixing layer deeper than the euphotic layer, and the period of spring phytoplankton blooms. Seasonal variability of the study region was well described in relationship with the development of phytoplankton, both in the Mar Cantábrico (Fernández & Bode 1991, 1994) and in Galicia (Bode et al. 1994a, 1996, Casas et al. 1997). According to these studies, phytoplankton blooms initiate generally in March, as a consequence of the increase in day length and subsurface irradiance and weak stratification of the upper surface layer, the latter caused by the presence of low-salinity water near the coast or by density gradients originated at fronts between different water masses.

In our study, salinity values of surface waters from Galicia were above the lower limit defined for ENACWt in Ríos et al. (1992), with salinity >36.0 psu in the southern part of the region and water-column salinity maxima located close to the surface. Therefore, ENACWt occupied most of the Galician shelf up to the western limit of the Bay of Biscay, constraining the original shelf water of lower salinity and temperature against the coast. The relative homogeneity of this water mass in the euphotic layer also restricted the development of phytoplankton blooms despite relatively high nutrient concentrations, particularly in the outer shelf. However, phytoplankton growth was favoured at mid-shelf fronts separating ENACWt from coastal water, where chlorophyll concentrations were high and nitrate concentration appeared greatly reduced. This effect of ENACWt fronts has been repeatedly reported in the region, both in the Mar Cantábrico (Bode et al. 1990, Fernández et al. 1991, 1993) and in Galicia (Bode et al. 1994b, Barquero et al. 1998). In contrast, during our study the Mar Cantábrico showed a significant accumulation of phytoplankton in the eastern part, associated with the presence of low-salinity water at the surface, probably of continental origin; ENACWt only affected the western part of this area. Also, the observed conditions in surface waters, with generally low DIN concentrations and relatively high temperature values near the coast, suggest that there was not a significant effect of the seasonal upwelling that affects mainly Galicia (Fraga 1981) but also the Mar Cantábrico (Botas et al. 1990).

Phytoplankton blooms were patchy throughout the studied region, with a larger extension and development in the Mar Cantábrico compared to Galicia. The dominant inorganic nitrogen species was nitrate, suggesting that these spring blooms used nitrate as the main nitrogen source, as was considered in the classical concept of new versus regenerated productivity, initially conceived for open ocean areas (Dugdale & Goering 1967). Shelf and coastal areas are also known to depend mainly on new nitrate during spring blooms (e.g. Kokkinakis & Wheeler 1988, Sahlsten et al. 1988, Goering 1967). Shelf and coastal areas are also known to depend mainly on new nitrate during spring blooms (e.g. Kokkinakis & Wheeler 1988, Bode & Dortch 1996) despite significant inputs of other nitrogen species from coastal sources. The dominance of diatoms during spring blooms in the study region (Fernández & Bode 1994, Casas et al. 1997) may explain the rapid use of nitrate, as these cells can use this form of nitrogen more efficiently than other phyto-

<table>
<thead>
<tr>
<th>Equation</th>
<th>a ± SE</th>
<th>b ± SE</th>
<th>r</th>
<th>n</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON = a + bchl a</td>
<td>0.261 ± 0.082</td>
<td>0.187 ± 0.030</td>
<td>0.604</td>
<td>114</td>
<td>***</td>
</tr>
<tr>
<td>DONtotal = a + bchl a</td>
<td>4.214 ± 0.787</td>
<td>1.180 ± 0.290</td>
<td>0.476</td>
<td>128</td>
<td>***</td>
</tr>
<tr>
<td>DONtotal = a + bDIN</td>
<td>10.667 ± 1.857</td>
<td>-1.167 ± 0.433</td>
<td>-0.430</td>
<td>137</td>
<td>***</td>
</tr>
<tr>
<td>DONtotal = a + bPON</td>
<td>3.173 ± 1.720</td>
<td>5.661 ± 2.403</td>
<td>0.644</td>
<td>120</td>
<td>***</td>
</tr>
<tr>
<td>DONexcess = a + bPP</td>
<td>5.461 ± 0.229</td>
<td>0.065 ± 0.006</td>
<td>0.302</td>
<td>128</td>
<td>**</td>
</tr>
<tr>
<td>DONexcess = a + bCSFPR</td>
<td>5.118 ± 0.341</td>
<td>0.146 ± 0.019</td>
<td>0.242</td>
<td>128</td>
<td>*</td>
</tr>
<tr>
<td>DONexcess = a + bchl a</td>
<td>0.371 ± 0.118</td>
<td>1.092 ± 0.057</td>
<td>0.310</td>
<td>91</td>
<td>**</td>
</tr>
<tr>
<td>DONexcess = a + bDIN</td>
<td>5.529 ± 0.855</td>
<td>-0.788 ± 0.195</td>
<td>-0.241</td>
<td>92</td>
<td>*</td>
</tr>
<tr>
<td>DONexcess = a + bPON</td>
<td>ns</td>
<td>4.792 ± 0.715</td>
<td>0.394</td>
<td>82</td>
<td>**</td>
</tr>
<tr>
<td>DONexcess = a + bCSFPR</td>
<td>ns</td>
<td>ns</td>
<td>0.157</td>
<td>91</td>
<td>ns</td>
</tr>
<tr>
<td>DONexcess = a + bCSFPR</td>
<td>ns</td>
<td>ns</td>
<td>0.178</td>
<td>91</td>
<td>ns</td>
</tr>
</tbody>
</table>
plankters (Glibert et al. 1982, Probyn 1985, Kokkinakis & Wheeler 1988). The coupling between nitrogen uptake and phytoplankton growth was more clearly shown in the Mar Cantábrico, where chl a and PON maxima were nearly coincident, while in Galicia distributions of both variables were less well related (Figs. 4 & 5). The presence of organic matter from the nearby rias, which are known to contribute significantly to the enrichment of the shelf (López-Jamar et al. 1992), may explain the accumulation of PON near the coast of Galicia. Also, high remineralization rates (Pérez et al. 1993) and heterotrophic production (Fernández et al. 1991) were predicted for ENACW in the region, both 1993) and heterotrophic production (Fernández et al. 1991) were predicted for ENACW in the region, both processes favoured by detrital organic particles in the water column of offshore waters transported from the south.

