Picoplankton community structure along the northern Iberian continental margin in late winter–early spring

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The surface distribution of autotrophic and heterotrophic picoplankton was assessed in 24 transects perpendicular to the coast along the N and NW Iberian peninsula shelf in late winter and early spring 2002. Community structure was analyzed by flow cytometry (FC) and found to be strongly influenced by hydrography. Typical late winter conditions were found during the survey, characterized by the presence of the poleward Portugal coastal counter current (PCCC) in the west and an increasing stratification eastwards. Cyanobacteria (mostly Synechococcus) dominated at low chlorophyll a (Chl a) concentration whereas both the total and relative abundance of picoeukaryotes generally increased with total phytoplankton biomass. Differences in the cell size of most FC-defined picoplanktonic groups were also observed along the longitudinal and coastal–offshore gradients. The presence of Prochlorococcus (<10^3 cells mL^-1) coincided with the core of the PCCC and its significant correlation with salinity suggests its possible use as a tracer of this current. Two groups of heterotrophic bacteria were distinguished according to their relative DNA content. High DNA bacteria dominated the community (60 ± 1% SE of total numbers), reaching maximum values in areas under riverine influence with presumed higher inputs of organic matter. Picoplankton biomass was dominated by heterotrophic bacteria in the western region (56 ± 3%) while autotrophic groups contributed on average 66 ± 2% in the southern Bay of Biscay. The heterotrophic bacteria to phytoplankton biomass ratio decreased significantly along the measured range. Yet showing regional differences, the estimated contribution of picophytoplankton to total algal biomass was high (mean 59 ± 4%), indicating the important role of small cells at the onset of the spring bloom in these temperate shelf waters.

INTRODUCTION
Functioning of planktonic communities is determined to a great extent by their size-structure (Kiørboe, 1993). Knowledge of the smallest-sized organisms or picoplankton (cells <2 μm) has been triggered by the development of new microbiological techniques. Among these, flow cytometry has permitted the rapid and routine discrimination of cells within three ecologically distinct groups: eukaryotic algae, cyanobacteria (Synechococcus and Prochlorococcus) and heterotrophic bacteria (Olson et al., 1993; Gasol and del Giorgio, 2000).

Picoplankton constitutes a ubiquitous component of pelagic ecosystems, usually dominating phytoplankton standing stock and production in the stratified, oligotrophic ocean [e.g. (Zubkov et al., 1998, 2000a; Li and Harrison, 2001)], although with a relatively lower contribution to primary production than to biomass (Fernández et al., 2003). Large-scale surveys in open-ocean waters have demonstrated an association between the composition of the picoplanktonic community and water mass properties (Li, 1995; Buck et al., 1996; Zubkov et al., 2000b; Li and Harrison, 2001; Tarran et al., 2001). Little is known about the picoplankton community structure along the N Atlantic Iberian coast, the northern limit of the NE Atlantic upwelling system, which is characterized by a remarkable hydrographic variability (Bode et al., 2002; Alvarez-Salgado et al., 2003). With some exceptions [e.g. (Morán et al., 2002; Rodríguez et al., 2003)] previous studies have largely focused on heterotrophic bacteria (Unanue et al., 1992; Barquero et al., 1998; Barbosa et al., 2001; Valencia et al., 2003), and most of them present a limited spatial coverage.
An extensive survey was conducted in the N and NW Iberian continental margin with the aim of assessing the relationship between the distribution of picoplankton and the water masses present in late winter and early spring 2002. The sampling time comprised the period of transition from prevailing downwelling (autumn–winter) to upwelling (spring–summer) conditions, coincident with a decreasing influence of the poleward Portugal coastal counter current [PCCC (Álvarez-Salgado et al., 2003)]. This current, also known as the Navidad current (Pingree and Le Cann, 1990; García-Soto et al., 2002) or saline intrusion (Botas et al., 1988), encountered the general Bay of Biscay anticyclonic circulation in the central Cantabrian Sea, causing a conspicuous longitudinal gradient in thermohaline properties. Sampling was performed along transects perpendicular to the coast from shallow to shelf-break waters. The effect of both scales of spatial variability (longitudinal and the coastal–offshore gradient) on picoplanktonic community structure were specifically addressed. The seasonal cycle in these temperate coastal waters indicates that heterotrophic bacteria play a major role well after the decline of the spring bloom (Serret et al., 1999; Valencia et al., 2003). It is also generally accepted that small algal cells become dominant under summer stratified conditions [e.g. (Tamigneaux et al., 1999)]. By estimating the biomass of autotrophic and heterotrophic components we show that picoplankton may also account for a substantial fraction of planktonic biomass at the early stages of the spring bloom.

