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Partitioning of phytoplanktonic organic carbon production and bacterial production along a coastal-offshore gradient in the NE Atlantic during different hydrographic regimes

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ABSTRACT: The rates of primary production of particulate (POC-pr) and dissolved organic carbon (DOC-pr), production of heterotrophic bacteria (BHP), and the abundance of autotrophic and heterotrophic picoplankton were estimated on 2 cruises in NE Atlantic coastal waters off the Iberian peninsula. Downwelling conditions prevailed during the spring cruise (1997), while a sequence of marked upwelling-relaxation-weak upwelling was found in late summer (1998). The standing stocks and activities of phytoplankton and bacteria displayed coastal-offshore gradients in both sampling periods. Whereas DOC-pr was similar on both cruises, POC-pr was generally higher in late summer, yielding lower values of percent extracellular release (PER): 6 ± 5% versus 9 ± 4% (mean ± SD) in spring. Short-term changes in hydrographic conditions strongly affected the relative release of photosynthate in late summer. PER tended to decrease with increasing production, with values below 5% at rates higher than 3 mg C m^{-3} h^{-1}. Chlorophyll *a* (chl *a*) normalized DOC-pr (DOC^B-pr) also increased significantly the higher the contribution of picoplankton (<3 µm) to total chl *a* concentration, confirming the importance of the size distribution of algae in the relative rates of extracellular release. Although BHP was significantly correlated with chl *a*, it bore no relationship to primary production rates, either dissolved or particulate, and released DOC was insufficient to meet estimates of bacterial demand of labile carbon, indicating that the activities of bacterioplankton and phytoplankton were uncoupled during the surveys. These results suggest that bacteria were mostly independent of algal DOC for growth in this coastal system.

KEY WORDS: Primary production · Percent extracellular release · Bacterial production · Phytoplankton-bacterioplankton coupling · Upwelling · Short-term changes

INTRODUCTION

Planktonic primary production is often considered as equivalent to particulate primary production (i.e. the amount of newly produced organic matter that is retained within the autotrophic organisms) although it is now well established that it also comprises an extracellularly released fraction (Mague et al. 1980, Fogg 1983) or dissolved primary production. These 2 fractions are ecologically very different. Whereas the photosynthesized particulate organic carbon (POC) is usually channelled by zooplankton to other trophic levels within the food web, the dissolved organic carbon (DOC) released by algae can be readily taken up by bacteria to fuel their growth and metabolism (Wolter 1982, Brock & Clyne 1984, Søndergaard et al. 1985, Norrman et al. 1995). The contribution of the released

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photosynthate to total primary production, commonly expressed as PER (percent extracellular release), is thought to be below 20% for most natural situations (Baines & Pace 1991, Nagata 2000). However, considerably higher PER values have been reported under special conditions, such as nutrient, irradiance or temperature stress (Zlotnik & Dubinsky 1989, Wood et al. 1992) or at the end of algal blooms (Lancelot & Billen 1984) and researchers have limited knowledge on how DOC production rates and PER change on short-term or seasonal scales (e.g. Lignell 1990). Among the factors affecting PER (see Nagata 2000 for a review), one of the most frequently claimed is the nutritional status of the algae (or its proxy, water nutrient concentrations), and thus, higher PER with increasing nutrient deficiency has been demonstrated in some studies (Myklestad 1977, Lancelot 1983, Obernosterer & Herndl 1995).

To test the effect of nutrient supply on the intra- or extracellular allocation of recently fixed photosynthate in natural phytoplankton communities, we chose a coastal environment affected by periodic, seasonal upwelling events. The effect of sporadic changes in the nutrient field could then be compared to the background of a coastal-offshore gradient of decreasing productivity. Experiments were performed in the vicinity of the Ría de Vigo, one of the Rías Baixas, coastal embayments located on the NW coast of the Iberian Peninsula. The continental shelf and shelf-break of the NW Iberian coast are affected by seasonal, wind-driven upwelling (usually from April to September) and downwelling (from October to March) events (Blanton et al. 1984, Álvarez-Salgado et al. 1993, Castro et el. 1997). During the periods of upwelling (usually lasting 10 to 15 d; Álvarez-Salgado et al. 1999), high primary production rates are commonly reported (Tenore et al. 1995, Hanson et al. 1986, Bode et al. 1996, Tilstone et al. 1999) as a result of nutrient fertilization. Except for a very recent study providing estimates of dissolved primary production during summer and autumn (Teira et al. 2001a), primary production surveys in this part of the Atlantic have focused only on the particulate fraction. These authors reported a considerable variability of PER in the region (from 3 to 60%), with different means for the photic zone in autumn (33%) and under summer upwelling conditions (6%). However, variability in dissolved primary production at the short-time scales of the upwelling pulses remains to be explored. Variations in the bulk DOC pool in shelf waters have been measured following changes in primary production (Álvarez-Salgado et al. 1999) suggesting that part of this DOC variability might be caused by changes in the rates of DOC release by primary producers.

The relative high concentrations of labile DOC near the NW Iberian coast (Doval et al. 1997, Álvarez-Salgado et al. 1999) might indicate that heterotrophic bacteria are not limited by general carbon availability; however, this hypothesis remains largely untested partially due to the scarce number of reports on bacterial abundance and activity published for this region (Hanson et al. 1986, Zdanowski & Figueiras 1997, Barbosa et al. 2001). In addition, the nutritional quality of the different DOC fluxes entering the system may markedly affect the bacterial responses. Specifically, growing bacteria very likely prefer recently released photosynthate (dissolved primary production) to other organic compounds present in the water (Coffin et al. 1993, Norrman et al. 1995), either refractory or requiring previous enzymatic degradation. The general coastal-offshore gradient in the concentration of inorganic nutrients in the study area is also paralleled by the contribution to the DOC pool of allochthonous sources, which becomes higher near the Ría de Vigo estuary; therefore, the strength of the possible trophic linkage between algae and bacteria via algal DOC or phytoplankton-bacterioplankton coupling could be expected to increase ocean-wards (Morán et al. 2001).

