Bacterial community composition and colored dissolved organic matter in a coastal upwelling ecosystem

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ABSTRACT: The aim of the present study was to link changes in dissolved organic matter (DOM) composition, as characterized through the intensity of humic- and protein–like fluorescence, with changes in the abundance of major bacterial groups (Alpha-, Beta-, and Gammaproteobacteria and Bacteroidetes) and bacterial bulk activity in the coastal transition zone of NW Spain during the low productivity season. Sampling was carried out under two contrasting periods characterized by extremely low (February) and high (October) precipitation. In October, the mean humic-like DOM fluorescence in surface waters (3.1 ppb eq QS) was higher than the annual average for this coastal zone (2.2 ppb eq QS) which was attributed to enhanced continental runoff. Alphaproteobacteria and Bacteroidetes were the most abundant groups during both sampling periods, accounting for about 13 and 16 % of total bacterial abundance, respectively. Gammaproteobacteria were significantly more abundant in October, when they constituted 10 % of total bacterial counts. Betaproteobacteria were detectable only during the high precipitation period accounting for 2-9 % of total bacterial abundance. The bulk dissolved organic carbon concentration similarly explained the relative abundance of Alpha-, Beta-, and Gammaproteobacteria (ca. 50% of total variability). By contrast, a strong correlation was found between the humic-like DOM fluorescence and the relative abundance of the Betaproteobacteria group, explaining 68 % of the total variability. Multi-variate linear regression revealed that the relative abundance of Betaproteobacteria has the greatest influence on bacterial carbon fluxes, explaining, together with that of Gammaproteobacteria, 74 and 67 % of bulk bacterial activity and biomass variability, respectively. Despite its relatively low abundance, Betaproteobacteria might play a relevant biogeochemical role in this coastal-transition ecosystem during the low productivity period as allochthonous –DOM consumers.
INTRODUCTION

The importance of dissolved organic matter (DOM) for carbon and nutrient fluxes is enhanced in coastal waters where river inputs, together with an intense physical and biological activity, contribute to the accumulation of large amounts of DOM from both marine and terrestrial origin (Cauwet 2002). Paradoxically, despite terrestrial DOM has traditionally been classified as refractory, a relatively large fraction appears to be consumed by heterotrophic bacteria (Moran & Hodson 1994; Kelley et al. 1998, McCallister et al. 2004).

Addressing the links between diversity and functions of natural bacterial communities is essential to understand their roles in biogeochemical cycles. To the date, very little information exists on the role that particular bacterial taxa play in DOM decomposition in marine planktonic systems (Kirchman 2004, Mou et al. 2008). Culture-independent molecular tools, such as the combination of microautoradiography and FISH (e.g. Cottrell & Kirchman 2000, Elifantz et al 2005, Malmstrom et al. 2005, Teira et al. 2006, Alonso-Sáez & Gasol 2007,) or metagenomic DNA analysis (e.g. Mou et al. 2008), have been used to unravel the links between bacterial function and diversity in a wide variety of marine pelagic environments.. This type of studies have revealed systematic differences in the patterns of uptake of specific organic compounds among major phylogenetic groups. Alphaproteobacteria seem to mostly contribute to low molecular weight (LMW) DOM utilisation (glucose, amino acids, DMSP), whereas Bacteroidetes appear to be proficient in the use of proteins and other plankton-derived high molecular weight (HMW) compounds. Several studies in lakes and estuaries have also reported changes in bacterial community structure and function associated to shifts in terrestrial organic matter influx. Comparatively, little is known about the patterns of utilization of allochthonous carbon sources, such as those derived from continental

The NW Iberian coastal transition zone, is an area affected by a marked seasonal cycle of coastal winds, which divides the annual cycle in an upwelling season (March to September), with short relaxation intervals that enhance productivity, and a downwelling season (from October to March), characterized by low phytoplankton biomass and primary production (Álvarez-Salgado et al. 2003). Continental runoff is most intense during this low productivity period, potentially transporting large amounts of allochthonous carbon into this coastal zone.

Fluorescence has proven to be a simple, sensible and quick tool for characterising DOM in marine systems (Nieto-Cid et al. 2005). There are two main classes of DOM which may be quantified through fluorescence analysis: (1) the labile protein-like material; and (2) the refractory humic-like substances from both terrestrial and marine origin. Nieto-Cid et al. (2005) reported seasonal variations in DOM fluorescence in shelf waters off NW Spain and attributed humic-like DOM fluorescence maxima in surface waters to enhanced continental runoff.