**METHOD**

Sampling was carried out on board R/V Thalassa during the PELACUS 0302 cruise, from 14 March through 2 April 2002. Water samples for picoplankton abundance were taken from the surface (3 m) of 171 stations located along transects perpendicular to the coast (Fig. 1). Sampling began in Galician waters and gradually moved eastwards. Data obtained during the last two days in an intensive sampling of a 38 stations grid in the vicinity of Cape Peñes (~6°W) were only included when examining general relationships between the abundance and biomass of picoplankton and other biological variables. In addition, vertical profiles were obtained at 11 selected stations covering the whole area (P1–P11, see Fig. 1).

**Hydrography and chlorophyll a**

At each station, temperature, salinity and fluorescence data were acquired with a CTD Sea Bird 25 equipped with a SeaPoint fluorometer from the surface to the near-bottom, or down to 200 m at deeper stations. CTD data were processed to derive 1 dbar averages. A thermal stratification index (SI) was calculated as the temperature gradient (°C m⁻¹) between the surface and 100 m depth, or between the surface and the maximum sampling depth for shallower stations.

Water samples were taken from the underway water sampling system (~3 m depth) or from 5 L Niskin bottles in a rosette sampler attached to the CTD at the vertical profile stations. Chlorophyll a (Chl a) was obtained from fluorescence values after calibration with acetonic extracts of phytoplankton from discrete samples. The latter were obtained from water samples (100 mL) filtered through glass fibre filters (Whatmann GF/F) and frozen until analysis in the laboratory. Pigments were extracted in 90% acetone for 24 h in the dark at 4°C and the Chl a concentration was measured by using a Perkin Elmer LB-50s spectrofluorometer (Neveux and Panouse, 1987).

For descriptive purposes the study area was divided into four zones (Fig. 1) on the basis of distinct
hydrographic properties (Table I). A similar approach was taken by previous studies in this region (Bode et al., 2001, 2002). The geographical limits were 42–43°N, 8.8–10°W for the western (W) zone; 43–44°N, 7.6–9°W for the north-western (NW) zone; 5.4–7.6°W for the western Cantabrian (WC) zone; and eastwards of 5.4°W for the eastern Cantabrian (EC) zone.

### Abundance and biomass of picoplankton

Samples (1.8 mL) for the analysis of picoplankton were fixed with 1% paraformaldehyde + 0.05% glutaraldehyde, frozen in liquid N2 and subsequently kept at 4°C. Samples (1.8 mL) for the analysis of picoplankton were taken by previous studies in this region (Bode et al., 1993) thus falling into the nanoplankton size-class. Two groups of heterotrophic bacteria were distinguished by their relative green fluorescence (FL1, 530 nm) after SYTO-13 staining. Following previous works (Gasol et al., 1999; Lebaron et al., 2002) these groups were called HDNA and LDNA bacteria, after high DNA and low DNA content, respectively.

Picoplankton abundance was converted to biomass by using the cellular carbon contents provided by Zubkov et al. (Zubkov et al., 1998, 2000a) for Atlantic samples: 32 fg C cell−1 for Prochlorococcus, 103 fg C cell−1 for Synechococcus, 1.496 pg C cell−1 for eukaryotes and 20 fg C cell−1 for heterotrophic bacteria. Total phytoplankton biomass was estimated using a C:Chl a ratio of 50 (Varela et al., 1988; Barquero et al., 1990).

Data were log-transformed in order to attain normality and homogeneity of variances. Statistical analyses were made with StatSoft (StatSoft). One-way ANOVAs and post-hoc Student–Newman–Keuls (SNK) tests were applied to assess differences in picoplankton distribution between the four zones considered and along the coastal–offshore gradient. For that purpose, stations were grouped according to their bathymetry as inner shelf (<100 m), outer shelf (100–200 m) and offshore (>200 m). Finally, since all variables were equally subject to measurement errors, Model II regression analysis (Ricker, 1973) was performed together with the more common Model I when testing the significance of log–log regression slopes (Model II slope = Model I slope/ r). Ninety-five percent confidence limits of Model II slopes were calculated according to Ricker (Ricker, 1975).

### RESULTS

#### Hydrography and chlorophyll a

Typically winter hydrographic conditions were found west of 7.6°W, with a well-mixed water column (SI < 0.001°C m−1). The gradual increase of the thermal
stratification index eastwards of that longitude (Table I) indicated the initial phase of spring stratification in the easternmost area. A marked thermohaline gradient was observed in the study area (Fig. 2), caused by waters of subtropical origin transported by the Portugal coastal counter current (PCCC). Although surface temperature displayed no clear pattern (Fig. 2A), its distribution at 50 m depth (Fig. 2B) tracked the extension of the PCCC, roughly defined by the 12.6°C isotherm. The PCCC extended from Galicia to the central Cantabrian Sea attaining maximum values of salinity of 35.8 whereas the eastern Cantabrian Sea was characterized by values generally below 35.7 (Fig. 2C and D). South of 43°N and in the easternmost zone, marked coastal–offshore salinity gradients were associated with continental runoff. Phytoplankton biomass, estimated as Chl a concentration, closely matched the distribution of thermohaline properties (Fig. 2E and F) with values usually <1 μg L⁻¹ in the western zones and reaching values typical of spring blooms (4 μg L⁻¹) in the easternmost area.