Here, we report results of photosynthesized organic carbon partitioning and bacterial production, and estimate the potential of algal-derived DOC in meeting bacterial carbon demand during 2 cruises aimed at meeting different hydrographic scenarios. The spring cruise was characterized by fairly constant downwelling conditions, while in late summer both intense upwelling and upwelling relaxation phases were encountered. Late summer short-term variability in hydrographic conditions allowed us to assess its effect on the relative contribution of dissolved primary production to total rates.

MATERIALS AND METHODS

Sampling strategy. Experiments were performed on board of the RV 'Cornide de Saavedra' during the INCOCEANO-1 (spring 1997, from 27 April to 3 May) and INCOCEANO-2 (late summer 1998, from 3 to 14 September) cruises. Three transects from 41.9 to 42.43°N perpendicular to the Galician coast (NW Spain) were repeatedly sampled (4 to 6 times) in order to measure diverse physico-chemical and biological variables. We carried out our experiments at stations located on the central transect (42.15°N) from the Ría de Vigo estuary to open ocean (>2000 m depth) waters (Fig. 1). Stations are hereafter referred to as deep (D_1 and D_2), slope (Sl), shelf (Sh) or Ría (R). Except for Stns Sl and Sh in spring, which were also sampled once at 30 and 10 m depth, respectively, all experiments were conducted with surface (5 m) water. This could reduce the chances of detecting downwelling/upwelling

effects. Water was taken from Niskin bottles attached to a hydrographic cable in spring and to a rosette-CTD system in late summer. Samples for analyzing dissolved inorganic nitrogen (DIN, $NO_2^- + NO_3^- + NH_4^+$), phosphate and silicate were immediately frozen and their concentrations determined on land with a Technicon autoanalyzer using the standard protocols of Hansen & Grasshoff (1983) with some minor modifications (Mouriño & Fraga 1985, Álvarez-Salgado et al. 1992).

Biomass and abundance of phytoplankton and heterotrophic bacteria. Chlorophyll *a* concentration (chl *a*) was measured fluorometrically in 90% acetone extracts of filters kept at 4°C for 24 h. At the start of the experiments, samples of 30 to 200 ml were filtered through 0.22 µm Millipore membrane (mixed cellulose esters) filters for in order to estimate total chl *a*. In most experiments, parallel samples were size fractionated by first filtering them through 1.2 µm Millipore membrane (spring) or 3 µm (late summer) filters and then recovering the filtrate onto 0.22 µm filters of the same type. The sum of the 2 fractions was not significantly different from the direct estimation of total chl *a* (paired *t*-tests, $p = 0.21$ in spring, $p = 0.58$ in late summer).

Samples for the determination of the abundance of heterotrophic bacterioplankton, cyanobacteria (*Synechococcus* spp. and *Prochlorococcus* spp.) and small photosynthetic eukaryotes were taken at each station. Samples (1.2 ml) were immediately fixed with 1% paraformaldehyde + 0.05% glutaraldehyde solution and stored frozen in liquid N_2 until analysis. Samples were counted with a FACSCalibur (Becton & Dickinson) flow cytometer, equipped with a laser emitting at 488 nm. Samples were stained with 2.5 µM Syto 13 (Molecular Probes) to detect heterotrophic bacteria. Aliquots of a solution of fluorescent 0.96 µm Polysciences latex beads, previously counted by epifluorescence microscopy, were added as internal standards. *Synechococcus* and *Prochlorococcus* fluorescence could be easily identified in plots of side scatter (SSC) versus FL2 (orange fluorescence) and FL3 (red fluorescence). Up to 10 000 events were acquired at high flow rate (~60 µl min^{-1}) in every sample. Two groups of heterotrophic bacteria, which were distinguished according to their relative FL1 signal (green fluorescence), were called high DNA (HDNA) and low DNA (LDNA) bacteria. HDNA bacteria generally correspond to the most active fraction of the total community (Gasol et al. 1999, Lebaron et al. 2001). The biovolume of heterotrophic bacteria was estimated following the algorithm described in Gasol & del Giorgio (2000), based on relative DNA fluorescence.

Partitioning of phytoplanktonic organic carbon production. Phytoplanktonic production of POC and DOC was estimated from the results of kinetic experiments of ^{14}C incorporation (Morán et al. 2001, Morán & Estrada 2002). Water samples (70 ml) were placed into light (transparent) and dark (aluminum foil-covered) sterile polystyrene tissue culture bottles (Corning), and spiked with 1.62 to 6.82×10^5 Bq (4.39 to 18.43 µCi) of ^{14}C-bicarbonate (VKI). Samples were incubated on deck under *in situ* simulated light conditions in a flowing-seawater bath at surface temperature, and 4 light and 4 dark bottles were taken at intervals for processing. Four dark bottles (t_0 bottles) were immediately processed at the beginning of each experiment. Aliquots of 5 ml were taken from 2 light and 2 dark bottles and placed in 20 ml scintillation vials for determination of labeled total organic carbon (TOC); the remaining 65 ml were filtered onto 0.22 µm membrane filters for determination of total labelled POC. Ideally, this fraction would include all photosynthetically produced POC contained in algal biomass plus the bacterial POC resulting from bacterial uptake of DOC