Within this context, the aim of the present study was to link changes in DOM composition, as characterized through fluorescence, with changes in the relative abundance of major bacterial groups (Alpha-, Beta-, and Gammaproteobacteria and Bacteroidetes, also known as CFB, Cytophaga-Flavobacteria-Bacteroides) and bacterial production and biomass in the coastal transition zone of the NW Iberian peninsula during the low productivity phase. Sampling was conducted during two contrasting periods, characterized by low (February) and high (October) continental runoff.
METHODS AND MATERIALS

Sampling area

The coastal transition zone of the NW Iberian Peninsula was sampled during two cruises conducted from 7-14 February and 23-30 October 2005 on board RV Cornide de Saavedra. The sampling stations are presented in Figure 1A. In February, we sampled along a latitudinal transect centered at 41.92 °N, close to the mouth of the River Miño. We did not complete the transect in front of the River Miño during October due to the very bad weather conditions. A total of 5-6 stations were occupied during each cruise. Temperature and salinity profiles were obtained with a SBE9/11 CTD device incorporated into a rosette sampler equipped with 24 12-L Niskin bottles. CTD Temperature and salinity sensors were calibrated using digital reversing thermometers and water samples drawn for salinity determinations.

At each station, we took samples at 5 depths within the photic zone (depth reached by 1% of surface irradiance) in order to measure chlorophyll-a concentration, bacterial abundance, bacterial production, and to study the taxonomic composition of the bacterial community using Fluorescence In Situ Hybridization (FISH) techniques. Samples for chemical analysis (nutrients, DOC concentration and DOM fluorescence) were also sampled at each station/depth, including one-two additional depths below the photic zone.

Dissolved inorganic nutrients

Water samples were collected in 50 mL polyethylene flasks, directly from the Niskin bottles, and kept frozen (-20 °C) until determination of ammonium, nitrite, nitrate, phosphate and silicate concentrations, using standard segmented flow analysis with standard colorimetric methods (Grashoff 1983).
**Chlorophyll-a (chla) concentration**

Chlorophyll-a was measured fluorimetrically after concentration of particulate matter by filtering 250 mL of seawater through 0.2 μm polycarbonate filters. The filters were immediately frozen and kept at –20 °C until further analysis ashore. In the laboratory, chla was extracted by adding 5 mL of 90% acetone to the filters. Extraction was done at 4 °C overnight. Chla fluorescence was determined with a TD700 fluorometer, calibrated with pure chla (sigma).

**DOC concentration**

Samples for dissolved organic matter (DOM) were collected into 500-mL acid-cleaned flasks and filtered through precombusted (450 °C, 4 h) 47 mm Ø Whatman GF/F filters in an acid-cleaned glass filtration system under low N₂ flow pressure. Aliquots for the analysis of DOC were collected into 10-mL precombusted (450 °C, 12 h) glass ampoules. After acidification with H₃PO₄ to pH < 2, the ampoules were heat-sealed and stored in the dark at 4 °C until analysis. DOC was measured with a Shimadzu TOC-5000 organic carbon analyzer, as described in Nieto-Cid et al. (2005). The system was standardized daily with potassium hydrogen phthalate. The concentrations of DOC were determined by subtracting the average peak area from the instrument blank area and dividing by the slope of the standard curve. The precision of measurement was ±0.7 μmol C L⁻¹. The accuracy was tested daily reference materials provided by D. Hansell (University of Miami).
DOM fluorescence

Samples for fluorescence of DOM (FDOM) determination were filtered through precombusted (450 °C, 4 h) 47 mm Ø Whatman GF/F filters in an acid-cleaned glass filtration system under low N₂ flow pressure, and preserved at 4 °C until determination with a Perkin Elmer LS 55 luminiscence spectrometer within 1 h of sample collection. The instrument was equipped with a xenon discharge lamp, equivalent to 20 kW for 8 μs duration, and a 1-cm quartz fluorescence cell. Milli-Q water was used as a reference for fluorescence analyses, and the intensity of the Raman peak was checked regularly.

Discrete excitation/emission (Ex/Em) pair measurements were performed at peaks M (marine humic substances, Ex/Em 320/410 nm; FDOMₘ) and T (aromatic amino acids, Ex/Em 280/350 nm; FDOMₜ). Basically we determined the fluorescence at the wavelengths proposed by Coble et al. (1990) and confirmed by Nieto-Cid et al. (2005) in the Iberian upwelling system. Four replicate measurements were performed for each Ex/Em pair. A four-point standard curve was prepared daily with a mixed standard of quinine sulphate (QS) and tryptophan (Trp) in sulphuric acid 0.05 M (Nieto-Cid et al. 2005). The equivalent concentration of every peak was determined by subtracting the average peak height from the blank height and dividing by the slope of the standard curve. Fluorescence units were expressed in ppb equivalents of QS (ppb QS) for FDOMₘ and ppb equivalents of Trp (ppb Trp) for FDOMₜ. The precision was ±0.1 ppb Qs and ±0.6 ppb Trp, respectively. The ratio between DOC and FDOMₘ, after several replicate measurements at different concentrations of a commercial fulvic acid, was 2.67 ± 0.06 μM C (ppb eq QS)⁻¹ (Nieto-Cid et al 2005).
Bacterial abundance and assemblage composition

Immediately after collecting the samples from the Niskin bottles, water samples of 5-10 mL were fixed by adding 0.2-μm filtered paraformaldehyde (2% final conc.) and subsequently, the samples were stored at 4°C in the dark for 4-12 h. Thereafter, the sample was filtered through a 0.2 μm polycarbonate filter (Millipore, GTTP, 25 mm filter diameter) supported by a cellulose nitrate filter (Millipore, HAWP, 0.45 μm), washed twice with Milli-Q water, dried and stored in a microfuge vial at -20°C until further processing in the home laboratory.