Differences between zones in the surface values of the aforementioned variables were highly significant (1-way ANOVAs, P < 0.001, Table I). The W zone presented a marked coastal–offshore salinity gradient in front of the Rías Baixas caused by continental runoff, with highest values of temperature and salinity in offshore waters and an opposite pattern for Chl a, which reached 1.4 μg L⁻¹ close to the Rías. The NW zone, which was uniformly under the influence of the PCCC, showed the highest salinity (35.7–35.8) and temperature (12.8–13.4°C) values, and lowest phytoplankton biomass (<0.6 μg Chl a L⁻¹). The WC zone presented a high variability in thermohaline properties (35.1–35.8, 12.5–14°C) since there was located the confluence of the PCCC and the southern Bay of Biscay general anticyclonic circulation (Fig. 2A), with Chl a values exceeding 1 μg L⁻¹ at roughly half the stations. Finally, the lowest values of salinity (<35.7) and temperature (<13.2°C) and highest Chl a (1.4 μg L⁻¹) found during the cruise characterized the EC zone.

**Picoplankton distribution**

A spatial segregation in the surface distribution of cyanobacteria and autotrophic eukaryotes was apparent (Fig. 3). *Synechococcus* (mean abundance 1.1 ± 0.2 x 10⁴ cells mL⁻¹) dominated picophytoplankton in the zones under a marked influence of the PCCC (W and NW), and reached maximum numbers (1.3 x 10⁵ cells mL⁻¹) in the frontal area between the PCCC and southern Bay of Biscay waters (Fig. 3B). The presence of *Prochlorococcus* in surface waters was confined to the westernmost area (>8.5°W) coincident with the PCCC core of highest salinity (Fig. 3A). Although it was also detected at depth further east (<7.4°W) its abundance never exceeded 1500 cells mL⁻¹, and it was significantly correlated with salinity (Fig. 4, r = 0.64, P = 0.002, n = 21) attaining maximum concentrations at ~35.8.
Conversely, relatively low abundances of both groups of autotrophic eukaryotes, usually below $5 \times 10^3$ cells mL$^{-1}$, were found in the W and NW zones (Fig. 3C and D). As for *Synechococcus*, maximum values ($6.1 \times 10^4$ cells mL$^{-1}$) were associated with the eastern limit of the PCCC within the WC zone. Their abundance remained high in the EC zone ($2.1 \pm 0.1 \times 10^4$ cells mL$^{-1}$). The distribution patterns of small and large eukaryotes were rather similar, although a weak increase of the large group concurrent with a decrease of the small one was observed in the EC zone.

Numbers of heterotrophic bacteria were less variable than those of picophytoplankton ($1.5 \times 10^5$-$2.9 \times 10^6$ cells mL$^{-1}$, Fig. 3E). However, significant differences among zones were still found (Table II). A distinct geographical pattern emerged from the distribution of the two bacterial groups, specifically the contribution of HDNA bacteria to total numbers (Fig. 3F). Maximum abundances of HDNA bacteria were recorded in the W and EC zones ($4.3 \pm 0.4$ and $7.2 \pm 0.7 \times 10^5$ cells mL$^{-1}$, respectively), while LDNA bacteria were significantly more abundant in the WC zone ($3.7 \pm 0.3 \times 10^5$ cells mL$^{-1}$). As a consequence, the percentage of HDNA cells was high (>70%) and significantly different in the W and EC zones (Table II).

As a proxy of cell size, Table III shows the mean light forward scatter (FSC) of each group in the four zones. Again, highly significant differences were found among zones (1-way ANOVA, $P < 0.001$). *Synechococcus* cells were larger in the W and NW zones and both groups of autotrophic eukaryotes tended to gradually decrease in size eastwards. Geographical patterns were less clear in the case of bacteria, but for both groups (HDNA and LDNA) the largest cells were found in the W and EC zones, coinciding with the highest contribution of HDNA to total counts (Table II).

At those stations where vertical profiles were also conducted (Fig. 1), mean abundances for the upper 100 m of the water column (except at the shallower P1 and P2 stations, 30 and 60 m respectively) were calculated. Water column and surface abundances were significantly correlated in all groups ($P < 0.01, n = 11$) except large eukaryotes. However, differences were observed in...
the vertical distribution of the analyzed groups (Fig. 5). Both eukaryotic groups and HDNA bacteria were more abundant at the surface than in deep waters (paired t-tests, P < 0.05, n = 11), while no significant differences were found between surface and mean 0–100 m concentrations of Synechococcus cells or LDNA bacteria, suggesting a uniform distribution within the water column.

Differences in abundance and cell size along the coastal–offshore gradient were also assessed with all data pooled. A 2-way ANOVA by zone and bathymetry had revealed that there was no significant interaction between these factors (P > 0.05). Whereas Synechococcus and large eukaryotes were found to be significantly more abundant offshore than in shelf waters (Table IV), the pattern of HDNA bacteria abundance displayed a clearly bimodal distribution, with higher abundances both in inner shelf waters and offshore. With regard to variations in cell size, differences were only significant for the two groups of eukaryotes, which were larger over the shelf than offshore (Table V).