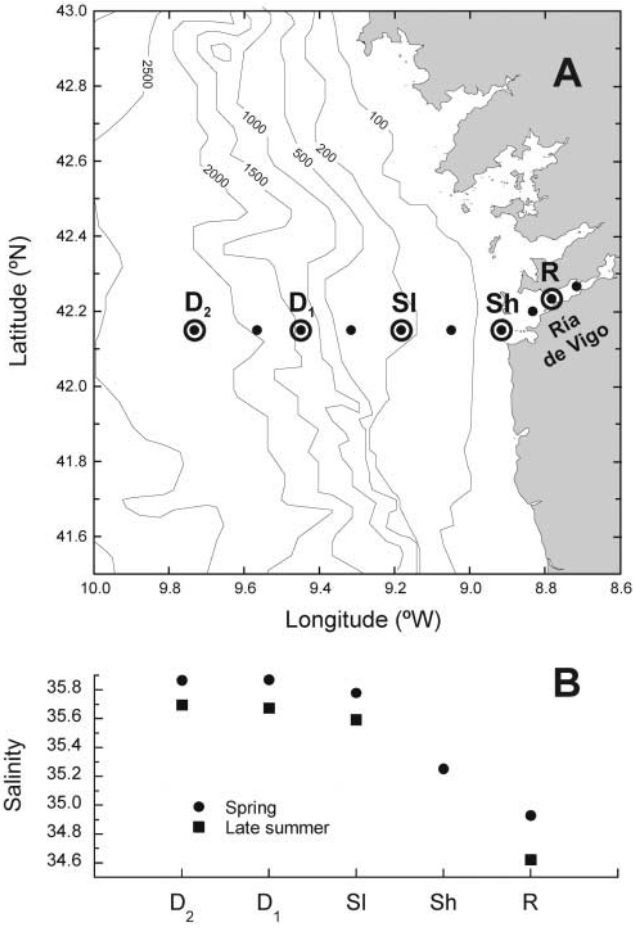

Fig. 1. (A) Southern part of Galician coast (NW Iberian Peninsula) showing the position of the stations sampled during the INCOCEANO cruises. Stations where POC and DOC primary production was estimated are surrounded with circles. (B) Average surface salinity at the sampled stations

released by algae. From the remaining 2 light and 2 dark bottles, 5 ml aliquots were also filtered through 0.22 µm filters and the filtrate collected for determination of labeled DOC. The remaining 65 ml were filtered onto membrane 1.2 µm filters (spring) or 3 µm filters (late summer) for determination of labeled POC greater than each filter pore size. This filtration was intended to retain most phytoplanktonic POC, but also to let through most $PO^{14}C$ due to heterotrophic bacterial uptake of $DO^{14}C$. During late summer, in order to check for autotrophic production of the fraction smaller than 3 µm, 4 additional bottles were filled with pre-filtered (<3 µm) water. Two bottles were processed at t_0, and the other 2 were incubated in parallel and filtered at the end of the kinetic experiments. Differential pressure was kept below 80 mm Hg in all filtrations to prevent cell breakage.

Filters were put into 6 ml scintillation vials, which were left open and fumed with concentrated HCl (35%) for ca. 12 h before the addition of 4.5 ml of Packard Ultima Gold XR liquid scintillation cocktail. Liquid samples (with labeled TOC or DOC) were acidified with 1 ml 1 M HCl and bubbled with air for a minimum 0.5 h before addition of 10 ml scintillation cocktail. Radioactivity (dpm) was measured on land in a Beckman LS6000LL liquid scintillation counter with the internal standard method. Dark bottle values throughout the experiments were comparable to t_0 values. The latter were subtracted from subsequent samples for correction of non-photosynthetic ^{14}C incorporation.

Compartmental carbon exchange models were used for obtaining steady state rates of production of POC, DOC and TOC (see Morán et al. 2001, Morán & Estrada 2002). Least-squares model fitting was performed with SAAM II software for kinetic analyses (SAAM Institute, Washington). The outputs of the model are fractional constant rates of C flux between compartments (h^{-1}), which were converted to DOC and POC production rates (mg C m^{-3} h^{-1}) using the average concentration of dissolved inorganic carbon (DIC) measured at each station (X. A. Álvarez-Salgado et al. unpubl. results). No isotopic discrimination factor was considered for the conversion to carbon units. Unexpectedly, the production rates of POC of the size fractions greater than 1.2 and 3 µm were not significantly lower than that greater than 0.22 µm. Thus, herein and to allow comparison of both cruises, for POC, we will always refer to POC greater than 0.22 µm. In the experiments with pre-filtered water (late summer), the percentage of POC production due to cells <3 µm was as low as 7.5 ± 4.7% on average. PER was calculated as the ratio of DOC production rate to the sum of POC and DOC production rates times 100.

Bacterial heterotrophic production. Bacterial heterotrophic production (BHP) was estimated by ^3H-leucine incorporation into bacterial protein as described in Smith & Azam (1992). Commercial leucine solution (Amersham) was brought to 1 µM with 0.2 µm filtered and autoclaved MilliQ water, and diluted 10 times with non-radioactive leucine. Next, 40 nM leucine (chosen after 2 saturation curves done in different water) was added to 1.2 ml seawater samples in Eppendorf vials. Four replicates and 2 immediately killed controls were always incubated. The vials were incubated in the dark at *in situ* temperatures. Incubations lasted between 1 and 3 h following 3 linearity experiments. Control and incubated samples were killed with 50% TCA (5% TCA, final concentration). Once killed, samples were stored at ambient temperature until processing as in Smith & Azam (1992). To each vial, 1 ml of scintillation cocktail was added before being counted in a Beckman LS6000LL liquid scintillation counter. Dpm were calculated with the internal standard method. Finally, BHP was calculated from leucine incorporation rates by multiplying it by an appropriate empirical conversion factor (CF). During late summer, CFs were empirically determined once at each station following common procedures (Kirchman & Ducklow 1993), showing an increasing offshore-inshore gradient ranging from 0.67 to 3.55 kg C mol Leu^{-1} (C. Pedrós-Alió & J. M. Gasol unpubl. results). These empirical values for the outermost stations (Sl, D_1 and D_2) were similar to those reported by Barbosa et al. (2001) for the same area in August (0.14 to 0.77 kg C mol Leu^{-1}). We applied the same factors for each of the spring cruise stations. For estimating the total flux of organic carbon through heterotrophic bacteria, or bacterial carbon demand (BCD), we used 2 approaches. In the first one (BCD_{model}), we used the empirical regression model of del Giorgio & Cole (1998) relating bacterial respiration and production; hence, making it possible to derive bacterial growth efficiency (BGE) from BHP values. BGE values thus obtained for our experiments ranged from 2 to 27%. In the second approach ($BCD_{0.15}$), we used a fixed BGE of 15% for all experiments. This value is close to the mean 16% found by Barbosa et al. (2001) for 2 experiments conducted in the same region in August. We also empirically calculated 16% at Stn D_2 in spring (X. A. Álvarez-Salgado & J. M. Gasol unpubl. results). Except for the experiments carried out at Stn R during late summer, the BGE values from del Giorgio & Cole's (1998) model were always lower than 15%; hence, BCD_{model} values are regarded as a maximum estimate of the amount of organic carbon processed by heterotrophic bacteria.