Filters for CARD-FISH were embedded in low-gelling-point agarose and incubated with lysozyme. Filters were cut in sections and hybridized with horseradish peroxidase (HRP)-labeled oligonucleotide probes (Eub338, for bacteria, Alf968, for Alphaproteobacteria, Gam42a, for Gammaproteobacteria, Bet42a, for Betaproteobacteria, CF319a, for Bacteroidetes, and NON-Eub338, as negative control) and tyramide-Alexa488 for signal amplification following the protocol described in Teira et al. (2008). It is important to mention that the FISH probe used for Bacteroidetes (CF319a) targets mainly the class Flavobacteria (formerly known as order Cytophagales), which comprises the most abundant class of planktonic marine Bacteroidetes (Alonso et al. 2007). Cells were counter-stained with a DAPI-mix (5.5 parts of Citifluor [Citifluor, Ltd.], 1 part of Vectashield [Vector Laboratories, Inc.] and 0.5 parts of PBS with DAPI (final concentration 1 µg mL⁻¹).

The slides were examined under a Leica microscope equipped with a 100-W Hg-lamp and appropriate filter sets for DAPI and Alexa488. More than 800 DAPI-stained cells were counted per sample. For each microscope field, 2 different categories were enumerated: (i) total DAPI-stained cells, and (ii) cells stained with the specific probe. Negative control counts (hybridization with HRP-Non338) were always below
0.5% of DAPI-stained cells. The counting error, expressed as the percentage of standard error between replicates, was < 2% for DAPI counts and < 9% for FISH counts.

Bacterial biomass was estimated using a conversion factor of 12 fg C cell\(^{-1}\) (Fukuda et al. 1998).

**Bacterial production**

Bulk prokaryotic activity was measured by incubating 5-10 mL of water in duplicate and one formaldehyde-killed blank with 40 nmol L\(^{-1}\) \([^3]H\)-leucine (final conc., SA 595.7 and 558.7 GBq mmol\(^{-1}\), Amersham) in the dark at in situ (±1°C) temperature for 1-1.5 h. Thereafter, the incubation was terminated by adding formaldehyde (2% final conc.) to the samples and filtering them through 0.2 µm cellulose nitrate filters (Millipore, 25 mm filter diameter). Subsequently, the filters were rinsed three times with 5% ice-cold trichloroacetic acid, placed in scintillation vials and stored at ∼20°C until counting in a liquid scintillation counter. The disintegrations per minute (DPM) of the formaldehyde-fixed blank were subtracted from the samples and the resulting DPM converted into leucine incorporation rates. Two previous experiments were conducted, one with coastal and one with oceanic water, in order to determine the saturation leucine concentration. Conversion factor to convert leucine to carbon was also determined experimentally once during each cruise, with water from a shelf station (stn 46 in February and stn 26 in October). The derived conversion factors were 2.34 and 2.03 Kg C mol Leu\(^{-1}\), in February and October, respectively.

**Meteorological variables**

Daily Ekman transport values (-Qx, m\(^3\) s\(^{-1}\) Km\(^{-1}\)) were calculated as described by Nieto-Cid et al. (2005). Precipitation data (mm) were obtained from the hydrological bulletin.
of the Spanish Ministerio de Medio Ambiente. The river Oitabén-Verdugo, the main tributary to the Ría de Vigo, is a combination of regulated and natural flows. The flow from the Eiras reservoir was provided by the company in charge of the management of urban waters (SERAGUAS S.A.) The river Miño flow was taken from the gauge station of Foz do Mouro, operated by the Portuguese Sistema Nacional de Informação de Recursos Hídricos (SNIRH).

**Statistical analysis**

Variables which did not comply with normality were logarithmically transformed. The Pearson coefficient was used to analyse correlations between bacterial community composition and both environmental and biological variables. Multivariate linear regression analysis was conducted in order to explore the relationship between bacterial community structure and both bacterial production and biomass.