**General relationships and biomass**

With all data pooled, the abundance of all groups except LDNA bacteria was significantly correlated with Chl a (Fig. 6, Table VI). The correlation coefficients (r) of the autotrophic groups detected in all samples (Prochlorococcus excluded) tended to increase with the average size of the group: −0.20 for Synechococcus, 0.54 for small eukaryotes and 0.71 for large eukaryotes, suggesting weaker responses of the smaller groups to increasing total biomass. As for eukaryotic cells, HDNA bacteria

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**Table II: Mean ± SE autotrophic and heterotrophic picoplankton abundance and percentage of high DNA bacteria in the four zones**

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>NW</th>
<th>WC</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechococcus (10³ cells mL⁻¹)</td>
<td>3.49 ± 1.36ᵃ</td>
<td>13.71 ± 1.74ᵇ</td>
<td>18.04 ± 5.22ᵇ</td>
<td>5.59 ± 0.95ᵇ</td>
</tr>
<tr>
<td>Prochlorococcus (10³ cells mL⁻¹)</td>
<td>0.06 ± 0.06</td>
<td>0.28 ± 0.07</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Small eukaryotes (10³ cells mL⁻¹)</td>
<td>1.39 ± 0.43ᵃ</td>
<td>4.38 ± 0.49ᵇ</td>
<td>24.15 ± 2.10ᵇ</td>
<td>16.43 ± 1.23ᵇ</td>
</tr>
<tr>
<td>Large eukaryotes (10³ cells mL⁻¹)</td>
<td>1.11 ± 0.16ᵃ</td>
<td>1.68 ± 0.11ᵇ</td>
<td>3.30 ± 0.31ᶜ</td>
<td>4.15 ± 0.29ᵍ</td>
</tr>
<tr>
<td>Heterotrophic bacteria (10⁵ cells mL⁻¹)</td>
<td>6.05 ± 0.42ᵇ</td>
<td>4.75 ± 0.25ᵃ</td>
<td>6.89 ± 0.51ᵇ</td>
<td>9.73 ± 0.83ᶜ</td>
</tr>
<tr>
<td>HDNA bacteria (%)</td>
<td>70 ± 3ᵃ</td>
<td>52 ± 2ᵇ</td>
<td>47 ± 2ᵇ</td>
<td>72 ± 2ᵃ</td>
</tr>
</tbody>
</table>

Differences between regions at the 5% significance level are indicated by different superscripts (Student–Newman–Keuls test, P < 0.05). If the same letter appears in two regions the difference is not significant.

**Table III: Mean ± SE relative light forward scatter (FSC, arbitrary units) as a surrogate of cell size of different picoplankton groups in the four zones**

<table>
<thead>
<tr>
<th></th>
<th>W</th>
<th>NW</th>
<th>WC</th>
<th>EC</th>
<th>P</th>
<th>SNK-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechococcus</td>
<td>1.38 ± 0.04</td>
<td>1.37 ± 0.02</td>
<td>1.07 ± 0.04</td>
<td>1.03 ± 0.04</td>
<td>***</td>
<td>W = NW &gt; WC = EC</td>
</tr>
<tr>
<td>Small eukaryotes</td>
<td>5.10 ± 0.39</td>
<td>3.85 ± 0.13</td>
<td>3.06 ± 0.17</td>
<td>2.65 ± 0.11</td>
<td>***</td>
<td>W &gt; NW &gt; WC = EC</td>
</tr>
<tr>
<td>Large eukaryotes</td>
<td>34.33 ± 3.19</td>
<td>19.21 ± 1.23</td>
<td>15.18 ± 0.86</td>
<td>14.86 ± 0.80</td>
<td>***</td>
<td>W &gt; NW &gt; WC = EC</td>
</tr>
<tr>
<td>LDNA bacteria</td>
<td>0.51 ± 0.03</td>
<td>0.35 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>0.45 ± 0.03</td>
<td>***</td>
<td>W &gt; NW = WC, W = EC, NW = EC</td>
</tr>
<tr>
<td>HDNA bacteria</td>
<td>0.79 ± 0.04</td>
<td>0.54 ± 0.02</td>
<td>0.54 ± 0.03</td>
<td>0.66 ± 0.03</td>
<td>***</td>
<td>W &gt; EC &gt; NW = WC</td>
</tr>
</tbody>
</table>

See the text for details. P: significance of 1-way ANOVA: ***, P < 0.001. SNK-test: Student–Newman–Keuls test, P < 0.05.