Except for time-course ^{14}C-uptake data fitting, all statistical analyses were performed with STATISTICA software. We performed 2-way ANOVAs for several variables with cruise and area as factors. Stations were grouped into 2 areas: 'coastal' (R, Sh and Sl) and 'off-

shore' (D_1 and D_2). For the late summer cruise, we used the average of the 3 experiments performed at Stn R from 6 to 7 September. With the exception of percentages, data were log-transformed for attaining normality and homoscedasticity in the correlation and regression analyses. Linear regressions were calculated following Model I, with Model II also being used when slope values were critical for inferring conclusions (Ricker 1973, 1975).

RESULTS

Hydrography and nutrients

Stable downwelling conditions were found during the spring cruise (Fig. 2A), with a marked salinity front in the inner slope, caused by the northward flux of the subtropical waters carried by the Portugal Coastal Counter Current (B. M. Míguez et al. unpubl.). In contrast, notable changes in the hydrographic conditions were observed during the late summer sampling (B. M. Míguez et al. unpubl.), with a sequence of intense coastal upwelling until 5 September 1998 (Fig. 2B), followed by upwelling relaxation from 5 to 10 September (Fig. 2C), and resumption of a weaker upwelling event from 10 September to the end of the sampling period (Fig. 2D). Average dissolved inorganic nitrogen and phosphate concentrations at the sampled stations are given in Table 1. The short-term hydrographic changes in late summer were accompanied by nutrient injections at shallow depths, detected mainly at Stn Sh during the first upwelling event, and a subsequent decrease in nutrient concentrations (see below) partially reversed by the late upwelling episode (F. G. Figueiras et al. unpubl.). In spite of this variability, the effect of the Ría de Vigo estuary was strong enough to show a clear ocean-wards increase in salinity along the sampled transect (Fig. 1). Therefore, salinity was chosen as the inshore-offshore gradient descriptor. Many variables of planktonic biomass and production, such as the concentration of chl *a*, also presented inshore-offshore patterns (Fig. 3).

Biomass and abundance of phytoplankton and heterotrophic bacteria

Albeit chl *a* also varied between consecutive samplings (Fig. 3), it decreased significantly in both cruises with distance from the shore, with coastal values being ca. 4 times greater than offshore ones (2-way ANOVA, area, $p = 0.02$). The contribution of picophytoplankton to total chl *a* was generally below 20%, as shown by the percentage of chl *a* passing through 1.2 µm (spring,

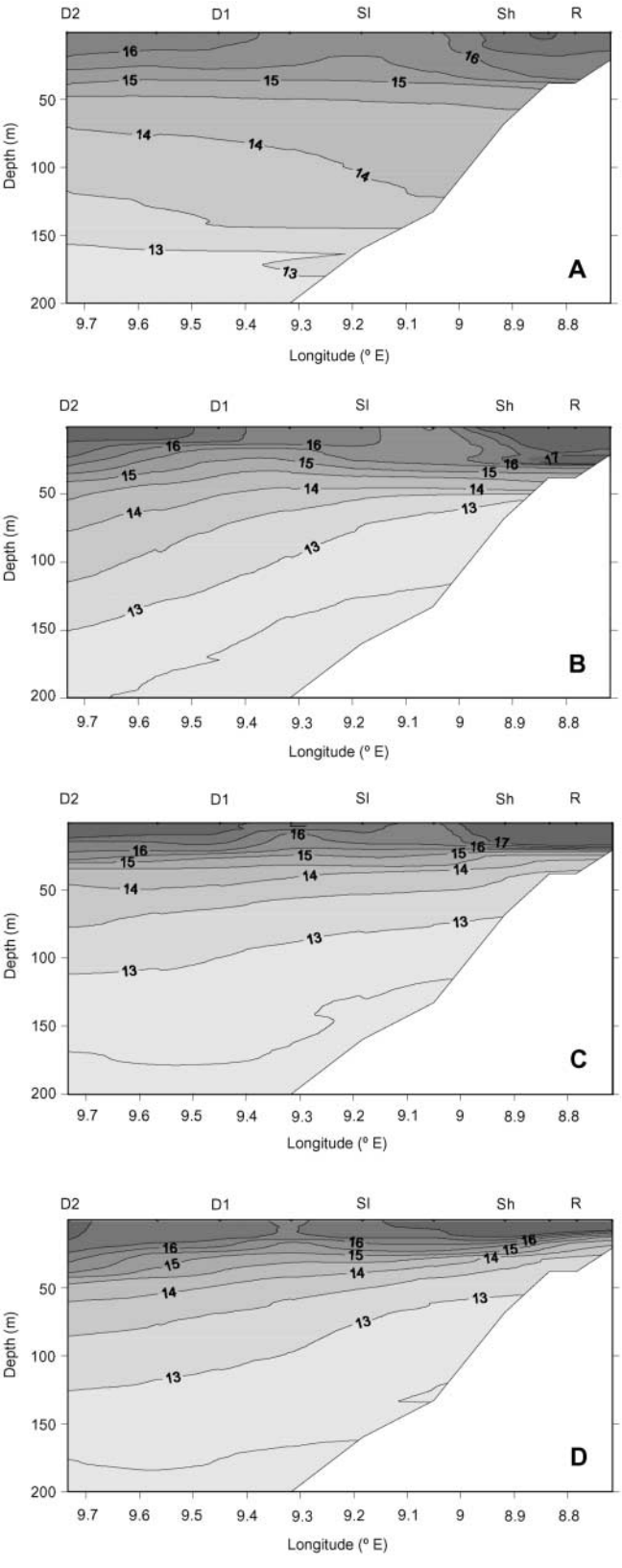

Fig. 2. Vertical distribution of temperature (ºC) across the sampled transect. (A) Spring 1997, 26 to 27 April. (B), (C) and (D) Late summer 1998, 2 to 3, 7 to 8 and 11 to 12 September, respectively