**RESULTS**

**Meteorological conditions and hydrography**

The February cruise took place under upwelling-favourable (northerly) winds (-Qx = 379 m$^3$ s$^{-1}$, Table 1), whereas the October cruise took place during downwelling conditions (-Qx = -2036 m$^3$ s$^{-1}$, Table 1). There was a period of northerly/relaxed winds during the 10 previous days to the October cruise. Rainfall was abnormally low, almost nil, during the February cruise, and extremely high in October (Table 1), about twice the historical mean for the region, which would imply enhanced continental runoff. The measured flow from the river Oitabén-Verdugo was, on average, 5 m$^3$ s$^{-1}$ during the February cruise, and 28 m$^3$ s$^{-1}$ during the October cruise (Table 1). In addition, the mean river flow during the 10 days previous to the October cruise was as high as 42.6 m$^3$ s$^{-1}$,
considerably higher than the annual mean (15.9 m$^3$ s$^{-1}$). The measured flow from the river Miño did not differ between sampling periods (Table 1) and was similar to the annual mean (156 m$^3$ s$^{-1}$). The meteorological conditions were reflected in the hydrography of the region during each sampling period. In February, the water column was well mixed with temperatures averaging 13 ºC (Fig. 2A & C, Table 1) and salinities averaging 35.81. The prevailing northerly winds are responsible for the upwelling of subpolar Eastern North Atlantic Central Water (ENACW, salinity 35.69, temperature 12.3 ºC) over the shelf (Fig 2 A&C). By contrast, in October, an upper mixed layer of 20-60 m, with a mean temperature of 16.9 ºC and a mean salinity of 35.77, was observed. Below the thermocline the temperature decreased to 13.5 ºC and the salinity increased to 35.9 (Fig. 2). Strong rainfall before and during the October cruise together with the previous period of northerly/relaxed winds, which favor positive Ekman transport, supports the freshwater origin of this low-salinity surface water layer.

**Vertical distribution of nutrients, chlorophyll-a, DOC, and DOM fluorescence**

The vertical distribution of nutrient salts (Fig 3) reflected the structure of the water column. The nitrate, phosphate and silicate concentrations in the mixed layer were significantly lower in October than in February (T-test, p<0.001). There were no significant differences in the ammonium concentration between both sampling periods.

The vertical profiles of DOC were very distinct between the two sampling periods (Fig. 4A & B). Significantly higher values (T-test, p<0.001) and higher variability were recorded in October compared to February. The same pattern was observed in the vertical profiles of FDOM$_M$ and FDOM$_T$ (Fig 4E-H). In February, the inner shelf stations showed a relatively high FDOM$_M$ signal associated to a very low FDOM$_T$ signal, which is characteristic of nutrient-rich, DOC-poor ENACW. In
October, the mean FDOM$_M$ showed an increase with depth while DOC, and FDOM$_T$ decreased with depth. Maximum variability of these parameters occurred in the upper 60 m (Fig 4BF & H). Highest FDOM$_M$ values were observed at station 26, whereas highest FDOM$_T$ values were found at st 41. Chlorophyll-a concentration, did not present significant differences in mean values between the two sampling periods (Fig 4C & D).

**Vertical distribution of bacterial biomass and production**

Both bacterial biomass and production were significantly higher in October than in February (T-test, p<0.001) (Fig 5). In October, a great variability was observed in the upper 60 m for both variables, showing higher values at the shelf than at the offshore station. Maximum biomass and production occurs at stn 26.

**Vertical distribution of major bacterial groups**

On average, 63% of DAPI-stained cells were detected by the general probe for bacteria (Eub338), being this proportion higher in October (68%) than in February (58%). The total percentage of bacteria recovered by the 4 selected probes was, on average, 41%, being higher in October (49%) than in February (33%). The most abundant groups in the area were Alphaproteobacteria and Bacteroidetes, accounting, on average, for 8.5 and 10.5% of total DAPI-stained cells (13.5 and 16.5 % of total bacterial abundance), respectively. The least abundant groups, Gamma- and Betaproteobacteria, accounted for ~4 and ~1% of total DAPI counts (~7 and ~2% of total bacterial abundance), respectively. The relative abundance of Bacteroidetes did not differ significantly between sampling periods (T-test, p>0.05), whereas the relative abundance of Alphaproteobacteria was significantly higher in October (11%) than in February (6%) (T-test, p>0.01). The relative abundance of Betaproteobacteria (0.2 % of DAPI counts)
in February was below the detection limit (0.5% of DAPI counts). By contrast, the relative abundance of Betaproteobacteria ranged from 0.7 to 4.6 % of DAPI counts (1.1 to 8.6 % of total bacterial abundance) in October. The highest relative abundance of Betaproteobacteria occurred at stn 26. The relative abundance of Gammaproteobacteria was also significantly higher in October (10.5 % of total bacterial abundance) than in February (3.4 % of total bacterial abundance).