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Fig. 5. Relationship between picoplankton surface abundance and mean values for the upper 100 m of the water column. The line represents the 1:1 relationship.
were also positively correlated with Chl $a$ (Fig. 6B). The lack of correlation of LDNA bacteria did not preclude finding a significant correlation between the percentage of HDNA bacteria and Chl $a$ ($r = 0.49$). The slopes of the Model I linear regressions between picoplankton abundance and Chl $a$ (Table VI) were significantly lower than 1.0 ($t$-test, $P < 0.01$) for all groups except for small eukaryotes. However, lower relative increases in abundance with increasing Chl $a$ were only consistently shown by *Prochlorococcus* and HDNA bacteria, since 95% confidence limits of Model II slopes in contrast, *Synechococcus* showed a weak trend of decreasing numbers with increasing Chl $a$.

Mean biomass of picoplankton in surface waters of the study area was $42 \pm 2 \mu g C L^{-1}$ although significant differences were observed between zones (ANOVA, $P < 0.001$). Picoeukaryotes largely dominated picoautotrophic biomass with a contribution $>88\%$ in all zones (Fig. 7). The relative contribution of eukaryotes to total picoplanktonic biomass (i.e. including heterotrophic bacteria) was significantly higher in the WC and EC zones (ANOVA, $P < 0.001$), where they dominated the community, accounting for $69 \pm 2$ and $61 \pm 3\%$, respectively. Biomass of *Synechococcus* cyanobacteria peaked in the NW and WC zones ($1.4 \pm 0.2$ and $1.9 \pm 0.5 \mu g C L^{-1}$, respectively), being on average $<7\%$ of total picophytoplanktonic biomass in all zones, and as low as $1\%$ in EC. *Prochlorococcus* contributed less than $0.05\%$ to picophytoplankton biomass in those zones where it was detected (W and NW). Heterotrophic bacteria accounted for a noticeable fraction of total picophytoplankton virtually contributed $99\%$ of PB in all zones (ANOVA, $P < 0.001$), being on average $7\%$ of PB in all zones (ANOVA, $P < 0.001$), being on average $7\%$ of total picoplanktonic biomass in all zones ($>25\%$), but dominated over picoautotrophic biomass only in W and NW ($76 \pm 4$ and $51 \pm 3\%$, respectively). In the WC and EC zones the ratio of picoplanktonic heterotrophic to autotrophic biomass was generally well below 1 ($0.46 \pm 0.07$ and $0.83 \pm 0.13$, respectively).

On average, picophytoplankton accounted for $59 \pm 4\%$ of total phytoplankton biomass (PB) estimated from Chl $a$ measurements, with significant differences between zones (ANOVA, $P < 0.001$). For instance, picophytoplankton virtually contributed $99\%$ of PB in the WC zone but accounted for only $12\%$ in W (Fig. 7). As expected, autotrophic picoplankton biomass was positive and significantly correlated with total

### Table IV: Mean ± SE abundance of autotrophic ($10^3$ cells mL$^{-1}$) and heterotrophic picoplankton ($10^4$ cells mL$^{-1}$) in three bathymetric zones along the coastal–offshore gradient

<table>
<thead>
<tr>
<th>Zone</th>
<th>1. Inner shelf (&lt;100 m)</th>
<th>2. Outer shelf (100–200 m)</th>
<th>3. Offshore (&gt;200 m)</th>
<th>$P$</th>
<th>SNK-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechococcus</td>
<td>4.16 ± 0.74</td>
<td>8.88 ± 1.35</td>
<td>14.6 ± 3.49</td>
<td>** 1 &lt; 2 = 3</td>
<td></td>
</tr>
<tr>
<td>Small eukaryotes</td>
<td>17.99 ± 2.43</td>
<td>15.55 ± 1.56</td>
<td>18.24 ± 1.89</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Large eukaryotes</td>
<td>2.99 ± 0.45</td>
<td>2.50 ± 0.16</td>
<td>4.28 ± 0.34</td>
<td>** 1 = 2 &lt; 3</td>
<td></td>
</tr>
<tr>
<td>LDNA bacteria</td>
<td>2.88 ± 0.24</td>
<td>3.14 ± 0.22</td>
<td>4.22 ± 0.47</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>HDNA bacteria</td>
<td>6.90 ± 0.77</td>
<td>5.24 ± 0.58</td>
<td>7.68 ± 0.79</td>
<td>** 1 = 3 &gt; 2</td>
<td></td>
</tr>
<tr>
<td>% HDNA</td>
<td>68 ± 2</td>
<td>57 ± 2</td>
<td>64 ± 2</td>
<td>** 1 = 3 &gt; 2</td>
<td></td>
</tr>
</tbody>
</table>

Also shown is the percentage of HDNA bacteria (%). $P$, significance of 1-way ANOVA: **, $P < 0.01$; ns, non significant. SNK-test: Student–Newman–Keuls test, $P < 0.05$.