Table 1. Average (SE) autotrophic and heterotrophic picoplankton abundances and nutrient concentrations at the stations sampled. DIN, dissolved inorganic nitrogen; PO_4, phosphate; Pico chl *a*, percentage of total chlorophyll *a* smaller than 1.2 µm (spring) and 3 µm (late summer); Syne, *Synechococcus* sp.; Proc, *Prochlorococcus* sp.; BN, heterotrophic bacteria; HDNA, percentage of high DNA heterotrophic bacteria. –: only 1 measurement

Stn	Sampling date	DIN (µM)	PO_4 (µM)	Pico chl *a* (%)	Syne (10^4 cells ml^{-1})	Proc (10^4 cells ml^{-1})	BN (10^6 cells ml^{-1})	HDNA (%)
Spring	**April–May 1997**							
D_2	27, 28	0.63	0.02	13	3.12	5.17	0.96	50
		(0.07)	(0.01)	(0)	(0.39)	(2.07)	(0.04)	(4)
D_1	29	0.18	0.02	13	8.60	35.42	0.68	33
		–	–	–	–	–	–	–
Sl	30[a]	0.21	0.02	7	4.37	1.07	1.00	49
		(0.01)	(0.00)	(2)	(1.27)	(0.66)	(0.07)	(5)
Sh	01[b], 03	0.48	0.09	14	2.71	0	1.27	59
		(0.22)	(0.03)	(4)	(0.07)	(0)	(0.14)	(7)
R	02	0.49	0.10	11	27.32	0	2.96	75
		–	–	–	–	–	–	–
Late summer	**September 1998**							
D_2	04,13	0.48	0.03	15	15.35	8.10	2.27	33
		(0.06)	(0.00)	–	(1.28)	(2.26)	(0.56)	(4)
D_1	03, 08, 09, 13	0.29	0.04	23	25.15	8.61	2.40	36
		(0.12)	(0.03)	(3)	(4.32)	(5.00)	(0.44)	(6)
SI	05, 10, 12, 14	0.84	0.06	15	29.45	0.73	2.03	30
		(0.39)	(0.03)	(4)	(7.11)	(0.43)	(0.37)	(2)
R	06, 06, 07, 11	1.81	0.18	10	0.29	0	1.97	75
		(0.54)	(0.03)	(4)	(0.12)	(0)	(0.16)	(6)

[a]5 and 30 m sampled. [b]5 and 10 m sampled

mean 12%) and 3 µm filters (late summer, mean 16%) (Table 1). In late summer, this percentage decreased with increasing total chl *a* ($r = -0.81$, $p = 0.007$, $n = 9$). The abundance of autotrophic picoplankton tended to be higher offshore than at the coast, especially under the upwelling conditions prevailing in late summer (Table 1). *Prochlorococcus* was generally detected only at the outermost stations (2-way ANOVA, area, $p = 0.02$). Except at Stn R, located within the Ría, *Synechococcus* abundance was higher in late summer (~10^5 cells ml^{-1}) than in spring (~10^4 cells ml^{-1}).

The abundance of heterotrophic bacteria (BN) in the experiments was generally above 10^6 cells ml^{-1}, with higher numbers in the coastalmost stations during spring (Table 1). Short-term variability in upwelling conditions markedly affected BN in late summer, precluding the appearance of any clear gradient. Overall, higher abundances were found during late summer (2-way ANOVA, cruise, $p = 0.012$). The percentage of high DNA cells was rather variable, with mean values slightly higher in spring (53%) than in late summer (45%) (Table 2). During spring BN was positively correlated with the percentage of HDNA cells ($r = 0.82$, $p = 0.007$, $n = 9$) but not during late summer. Bacterial biovolumes (data not shown) were slightly higher at Stn R (>0.070 $µm^3$) than at the rest of the stations, but were similar for both sampling periods. However, while in spring the mean bacterial biomass was much higher at coastal stations (33.1 mg C m^{-3}) than offshore (15.5 mg C m^{-3}), values were much more similar in late summer (36.2 and 40.5 mg C m^{-3}, respectively).

Partitioning of phytoplanktonic organic carbon production

Phytoplanktonic POC and DOC production rates obtained with the above-described time-course experiments should lie closer to gross than to net rates, because losses of linearity in labeled POC (due to respiration) or DOC (due to bacterial utilization) are accounted for in the compartmental model used (Morán & Estrada 2002). Following a distribution similar to that of chl *a*, the rate of particulate primary production (POC-pr) generally increased towards the coast as indicated by its negative correlation with salinity ($r = -0.46$, $p = 0.037$, $n = 21$), while the rate of dissolved primary production (DOC-pr) did not show any significant trend. As expected from the higher chl *a* found during the second cruise (Fig. 3), POC-pr was generally higher in late summer (Table 2), with the

exception of a notably high value measured at Stn R in spring (20 mg C m^{-3} h^{-1}). If this station is excluded, DOC-pr was similar in both periods, with an average value equalling 0.26 mg C m^{-3} h^{-1}.

To better compare different values of dissolved primary production, DOC-pr can be normalized with respect to algal biomass as estimated by chl *a* (i.e. DOC^B-pr) or to the total primary production rate (i.e. PER). Both variables, DOC^B-pr and PER, were significantly correlated for the whole data set (r = 0.54, p = 0.010, n = 0.22). DOC^B-pr tended to increase offshore (correlation with salinity: r = 0.58, p = 0.006, n = 21). While chl *a* normalized POC-pr (POC^B-pr) was similar during both cruises (Table 2), with mean values of 4.70 and 5.73 mg C (mg chl *a*)$^{-1}$ h^{-1}, mean spring DOC^B-pr was 1.6-fold greater than the late summer value (0.44 and 0.27 mg C (mg chl *a*)$^{-1}$ h^{-1}, respectively). This difference was significant (2-way ANOVA, cruise, p = 0.021). Except on 3 occasions, PER values were always below 15% (Table 2), with mean values of 9% in spring and 6% in late summer.