Relationship between major bacterial groups, chemical variables and bulk bacterial biomass and production

Using the entire data set, we conducted a correlation analysis to identify any potentially relevant chemical variable explaining the observed variability in the abundance of major bacterial groups (Table 2). None of the groups showed a significant correlation with ammonium or silicate. Both Betaproteobacteria and Gammaproteobacteria showed negative correlations with nitrate and phosphate. Only the Bacteroidetes groups correlated positively with chlorophyll-a. All the groups correlated positively with DOC, which similarly explained ca. 50 % of the observed variability in the relative abundance of Alpha-, Beta-, and Gammaproteobacteria, and only 18 % in the case of Bacteroidetes. All the groups showed positive correlations with both FDOMM and FDOMT, although explaining a variable percentage of their respective relative abundances. FDOMM explained only 11-32% of the variability in Alpha-, Gammaproteobacteria and Bacteroidetes, but as much as 68% in Betaproteobacteria. FDOMT, explained 40 % of the variability in the relative abundance of Alphaproteobacteria. All the groups showed positive correlations with both bacterial biomass and bacterial production (Table 2). We conducted a multi-variate linear regression using the bulk bacterial production and bacterial biomass as dependent variables and the abundance of the 4 major bacterial
groups as independent variables to explore the relationship between bacterial community structure and bacterial carbon fluxes (Table 3). Betaproteobacteria has the greatest influence on bacterial carbon fluxes, explaining, together with that of Gammaproteobacteria, 74 and 67% of bulk bacterial activity and biomass variability, respectively.

**DISCUSSION**

Over the last two decades, growingly efforts have been devoted to the analysis of DOM utilization by specific prokaryotic groups, following a variety of methodologies. Most of them implied the use of single compounds (leucine, glucose, N-acetylglucosamine, D- and L-Aspartic acid, chitin, ATP, etc) (Cotrell & Kirchmann 2000, Malmstrom et al. 2005, Teira et al. 2006, Alonso-Saéz & Gasol 2007) or different DOM components or fractions (e.g. low and high molecular weight fractions) (Covert & Moran 2001). The use of a single compound has the constraint that it may not model a given component of the DOM. On the other hand, the different size-fractions of DOM have a variable composition (Kirchman 2004). In addition, the isolation of a given component from natural DOM has several technical difficulties (very large sample volumes, $10^2$-$10^3$ liters) and limitations (changes in physical and chemical properties during the procedure) (Benner et al. 1992). A complementary approach comes from the combination of data on DOM composition and bacterial community structure. There are several direct chemical approaches to study DOM composition that do not involve isolation such as the analysis of elemental composition, the molecular characterization of DOM, or fluorescence spectroscopy which provide valuable information about the chemical nature (fluorescence functional groups) of DOM (Nieto-Cid et al. 2005).
To face our objective of linking changes in DOM optical properties and bacterial community structure we studied the coastal transition zone of NW Iberian Peninsula during the low productivity period (from October to March). This coastal zone is particularly interesting during this season since the sources of DOM can greatly vary (e.g. semi-labile DOM accumulated during the previous productive period, refractory DOM of terrestrial origin introduced via enhanced runoff) (Doval et al. 1997), which ensures a wide range of variation in DOM quantity and quality.

Primary production (~380 mg C m$^{-2}$ d$^{-1}$) and chlorophyll-a concentration (0.45 mg m$^{-3}$) were low and did not differ between both sampling periods (Teira et al. submitted). By contrast, rainfall was intense before and during the October cruise, and almost nil before and during the February cruise, and the mean flow from the River Oitabén, main tributary to the Ria de Vigo, was 5 to 8 fold higher in October than in February (Table 1). As a consequence, a higher influence of freshwater inputs was expected in October than in February. The hydrographic data support the influence of freshwater in October (Fig 2, for details see Teira et al, submitted). On the other hand, humic substances in the marine environment can be either of terrestrial origin or being generated in situ as a by-product of microbial respiration processes. Both are sensitive to natural UV radiation, which provokes intense photobleaching of these substances in the surface layer. Therefore, a vertical profile of humic-like fluorescence in a coastal area depends on the relative importance of continental runoff (that increases the fluorescence of surface waters), UV radiation intensity (that decreases the fluorescence of surface waters) and apparent oxygen utilisation (that increases the fluorescence of subsurface waters) (Nieto-Cid et al, 2005, 2006). Therefore, the high mean FDOM$_M$ measured in the upper layer (down to 60 m depth) in October (~3.1 ppb QS), as compared with the mean annual value reported for this area (~2.2 ppb QS, Nieto-Cid et al. 2005) reinforces the
hypothesis of a continental origin of surface humic-like DOM. Furthermore, the Betaproteobacteria group, which is typically abundant in freshwater environments (Methé et al. 1998, Bouvier & del Giorgio 2002, Kirchman et al. 2005) accounted for 2-9% of the total bacterial abundance in October, and were undetectable in February. The presence of these bacteria in marine waters has been typically regarded as an indicator of freshwater inputs. Garneau et al. (2006) also found a significant contribution of Betaproteobacteria (6% of DAPI counts) in a river-influenced offshore coastal Arctic station. The relative abundance of Betaproteobacteria in our study was not correlated to salinity, which was overall higher than 35.5, indicating an important dilution of the freshwater inputs along the coastal transition zone. Kisand et al (2008), demonstrated that the bacterial degradation of terrestrial humic might preferentially occur at high salinities (in polyhaline estuarine regions and coastal adjacent seas), with only a minor fraction of riverine DOC degradation occurring during the estuarine mixing.