### Table V: Mean ± SE relative FSC (arbitrary units) of the different picoplankton groups along the coastal–offshore gradient

<table>
<thead>
<tr>
<th>Zone</th>
<th>1. Inner shelf (&lt;100 m)</th>
<th>2. Outer shelf (100–200 m)</th>
<th>3. Offshore (&gt;200 m)</th>
<th>$P$</th>
<th>SNK-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechococcus</td>
<td>1.18 ± 0.04</td>
<td>1.20 ± 0.03</td>
<td>1.09 ± 0.05</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Small eukaryotes</td>
<td>3.51 ± 0.16</td>
<td>3.40 ± 0.14</td>
<td>2.63 ± 0.13</td>
<td>** 1 = 2 &gt; 3</td>
<td></td>
</tr>
<tr>
<td>Large eukaryotes</td>
<td>19.65 ± 1.49</td>
<td>18.46 ± 0.93</td>
<td>13.97 ± 0.73</td>
<td>** 1 = 2 &gt; 3</td>
<td></td>
</tr>
<tr>
<td>LDNA bacteria</td>
<td>0.43 ± 0.03</td>
<td>0.39 ± 0.02</td>
<td>0.43 ± 0.02</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>HDNA bacteria</td>
<td>0.63 ± 0.03</td>
<td>0.60 ± 0.02</td>
<td>0.64 ± 0.03</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

$P$, significance of 1-way ANOVA: **, $P < 0.01$; ns, non significant. SNK-test: Student–Newman–Keuls test, $P < 0.05$. 

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autotrophic biomass ($r = 0.59, P < 0.0001, n = 224$), with a Model I linear regression slope significantly lower than 1.0 ($t$-test, $P < 0.01$) but not in Model II due to high dispersion of data (Table VI). The biomass of heterotrophic bacteria (BB) was seldom higher than PB: only in 13% of the samples, located mostly at depth but also in surface waters of an anticyclonic eddy found during the intensive sampling (data not shown). The BB:PB ratio significantly decreased with increasing phytoplankton biomass, as indicated by the significantly lower than 1.0 slope in the log–log regression models between both variables (Table VI).

**DISCUSSION**

Three distinct water masses were sampled during the survey, as previously described for the area [e.g. (Botas et al., 1989; Bode et al., 2002)]: surface water, Bay of Biscay central water (BBCW) and Eastern North Atlantic central water of subtropical origin (ENACW$_{st}$) transported by the poleward Navidad current (Pingree and Le Cann, 1990; García-Soto et al., 2002), recently renamed...
as Portugal coastal counter current or PCCC (Álvarez-Salgado et al., 2003). The influence of freshwater, detected as low surface salinities (Fig. 2C and D), appeared in the same locations reported by Bode et al. (Bode et al., 2002). The eastwards decreasing influence of the PCCC, reaching the central Cantabrian Sea, is a common feature in the region in late winter–early spring (Botas et al., 1988, 1989; Fernández et al., 1991, 1993; Pingree, 1994). Low Chl _a_ concentrations in areas markedly influenced by the PCCC are in accordance with previous observations. Some studies (Fernández et al., 1991; Álvarez-Salgado et al., 2003) described also a dominance of small-sized organisms in PCCC waters, which agrees well with our results of cyanobacteria outnumbering the larger eukaryotes in those zones (Fig. 3, Table II). Conversely, high Chl _a_ zones would be associated with a dominance of larger phytoplanktonic cells (Fernández and Bode, 1991). Accordingly, highest abundances of both small and large eukaryotes (Fig. 3) were generally coincident with high Chl _a_ values, especially in the WC and EC zones where vernal stratification was already in progress. Changes in the nutrient field due to mesoscale physical processes such as upwellings or fronts are also known to affect planktonic standing stocks and community structure [e.g. (Fernández et al., 1991, 1993; Casotti et al., 2000; Arin et al., 2002)]. In this work, maximum abundances for most groups were associated with the frontal area originated by the confluence of the PCCC with less salty Bay of Biscay waters, in agreement with Bode et al. (Bode et al., 2002), who found that fronts increased the variance of phytoplankton biomass in comparison with surrounding waters.

In spite of the few samples, surface picoplankton abundance was significantly correlated with water column-averaged values for all but one group, thus rendering possible the use of surface samples to estimate integrated abundances during this period. However, not all picoplankton groups presented the same vertical distribution. LDNA bacteria were the most homogeneously distributed group, while HDNA bacteria were more abundant at the surface. Autotrophic picoplankton also showed differing patterns. Thus, _Synechococcus_ numbers presented low vertical variability as found in other sites in the Atlantic Ocean (Li and Wood, 1988; Olson et al., 1990; Partensky et al., 1996), while picoeukaryotes showed surface or near-surface peaks and consistently decreased with depth (Li and Wood, 1988; Zubkov et al., 1996).