As a consequence of the hydrodynamic changes observed during the late summer sampling, a notable short-term variability in primary production rates was found in subsequent visits to some stations. Fig. 4 shows the effect that the sequence of strong upwelling - upwelling relaxation - upwelling resumption had on PER and DOC^B-pr at Stns D_1 and Sl, both sampled 4 times during the cruise. The upwelling relaxation, detected as ~1°C warming of surface water, was followed by marked increases in the relative rates of dissolved primary production from 2% PER to 15 and 9%, respectively and 1.5- to 2-fold increase in DOC^B-pr (Table 2), presumably due to changes in nutrient availability. At Stn D_1, DIN concentration decreased from 0.37 to 0.01 µM with constant phosphate concentration (0.01 µM) from Day 3 to 8, whereas at Stn Sl, both DIN and phosphate decreased by >80%, from 2.01 to 0.40 µM and from 0.15 to 0.02 µM, respectively, from Day 5 to 10.

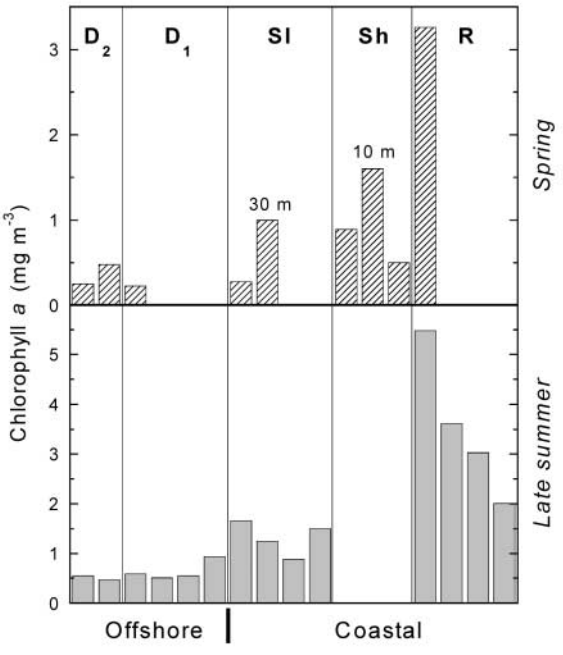

Fig. 3. Chlorophyll *a* concentration at the sampled stations. When a station was sampled more than once, subsequent samplings are represented from left to right (see Table 1 for dates). Note the different scale used

In the spring experiments, the rate of dissolved primary production was positively correlated with the rate of particulate primary production (r = 0.85, p = 0.007, n = 8) and with chl *a*, for both the total (r = 0.76, p = 0.028, n = 8) and the fraction <1.2 µm (r = 0.80, p = 0.018, n = 8). In contrast, in late summer DOC-pr was

Table 2. Mean rates (and ranges) of particulate and dissolved primary production, both total (POC-pr, DOC-pr) and chl *a* normalise (POC^B-pr, DOC^B-pr), and bacterial production (BHP) at the surface of the 2 groups of stations. Also shown is the percent extracellular release (PER) and the bacterial carbon demand as estimated from del Giorgio & Cole's model (1998) (BCD_{model}) and with a constant BGE of 15% ($BCD_{0.15}$)

Area	POC-pr (mg C m^{-3} h^{-1})	DOC-pr (mg C m^{-3} h^{-1})	PER (%)	POC^B-pr (mg C [mg chl *a*]$^{-1}$ h^{-1})	DOC^B-pr (mg C [mg chl *a*]$^{-1}$ h^{-1})	BHP	BCD_{model} (mg C m^{-3} h^{-1})	$BCD_{0.15}$
Spring								
Coastal	7.09 (1.15–19.98)	0.58 (0.19–1.55)	10.3 (3.7–16.3)	4.99 (3.79–6.13)	0.52 (0.23–0.54)	0.129 (0.016–0.348)	1.69 (0.60–2.84)	0.86 (0.10–2.31)
Offshore	1.81 (1.64–1.99)	0.17 (0.09–0.26)	8.4 (5.2–11.5)	5.61 (4.19–7.03)	0.47 (0.39–0.55)	0.010 (0.005–0.016)	0.42 (0.24–0.61)	0.07 (0.04–0.11)
Late summer								
Coastal	6.66 (1.66–10.87)	0.31 (0.23–0.46)	6.8 (2.1–18.0)	4.04 (0.83–6.86)	0.20 (0.08–0.37)	0.258 (0.029–1.018)	1.97 (0.94–4.10)	1.72 (0.19–6.78)
Offshore	4.67 (1.45–10.01)	0.20 (0.10–0.34)	6.1 (2.1–15.3)	7.42 (3.07–16.89)	0.35 (0.20–0.62)	0.063 (0.009–0.264)	1.07 (0.39–2.61)	0.42 (0.06–1.76)