The observed relationships suggested that DIN taken up by phytoplankton was converted into PON and a significant fraction rapidly appeared as DON. Both the coincidence of high DON and chl a concentrations in the low-DIN surface layer and the regression functions computed between these variables considering all observations support the contribution of phytoplankton to DON. Several experimental studies reported the rapid production of DON from phytoplankton (Bronk et al. 1994), but particularly when growing on nitrate (Collos et al. 1996, Hu & Smith 1998) and dominated by diatoms (Collos et al. 1992, Lara et al. 1997). However, some recent studies indicate that rapid DON excretion may not be of general occurrence in the ocean (Slawyk et al. 1998). The concentrations of DON measured during our study are within the range of reported values, even when different analytical techniques are involved. Fraga (1960, 1967) and Fraga & Vives (1961) measured average values of 8 to 10 mmol N m$^{-3}$ for the Galician Ria de Vigo, and recently Doval et al. (1997a) and Álvarez-Salgado et al. (1999), using the same Kjeldahl modified method (Doval et al. 1997b), measured DON in a range from 3 to 11 mmol N m$^{-3}$ at the same location. Generally lower DON concentrations have been found in oligotrophic seas, such as the range of 3.3 to 6.2 mmol N m$^{-3}$ found in the upper 100 m of the northwestern Mediterranean by Doval et al. (1999), practically coincident with the range of DON measured with the UV-oxidation method by Coste et al. (1988) in the Alboran Sea (southwestern Mediterranean). Larger DON concentrations have been reported for phytoplankton blooms in some estuarine systems (Bronk et al. 1998) and during cyanobacteria blooms in the Baltic (Sörensson & Sahlsten 1987).

Even when in our study DON was significantly correlated with chl a and primary production, and particularly with PON, the computed regression functions did not account for a large fraction of DON variability. Taking into account the correlation coefficients of Table 2, only 23 and 41 % of the variability in DON was explained by the regression between DON and chl a and PON, respectively. Similarly, only 9 % of DON variability could be related to the measured short-term rates of primary production. These low values may be a consequence of the uncoupling between production of DON and primary production also found in other studies (eg. Doval et al. 1997a, Álvarez-Salgado et al. 1999). Grazing of PON (including phytoplankton, bacteria and detritus) by microheterotrophs may be one of the major sources of DON (Legendre & Rassoulzadegan 1995, Ferrier-Pages et al. 1998, Glibert 1998). Another possible cause of the low correlation may be the existence of additional inputs of DON to surface layers. For instance, Cornell et al. (1998) showed that atmospheric deposition may account for a significant concentration of DON at some locations in the Atlantic and the Pacific. In addition, DON near the coast may be related to runoff and export from estuaries and rias, the latter well known as sites of DON production and export (Fraga 1960, 1967, Fraga & Vives 1961, Doval et al. 1997a, Álvarez-Salgado et al. 1999). Considering the salinity values measured in our study at a station close to the Ria de Vigo (Stn 79, 35.481 psu at the surface, 35.818 psu at 80 m depth) we estimated a 1% contribution of continental water to the surface layer, which would correspond to DON concentrations of 0.2 to 0.3 mmol N m$^{-3}$, according to the range of DON values reported by Doval et al. (1997a) for continental water in this ria (20 to 30 mmol m$^{-3}$). This means that the contribution of continental waters to total DON concentrations would be between 4 and 6%, since the average value of DON in coastal surface waters of Galicia was 5 mmol m$^{-3}$. Assuming concentration values of DON in continental waters of the Mar Cantábrico region similar to those reported by Doval et al. (1997a) for Galicia, the expected contribution of runoff at the lowest salinity values on the southeastern coast of the Bay of Biscay (Stn 39, 34.715 psu at the surface, 35.636 psu at 50 m) would be between 0.5 and 0.8 mmol m$^{-3}$, which would represent from 6 to 10% of the total DON. The low variability observed in the estimated DON$_{conserv}$ further confirms the low significance of DON inputs by runoff during the study (Fig. 6). Furthermore, Gardner & Stephens (1978) showed that most of DON released by rivers to the sea was stable over several months, and only a fraction of ca 20% could be related to amino acids that can be used up by organisms.