**Picophytoplankton**

The latitudinal limit of _Prochlorococcus_ distribution in open-ocean Atlantic waters was found around 60°N (Buck et al., 1996) but little is known about its distribution in coastal waters. With regard to N Iberian Atlantic waters, _Prochlorococcus_ had only been reported to our knowledge in shelf waters off the Galician Rías Baixas (Morán et al., 2002), and is apparently absent from the Celtic Sea (Zubkov et al., 2000a), the English Channel (D. Vaulot et al., unpublished data) and the French shelf (Lampert, 2001). Its persistent observation in the coastal transition zone of the central Cantabrian Sea from autumn to early winter (A. Calvo-Díaz et al., unpublished data) suggests that its northern limit of distribution along the Atlantic coast could be located somewhere in the southern Bay of Biscay. The absence of _Prochlorococcus_ northwards could be related to regional topography. Shelf-break fronts originated by the strong shelf currents found over the extensive Armorican and Celtic shelves (Koutsikopoulos and Le Cann, 1996) may prevent the advection of ENACW<sub>w</sub>, and hence passively transported _Prochlorococcus_, onto the shelf.

The abundance of _Prochlorococcus_ was 1–3 orders of magnitude lower than that found in subtropical waters (Chisholm et al., 1988; Buck et al., 1996; Partensky et al., 1999; Zubkov et al., 2000b; Tarran et al., 2001). Therefore _Synechococcus_ was always more abundant than _Prochlorococcus_ (Table II), contrary to common observations in areas where both cyanobacteria coexist [see review in (Partensky et al., 1999)]. Although our results agree with those found by Buck et al. (Buck et al., 1996) in the subarctic, at latitudes similar to this study (~45°N) _Prochlorococcus_ was significantly more abundant than _Synechococcus_ at abundances of the latter of the same order as ours (Buck et al., 1996; Tarran et al., 2001). A positive relationship between _Prochlorococcus_ numbers and temperature has been described (Vaulot et al., 1990; Buck et al., 1996). However, the low concentrations we have found (10<sup>3</sup> cells mL<sup>−1</sup>) could not be readily attributed to cold conditions, since prochlorophytes were absent later in the growth season (A. Calvo-Díaz and X. A. G. Morán, unpublished data) when temperatures rose above 15°C. This cyanobacterium has also been reported to disappear completely under severe winter mixing in temperate waters (Lindell and Post, 1995), so its presence in well-mixed shelf waters from Galicia to the western Cantabrian sea and its positive correlation with salinity (Fig. 4) suggest an association with PCCC waters and the possible use of _Prochlorococcus_ as a tracer of the extent and intensity of this current.

An increase in the relative abundance of picoeukaryotes has been generally observed in temperate meso- and eutrophic oceanic regions (Partensky et al., 1996), although _Synechococcus_ still dominated both in the absence (Zubkov et al., 2000b; Tarran et al., 2001) and presence of _Prochlorococcus_ (Li, 1995; Buck et al., 1996; Partensky et al., 1996; Tarran et al., 2001). As stated, we found a clear dominance of eukaryotic cells over cyanobacteria in
the Cantabrian Sea zones (Table II). This dominance held from late winter to early spring (A. Calvo-Díaz and X. A. G. Morán, unpublished data), suggesting a different structure of the picoplanktonic community over the shelf as compared with open-ocean waters. Although we cannot provide any hint into the taxonomic composition of the FC-defined large and small eukaryotes, HPLC analysis of the picoeukaryotic community in the Ría de Pontevedra (Rodríguez et al., 2003), located within the W zone, suggests it to be mainly composed of both Chl b- (prasinophytes and chlorophytes) and Chl c-containing organisms (small diatoms).

The association of distinct picophytoplankton species and/or groups with either subtropical (ENACW) or Bay of Biscay waters (BBCW, surface water) is not only suggested by the aforementioned differences in abundance, but also by a noticeable eastward trend of decreasing cell size for most groups (Table III), although it could as well indicate increased growth rates [e.g. (Harris, 1986)] during the initial phases of the spring bloom in the Cantabrian Sea. Further studies will be needed to test the validity of the use of cell size, the abundance of prochlorophytes or the ratio of cyanobacteria to eukaryotes abundance to track the PCCC in the area of study. High values of the latter have been successfully used to characterize saline intrusions of subtropical origin (Gradinger and Lenz, 1989; Jochem and Zeitzschel, 1993) while distinct communities of pico-autotrophs can develop in adjoining water masses [e.g. (Casotti et al., 2000)].

**Heterotrophic bacteria**

The abundance of heterotrophic bacteria ranged from $1.5 \times 10^3$ to $2.8 \times 10^6$ cells mL$^{-1}$, in agreement with reports on inshore bacterial abundance within the EC zone at similar temperatures (Unanue et al., 1992). Our extensive geographical coverage yielded a wider range of variation than more spatially constrained studies (Barquero et al., 1998; Valencia et al., 2003), although Barbosa et al. (Barbosa et al., 2001) observed a greater variability under upwelling conditions in the W zone. Nevertheless, the overall variability of heterotrophic bacteria was low compared with autotrophic groups, in accordance with the accepted view of a higher uniformity of bacterial biomass over other planktonic compartments (del Giorgio and Scarbrough, 1995). Yet, bacterial numbers responded to increased Chl $a$ (Fig. 6).