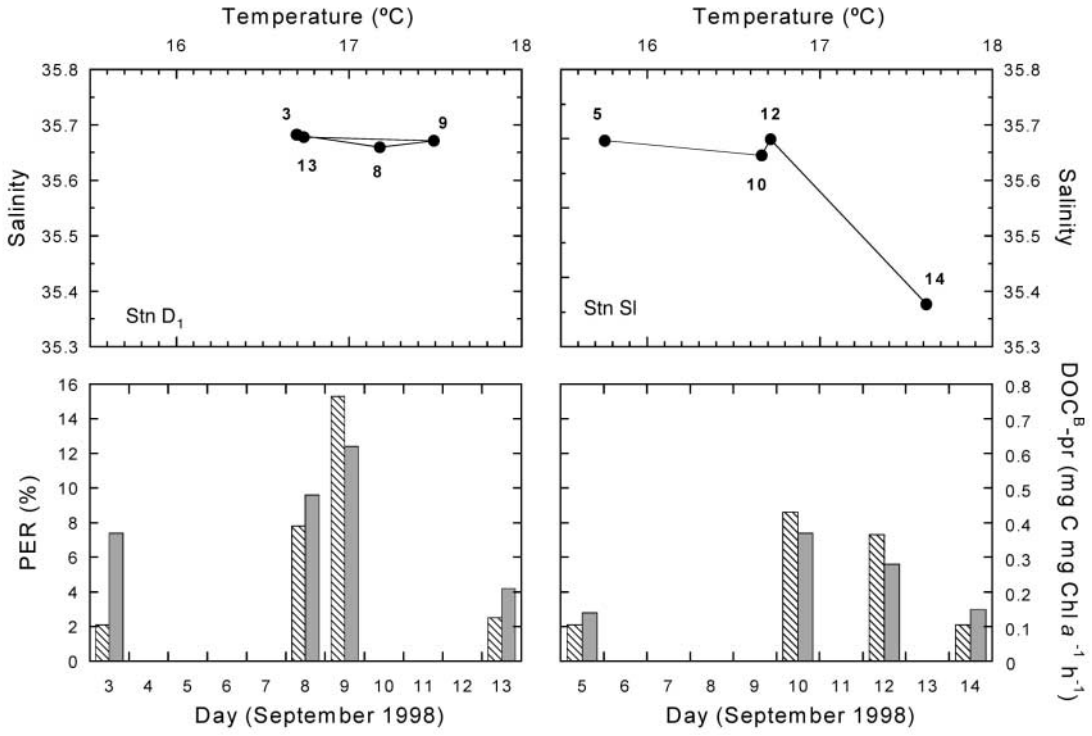

Fig. 4. Variability of temperature and salinity (upper panels), and percent extracellular release (PER, hatched bars) and chl *a* normalized DOC production rate (DOC^B-pr, shaded bars) (lower panels) along successive visits to Stns D_1 and Sl in late summer. Numbers in the upper panels represent sampling date

only significantly correlated with the fraction of chl *a* < 3 µm (r = 0.67, p = 0.048, n = 9). With all data pooled, DOC-pr was weakly but significantly correlated with POC-pr (r = 0.49, p = 0.019, n = 22) and TOC-pr (r = 0.56, p = 0.007, n = 22) but not with total chl *a* (Fig. 5). The linear regression (Model I) between POC-pr and DOC-pr (Fig. 5A) had a slope (0.38) which was significantly lower than 1.0 (*t*-test, p = 0.0045), meaning that the increment in POC-pr throughout the measured productivity range was not followed by a constant increment in DOC-pr but a decreasing one; in other words, PER decreased with increasing productivity. However, the Model II slope (0.76) was not significantly different from 1 (95% confidence limits 0.52 to 1.14, Ricker 1975) due to the high dispersion of the data.

Since different chl *a* size-fractions were used for the 2 cruises, a general relationship between picophytoplankton biomass and dissolved primary production cannot be derived. Nevertheless, a clear relationship was found between the size distribution of primary producers and the relative rate of dissolved primary production in late summer. Fig. 6 shows that the higher the percentage of picoplanktonic (i.e. <3 µm) chl *a*, the higher the chl *a* normalized dissolved primary production rate (r = 0.95, p < 0.001, n = 9). This finding was further supported by the significant correlation found between the abundance of *Synechococcus* and DOC^B-pr (r = 0.74, p = 0.002, n = 14 in summer; r = 0.63, p = 0.002, n = 21 for all data pooled).

Bacterial production

BHP ranged over 3 orders of magnitude, from 0.005 to 1.02 mg C m^{-3} h^{-1}, with values generally higher in late summer (Table 2). Differences between stations followed the coastal-offshore gradient, as indicated by the negative correlation of BHP with salinity (r = –0.88, p < 0.001, n = 22). With all data pooled, BHP was significantly correlated with bacterial abundance (r = 0.45, p = 0.031, n = 23). The correlation was higher (r = 0.65, p = 0.001, n = 23) if only the cells with high DNA content were considered, providing further evidence that HDNA cells are the most active fraction of the bacterial community. Availability of inorganic nutrients for bacterial growth seemed to play an important role in determining production rates: BHP was positively correlated to DIN (r = 0.47, p = 0.023, n = 23) and phosphate (r = 0.66, p = 0.001, n = 23) for the whole data set.

Cell-specific BHP (data not shown) presented the same pattern as the absolute values, with higher values in late summer (mean 0.14 fg C $cell^{-1}$ h^{-1}) than

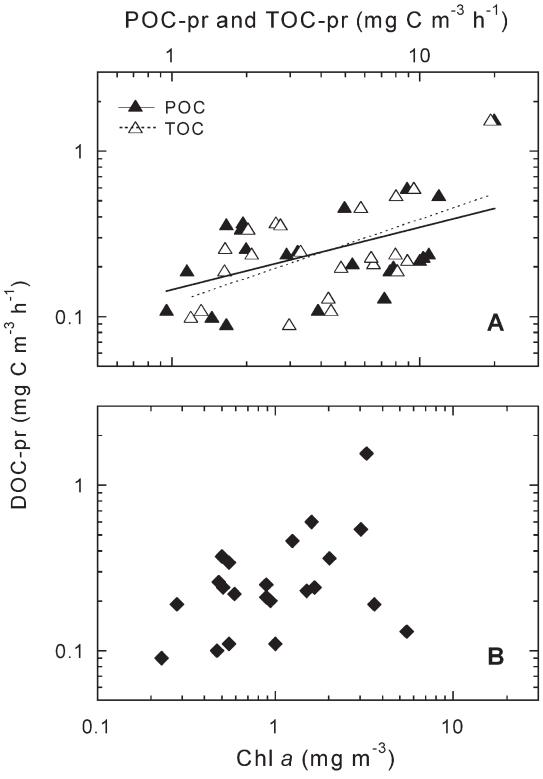

Fig. 5. DOC production rate versus (A) POC and TOC production rates and (B) chl *a* concentration for the whole data set. Fitted lines are linear regressions: log DOC-pr = –0.84 + 0.38 log POC-pr (r^2 = 0.24, p = 0.019, n = 22) and log DOC-pr = –0.91 + 0.51 log TOC-pr (r^2 = 0.31, p = 0.007, n = 22)

in spring (mean 0.04 fg C cell^{-1} h^{-1}). Differences in bacterial carbon demand were less marked, with BCD_{model} averages of 1.15 ± 0.82 mg C m^{-3} h^{-1} in spring and 1.89 ± 1.38 mg C m^{-3} h^{-1} in late summer, although mean late summer values were always higher than spring ones both at coastal and offshore stations (Table 2).