Other studies partitioned DON into labile or recently produced DON and refractory fractions using simple diffusion models when a clear source of refractory DON could be identified. Doval et al. (1999), assuming that DON from waters below 500 m in the heterotro-
The lack of a significant correlation between DON excess in Galicia during this study and, taking into account are consistent with the low phytoplankton production and 43% in the Mar Cantábrico region. These values data were not available for this cruise, we could expect Packard 1989). Although microplankton abundance and erotrophs at high chlorophyll concentrations (Dortch & Packard 1989). Even in some eutrophic waters, as in the Peru upwelling, nitrogen biomass can be dominated by heterotrophs at high chlorophyll concentrations (Dortch & Packard 1989). Although microplankton abundance data were not available for this cruise, we could expect a dominance of microheterotrophs in the study region, because of the rapid response of these organisms to increases in phytoplankton biomass. Labile DON is a primary nutrient source for bacterial growth and the basis of the microbial food web. Higher DON concentrations in the Mar Cantábrico compared to Galicia would support a higher biomass of microplankton. Also, DON is released by grazing of microplanktonic cells and bacteria (Legendre & Rassoulzadegan 1995, Ferrier-Pages et al. 1998). However, the actual importance of the measured DON for microheterotrophs, and particularly for bacteria, would depend on the relative availability of other required substrates, such as dissolved organic carbon (Legendre & Rassoulzadegan 1995). In addition, the fate of DON is more difficult to predict, because some forms of DON may be taken up by phytoplankton (Antia et al. 1991, Collos et al. 1992).

Our study provides the first data on nitrogen speciation in surface waters covering the whole northwestern Iberian shelf, taking into account inorganic/organic, dissolved/particulate forms. The results obtained agree with those of earlier studies (Fraga 1960, 1967, Fraga & Vives 1961) and recent measurements also including all carbon species (Doval et al. 1997a, Álvarez-Salgado et al. 1999) in the Ria de Vigo area (Galicia). Nearly 95% of nitrogen was dissolved, particularly as organic molecules that comprised more than 50% of total nitrogen concentration. In a similar way, Nixon & Pilson (1983) reviewed several studies of nitrogen partition in coastal, estuarine and open marine waters and found that DON represented from 54 to 64% of total nitrogen; Mantoura et al. (1988) reported for Carmarthen Bay (South Wales) DON variations between 27 and 65% of total nitrogen, while DIN conversely varied between 65 and 7% of total nitrogen in winter and summer, respectively. According to these values, it can be hypothesized that DON can accumulate to a maximum value close to 65% of total nitrogen, because above that value production and uptake of organic dissolved compounds are in balance. Despite the fact that most DON in marine open waters may be refractory to photochemical or biological degradation, as indicated by the relatively narrow range of variability compared to total nitrogen, up to 40% of DON may be available for bacteria and other microheterotrophs in the water column. As for dissolved organic carbon, reviewed by Sondergaard & Middleboe (1995), the concentration of labile DON may be determined by the affinity of bacterial populations for dissolved organic substrates, which would saturate uptake at higher concentrations in eutrophic compared to oligotrophic waters. In this way, our results and recent studies (Cherrier et al. 1996, Jørgensen et al. 1999) seem to confirm the trend of increasing concentrations of labile dissolved organic matter in eutrophic waters.
In addition to upwelling and export of dissolved organic matter from the Galician rias (Doval et al. 1997b, Álvarez-Salgado et al. 1999), spring phytoplankton blooms on the northern Spanish shelf appear to be a large source of DON in the study region. The accumulation of DON during the productive spring bloom period may fuel the observed high respiration rates of the microplanktonic community during summer and would explain the net heterotrophic annual balance of the Mar Cantábrico (Serret 1997).

Acknowledgements. The authors thank the crew of the RV ‘Cornide de Saavedra’ and all the participants in the PROSARP-0392 cruise for their help in sampling and data collection. E. López-Jamar, as chief scientist of the cruise, provided all the logistic facilities for this study. We are indebted to B. Casas and J. Lorenzo for phytoplankton analysis and R. Carballo for nutrient determinations. The Comments by M. D. Doval, X. Á. Álvarez-Salgado, G. Spyres, A. Miller and 3 anonymous referees to the various versions of the manuscript are greatly appreciated. This work was funded in part by projects SARP (MA 1.96, DGXIV) and OMEM-II-Part II (MASC-CT97-0076, MAST-III) of the EU, and by additional funds of the Instituto Español de Oceanografía (Grant IEO-1007). M.C. received a FPI fellowship from the Ministerio de Agricultura, Pesca y Alimentación (Spain).

LITERATURE CITED


Hansell DA (1993) Results and observations from the measurement of DOC and DON in seawater using a high-temperature catalytic oxidation technique. Mar Chem 41:195–202


Submitted: July 12, 1999; Accepted: December 8, 2000

Proofs received from author(s): March 8, 2001