It has long been established that heterotrophic bacteria are not all equally active (Stevenson, 1978; Gasol and del Giorgio, 2000; Servais et al., 2003). The so-called HDNA bacteria would correspond to the metabolically active fraction while LDNA bacteria, comprising dormant or dead cells, are usually characterized by significantly lower growth rates (Gasol et al., 1999; Lebaron et al., 2002; Servais et al., 2003). In marked contrast to oceanic or oligotrophic waters (del Giorgio and Scarbrough, 1995; Morán et al., 2004), most bacteria (60 ± 1%) were active in the study area. They clearly dominated the community in the W and EC zones, likely due at least partly to allochthonous inputs of dissolved organic matter and nutrients from the Rías Baixas and the Adour and Gironde rivers on the French coast. Higher concentrations of dissolved organic nitrogen have been recently reported in these areas, where inputs from continental waters can make up to 10% of the total (Boyle et al., 2001). The greater overall variation of HDNA bacteria (CV 77%) when compared with LDNA (CV 57%) suggests the existence of a relatively constant pool of LDNA bacteria and that HDNA bacteria are the ones most ready to respond to environmental changes (Servais et al., 2003). This hypothesis would be further supported by the finding that only the abundance of HDNA cells and its percentage contribution to total bacterial biomass were significantly and positively correlated with Chl $a$, as previously reported [e.g. (del Giorgio and Scarbrough, 1995)].

The larger size of coastal bacteria when compared with open-ocean waters (Fukuda et al., 1998; Li and Harrison, 2001) is likely associated with higher inputs of organic matter close to the coast (Schlesinger, 1991; Smith and Hollibaugh, 1993). This in turn would result in a relative increase of the active fraction, which usually presents larger sizes (Stevenson, 1978; Gasol et al., 1995). Our results would be consistent with that hypothesis since the size of HDNA bacteria was always higher than that of LDNA (Tables III and V) and their percentage in shelf waters was significantly higher coastal waters (Table IV). The high values of both variables found offshore remain to be explained, though. They may indicate the presence of different bacterial lineages in shelf and open-ocean waters (Rappé et al., 1997). In addition, clear geographical differences were observed in the size of HDNA bacteria (Table III), with larger cells in those zones (W and EC) with an overall higher contribution to total numbers (Table II), suggesting differences in cell-specific activity within this broadly-defined group (Lebaron et al., 2002).

**Biomass estimates**

Ignorance about true cellular sizes and the use of a single constant conversion factor for each group when changes in the size of autotrophic and heterotrophic cells were observed (Tables III and V), advise for caution in biomass estimates [see discussion in (Li et al., 1993)] and the subsequent comparisons among areas (Fukuda et al., 1998). Nevertheless, some general considerations can be made. Although marked longitudinal patterns were observed in
picophytoplankton community structure, eukaryotes clearly dominated picoautotrophic biomass (>88%) in the whole area. This dominance in biomass although not necessarily in abundance (e.g. the W zone) is in accordance with previous reports both in the North Atlantic (Li and Wood, 1988; Li et al., 1993; Li, 1995; Zubkov et al., 1998) and in the subarctic Pacific (Liu et al., 2002). The decreasing relative contribution of picophytoplankton to total algal biomass along a gradient of productivity (Agawin et al., 2000; Bell and Kalf, 2001) was not firmly supported by our data due to the relatively low correlation coefficient between both variables, which yielded a Model II log-log regression slope higher than 1.0 (Table VI).

Biomass of heterotrophic bacteria was less variable, reaching maximum values in the EC zone (20 ± 2 μg C L⁻¹). The heterotrophic to autotrophic picophytoplankton biomass ratio was >1 in the W and NW zones, probably associated with freshwater inputs, described as an important flux of allochthonous organic matter in the W zone (Doval et al., 1997). Bacterial biomass tends to be lower than picophytoplankton biomass in meso- to eutrophic waters (Gasol et al., 1997). Our estimated ratios of bacterial (BB) to phytoplankton (PB) biomass only exceeded unity at the surface of 10 stations, but they were also generally higher than 0.2, in disagreement with most values found by Valencia et al. (Valencia et al., 2003) in the Ria de A Coruña and the suggested BB:PB ratio for coastal areas (Ducklow and Carlson, 1992). The heterotrophic to autotrophic biomass ratio is also expected to decrease with increasing algal biomass (Gasol et al., 1997). Although we only included the biomass of bacteria within the heterotrophic compartment [see implications in (Gasol et al., 1997)], our data demonstrated an overall significant decrease of the BB:PB ratio at increasing phytoplankton biomass (Table VI). The lowest values (0.27 ± 0.03) were observed at offshore stations associated with maximum values of algal biomass (80 ± 6 μg C L⁻¹). However, maximum ratios (~0.5) were found in the EC zone, which showed also the highest autotrophic biomass, and in the inner shelf. As previously suggested (Morán et al., 2002), there should exist other sources of organic matter for bacterioplankton in addition to primary production in these areas.

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