Bacterial community structure and CARD-FISH: methodological considerations

Both the percentage of DAPI-stained cells detected by the general bacterial probe (Eub338) and the total percentage of bacteria identified with the set of probes were remarkably low in our study. Low detectability may be attributed to both a poor coverage of the oligonucleotide probes (see details in Amann & Fuchs 2008) or to a very low level of bacterial activity. The CF319a probe used for the quantification of Bacteroidetes has a coverage of 38%. This probe mainly fails to detect the class Bacteroides, which members are strictly anaerobic intestinal bacteria. Considering this, the coverage of the used probes, as assessed by Amann & Fuchs (2008) is reasonably good, ranging from 76 to 90%. On the other hand, the fact that the % of Eub338 positives is higher in October (68%) than in February (58%), in accordance with a
higher bacterial production, supports the second explanation. The sum of the 4 groups accounted for only 15 to 70% of total detected bacteria. A similar mean recovery rate (15 to 91%) was also obtained by Alonso-Sáez et al. (2008) in the Arctic, using CARD-FISH and the same set of probes.

**Major bacterial groups and DOM quality assessed by fluorescence**

The fact that the group Bacteroidetes did not show strong correlations with DOC, FDOM$_{M}$, and FDOM$_{T}$ (Table 2), agrees with the widely reported tendency of this group to proliferate during the decay of phytoplankton blooms (Pinhassi et al., 2004; Abell et al., 2005; Alderkamp et al., 2006; Teira et al., 2008). In fact, despite the very low levels of phytoplankton biomass found in the region during the downwelling period, only this group showed a significant positive correlation with chlorophyll-a.

The bulk DOC concentrations strongly correlated with Alpha-, Beta- and Gammaproteobacteria, explaining a similar percentage of their variability (ca. 50%). Therefore, the quantity of DOC does not explain the observed changes in the bacterial community structure. More interesting insights come from the analysis of the relationship between bacterial community structure and the fluorescence of DOM.

The group Alphaproteobacteria showed a strong positive correlation with the protein-like fluorescence (FDOM$_{T}$), explaining 40% of its variability. The FDOM$_{T}$ only explains 28% of the variability in Gammaproteobacteria and <17% in Betaproteobacteria and Bacteroidetes. The fluorescence of DOM at peak T has been found to be a tracer of labile dissolved amino acids (Yamashita & Tanoue 2003), which are liberated mainly during phytoplankton exudation, cell lysis and zooplankton grazing (Nagata 2000). Studies combining microautoradiography and Fluorescence in situ hybridization (Micro-FISH) and selected radiolabeled components of DOM, revealed
that Alphaproteobacteria (both SAR11 and *Roseobacter*) tend to dominate the
utilization of labile LMW-DOM (glucose and free amino acids) (Cotrell & Kirchmann
2000, Malmstrom et al. 2005, Alonso-Sáez & Gasol 2007) which agrees with our
results.

The strong correlation between Betaproteobacteria and FDOM$_M$ suggest the
potential immigrant nature of this group, and that they are likely living on allochthonous
DOM. The fact that this group is absent in February, when the continental runoff is
extremely reduced, further support the freshwater origin of this bacterial group. DOC of
freshwater origin in the coastal transition of the NW Iberian Peninsula is likely a minor
fraction of the bulk DOC, and thus it is not surprising that the bulk DOC similarly
explains the abundance of the three proteobacterial groups.

Pernthaler et al. (1998) showed that the abundance of Betaproteobacteria was
linked to the input of allochthonous organic carbon in lakes. It has been recently
demonstrated that only a few species of bacteria, belonging to Bacteroidetes and
Betaproteobacteria, are important players in the decomposition of riverine DOM in the
northern Baltic Sea (Kisand et al., 2002; Kisand and Wikner, 2003). Some subsequent
studies suggested that riverine bacterioplankton may successfully immigrate to seawater
and play an important biogeochemical role by decomposing allochthonous DOM
ultrafiltration to identify groups of estuarine bacteria using high- and low-molecular
weight fractions of surface DOM. They found that, Betaproteobacteria were only
present in the HMW enrichment. In surface waters, the HMW is composed mainly by
labile polysaccharides (Benner et al. 1992) and refractory humic substances, which
proportions may vary depending on environmental factors. For example,
polysaccharides accumulate after upwelling episodes in the NW Iberian upwelling
system (Nieto-Cid et al. 2004), whereas humic substances may accumulate in surface seawater as a result of intense freshwater discharge (Chen et al. 2002; Nieto-Cid et al. 2005). Covert & Moran (2001) speculated that HMW-DOM in their study was mainly composed by humic substances. Therefore our finding of a strong positive correlation between the relative abundance of Betaproteobacteria and FDOM\textsubscript{M} (Table 3), agree with the important presence of Betaproteobacteria on the HMW enrichment observed by Covert & Moran (2001).

**Bacterial community structure and bulk bacterial biomass and production.**

Although several recent studies suggest the influence of bacterial community composition, even as depicted from the abundance of major bacterial groups, on bacterial carbon cycling (Kirchman 2004, Bertilsson et al. 2007, Teira et al. 2008), the links between the distribution and abundance of bacterial taxa and ecosystem function is still poorly understood for natural microbial plankton communities. Multivariate linear regression analysis (Table 3) indicates that bacterial community structure explains a significant amount of the variation in bacterial production and biomass. It is remarkable that the most abundant groups, Alphaproteobacteria and Bacteroidetes, did not significantly explain variations in bulk bacterial activity or biomass. This is in agreement with several studies in both temperate and polar pelagic systems, which have shown that the most abundant groups are not the most active ones (Elifantz et al 2005, Alonso-Sáez & Gasol 2007, Malmstrom et al 2007, Alonso-Sáez et al 2008). Betaproteobacterial abundance was the most significant variable included in the regression models, suggesting a potentially relevant role of these bacteria in the carbon cycling during the sampling period. The strong positive correlation between Betaproteobacteria and the humic-like DOM fluorescence further support an important
contribution of allochthonous DOM remineralization to the carbon cycling in this coastal zone. A similar relationship between Betaproteobacterial abundance and bacterial production was also reported by Garneau et al. (2006) and Zhang et al (2006). Grossart et al. (2008) observed that both the abundance and the specific growth of Betaproteobacteria in lakes were largely controlled by the availability of humic substances. Bano et al. (1997) estimated that bacterial growth efficiency on humic substances was as high as 22%. Unfortunately, we do not have estimates of bacterial growth efficiencies, however, a highly efficient use of terrestrial humic substances by Betaproteobacteria, agree with its high contribution at explaining bulk bacterial production and biomass.

To summarize, a strong relationship has been found in the present study between the Alpha- and Betaproteobacteria groups and DOM quality as assessed by fluorescence analysis. Whereas the bulk DOC concentration does not provide relevant information to explain the variability in the relative abundance of different major groups, humic- and protein-like substances fluorescence provided insightful information. The humic substances explained 68% of the variability on Betaproteobacterial abundance, whereas the protein-like substances explained 40 % of the variability on Alphaproteobacterial abundance. Moreover, the abundance of Betaproteobacteria was the dominant variable in the regression models for bacterial production and biomass, which explained, respectively, 74 and 67 % of the total observed variability. Our observations, strongly suggest, despite its relatively low abundance, a potentially relevant biogeochemical role of this bacterial group in the coastal-transition zone of NW Iberian Peninsula during the low productivity period, as allochthonous-DOM consumers.

Although it is difficult to extrapolate from correlation analysis to causal relationships, our data add evidence to the rising conception that members of major
bacterial groups (Alphaproteobacteria, Betaproteobacteria, Bacteroidetes) could actually represent functionally relevant units in aquatic environments (Kirchman et al. 2005, Bertilsson et al. 2007, Teira et al. 2008).

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We thank all the colleagues involved in the project ZOTRACOS who helped with the preparation and sampling of the two cruises, particularly the principal investigator R. Varela. Special thanks to J.M. Cabanas who provided the meteorological data. We also would like to thank the captain and crew on board the RV Cornide de Saavedra. This research was supported by the CICyT contract nº REN2003-06633 (ZOTRACOS). E.T. was funded by a European Community Marie Curie Reintegration Fellowship (MERIC-CT-2004-511937) and a Juan de la Cierva-MEC contract.
LITERATURE CITED


Table 1. Summary of mean meteorological and hydrological conditions during the two sampling periods.

<table>
<thead>
<tr>
<th>Variable</th>
<th>February 2005</th>
<th>October 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Qx (m$^3$ s$^{-1}$ Km$^{-1}$)</td>
<td>379</td>
<td>-2036</td>
</tr>
<tr>
<td>Precipitation (mm)</td>
<td>0.4</td>
<td>105.0</td>
</tr>
<tr>
<td>River Miño flow (m$^3$ s$^{-1}$)</td>
<td>157</td>
<td>148</td>
</tr>
<tr>
<td>River Oitabén flow (m$^3$ s$^{-1}$)</td>
<td>5.0</td>
<td>28</td>
</tr>
<tr>
<td>Mixed Layer (ML) (m)</td>
<td>40-140</td>
<td>20-60</td>
</tr>
<tr>
<td>ML Temperature (°C)</td>
<td>13.0</td>
<td>16.9</td>
</tr>
<tr>
<td>ML Salinity</td>
<td>35.81</td>
<td>35.77</td>
</tr>
</tbody>
</table>
Table 2. Pearson correlation coefficients between the abundance of major bacterial groups (expressed as % of DAPI-stained cells) and several chemical and biological variables. FDOM$_M$, fluorescence at peak M; FDOM$_T$, fluorescence at peak T; Chla, chlorophyll-a concentration; DOC, dissolved organic carbon concentration; BP, bacterial production; BB, bacterial biomass; ALPHA, Alphaproteobacteria, BETA, Betaproteobacteria, GAMMA, Gammaproteobacteria; and CFB, Bacteroidetes.

Numbers in bold represent correlations higher than 0.6.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>NH$_4$</th>
<th>NO$_3$</th>
<th>PO$_4$</th>
<th>SiO$_4$</th>
<th>Chla</th>
<th>DOC</th>
<th>FDOM$_M$</th>
<th>FDOM$_T$</th>
<th>BP</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALPHA</td>
<td>47-50</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.713</td>
<td>0.567</td>
<td>0.633</td>
<td>0.600</td>
<td>0.520</td>
<td></td>
</tr>
<tr>
<td>BETA</td>
<td>47-50</td>
<td>NS</td>
<td>-0.506</td>
<td>-0.423</td>
<td>NS</td>
<td>0.703</td>
<td>0.825</td>
<td>0.411</td>
<td>0.758</td>
<td>0.754</td>
<td></td>
</tr>
<tr>
<td>GAMMA</td>
<td>47-50</td>
<td>NS</td>
<td>-0.427</td>
<td>-0.399</td>
<td>NS</td>
<td>0.714</td>
<td>0.525</td>
<td>0.534</td>
<td>0.686</td>
<td>0.618</td>
<td></td>
</tr>
<tr>
<td>CFB</td>
<td>47-50</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.386</td>
<td>0.428</td>
<td>0.333</td>
<td>0.389</td>
<td>0.428</td>
<td>0.330</td>
</tr>
</tbody>
</table>

***, p<0.001; **, p<0.01; *, p<0.05
Table 3. Linear regression analysis of the contribution of the major bacterial groups to the variability in bacterial production (BP) and bacterial biomass (BB). NS, not significant. Standardized coefficients measure the contribution by the bacterial group to explaining variation in the dependent variables.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>N = 48</th>
<th>Standardized coefficients</th>
<th>Adjusted</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alpha</td>
<td>Beta</td>
<td>Gamma</td>
</tr>
<tr>
<td>BP</td>
<td>NS</td>
<td>0.614</td>
<td>0.318</td>
<td>NS</td>
</tr>
<tr>
<td>BB</td>
<td>NS</td>
<td>0.645</td>
<td>0.318</td>
<td>NS</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. A) Map of the study area showing the locations of the stations sampled during the cruises conducted in February and October 2005.

Figure 2. Vertical distribution of temperature (A, B) and salinity (C, D) in February 2005 (A, C) and October 2005 (B, D). Black and open symbols represent shelf and offshore stations, respectively.

Figure 3. Vertical distribution of ammonium (A, B) nitrate (C, D), phosphate (E, F), and silicate (G, H) in February 2005 (A, C, E, G) and October 2005 (B, D, F, H). Black and open symbols represent shelf and offshore stations, respectively.

Figure 4. Vertical distribution of DOC (A, B), chlorophyll-a (chla) (C, D), humic-like DOM fluorescence, FDOM\textsubscript{M} and (E, F), protein-like DOM fluorescence, FDOM\textsubscript{T} (G, H) in February 2005 (A, C, E, G) and October 2005 (B, D, F, H). Black and open symbols represent shelf and offshore stations, respectively.

Figure 5. Vertical distribution of bacterial biomass (BB) (A, B) and bacterial production (BP), (C, D) in February 2005 (A, C) and October 2005 (B, D). Black and open symbols represent shelf and offshore stations, respectively.

Figure 6. Vertical distribution of the relative abundance (expressed as % of DAPI-stained cells) of Alphaproteobacteria, ALPHA (A, B), Betaproteobacteria, BETA (C, D), Gammaproteobacteria, GAMMA (E, F) and Bacteroidetes, CFB (G, H) in February 2005 (A, C, E, G) and October 2005 (B, D, F, H). Black and open symbols represent shelf and offshore stations, respectively.
Fig. 2

A

B

C

D

Temperature (ºC)

Salinity

Depth (m)
Fig. 3

Ammonium (μM)

Nitrate (μM)

Silicate (μM)

Phosphate (μM)

A

B

C

D

E

F

G

H

0
20
40
60
80
100
120
140

0.0
0.5
1.0
1.5

0 0.5 1.0 1.5

0 2 4 6 8 10 12

0 2 4 6 8 10

0.0 0.2 0.4 0.6 0.8 1.0

0 1 2 3 4 5 6 7
Fig. 5

![Graphs showing variations in BB (μg C L⁻¹) and BP (μg C L⁻¹ d⁻¹) across different depths.](image)

**A** and **B** illustrate the changes in BB across various depths, with different symbols representing different samples (e.g., 48, 46, 44, 41, 39, 37, 26, 39, 41, 43, 43, 10).

**C** and **D** depict similar changes in BP, with similar sample representations.
Fig. 6

A) ALPHA (%DAPI) vs. Depth (m)
B) BETA (%DAPI) vs. Depth (m)
C) GAMMA (%DAPI) vs. Depth (m)
D) CFB (%DAPI) vs. Depth (m)