The relationship between bacterial biomass and bacterial production rate is an indication of the degree of control of biomass by resource supply (Billen et al. 1990, Ducklow 1992) or bottom-up control. This relationship was different in the 2 seasons. In spring, weak resource limitation was observed (slope 0.28, r^2 = 0.62, p = 0.02, n = 8), while in late summer, bacterial biomass seemed not to be limited by resources (slope 0.06, r^2 = 0.11, p = 0.25, n = 14). This temporal evolution of a decrease in resource control is similar to that hypothesized by Ducklow (1992).

Relationships between phytoplankton and heterotrophic bacteria

Bacterial abundance was significantly correlated with chl *a* (r = 0.85, p = 0.003, n = 9) during spring, but not in late summer. With all data pooled, the correlation (r = 0.47, p = 0.023, n = 23) between both variables was slightly higher if the percentage of HDNA bacteria were used instead of BN (r = 0.55, p = 0.007, n = 23), suggesting that bacteria were more active in waters containing higher algal biomass. The ratios of bacterial to algal biomass (with a C:chl *a* ratio of 68, Barbosa et al. 2001) were significantly lower (2-way ANOVA, area, p = 0.01) in coastal (mean 0.42) than in offshore stations (mean 0.93). The bacterial production versus chl *a* linear regressions for both seasons were not significantly different, neither in the slope (ANCOVA, F = 1.05, p = 0.31) nor the intercept (ANCOVA, F = 0.05, p = 0.82); therefore, a common regression was calculated for the whole data set (Fig. 7), which explained 62% of the variance in BHP, suggesting that the same factor could be controlling BHP during both periods. In fact, a significant correlation (r = 0.74, p <

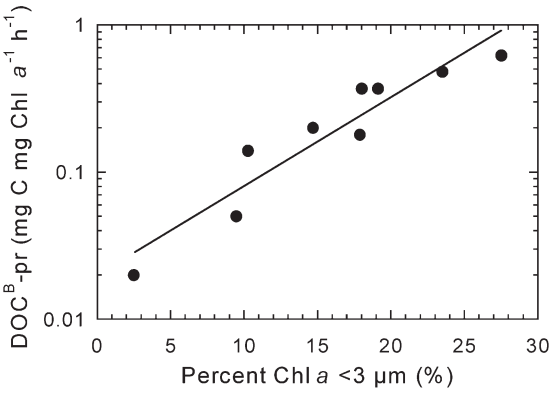

Fig. 6. Relationship between chl *a* normalized DOC production rate (DOC^B-pr) and the percentage of picoplanktonic (<3 µm) chl *a* in the late summer experiments. Linear regression: log DOC^B-pr = –1.70 + 0.06 (percent chl *a* < 3 µm) (r^2 = 0.89, p < 0.001, n = 9)

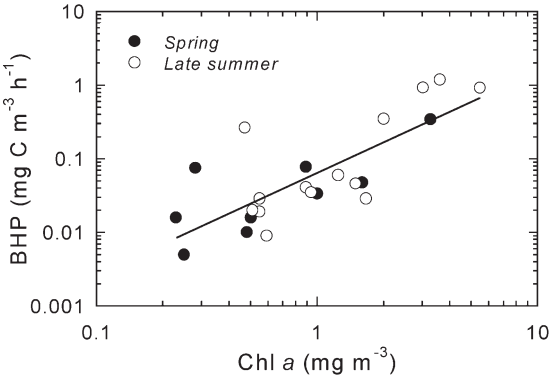

Fig. 7. Relationship between bacterial production (BHP) and chlorophyll *a* concentration. Solid line is the overall linear regression: log BHP = –1.20 + 1.37 log chl *a* (r^2 = 0.60, p < 0.001, n = 23)

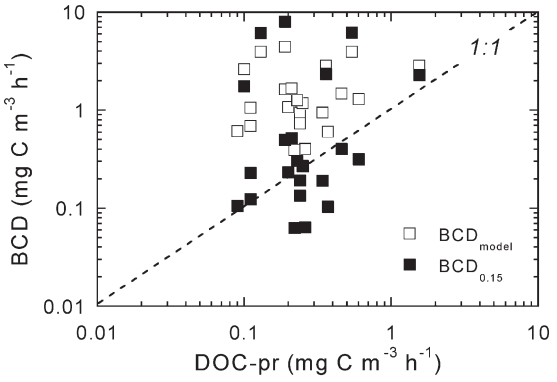

Fig. 8. Bacterial carbon demand (BCD) and dissolved primary production (DOC-pr). BCD was estimated with 2 different methods, the empirical model by Del Giorgio & Cole (1998) (BCD_{model}) and assuming a fixed bacterial growth efficiency of 15% ($BCD_{0.15}$). See text for details. The dashed line represents the 1:1 relationship

0.001, n = 21) was found between bulk DOC concentration (X. A. Álvarez-Salgado et al. unpubl.) and BHP for the whole data set. However, when BHP was compared with the current DOC supply by phytoplankton, the 2 variables were not correlated either for individual cruises or for the whole data set. BHP was not correlated either with particulate or total primary production.

Consequently, the estimates of BCD (BCD_{model} and $BCD_{0.15}$) bore no significant relationship with DOC-pr (Fig. 8). BHP generally amounted to less than 10% of the total (particulate plus dissolved) primary production (see Table 2), but DOC-pr alone was not enough to sustain BCD_{model} in any of the experiments, and could meet $BCD_{0.15}$ only in roughly one-third of the experiments (Fig. 8). These results strongly suggest that algae and bacteria were uncoupled in the region in the sampled periods, i.e. bacteria did not depend on substrates directly supplied by primary producers. However, some considerations can be made at this point. Offshore, a higher importance of DOC-pr in meeting BCD would be suggested with a fixed BGE, but not with the varying BGE estimates obtained with the model (Fig. 9). With a BGE of 15%, DOC-pr would on average have sufficed to fulfill bacterial requirements of carbon in offshore waters in spring. In spite of the large variability, dissolved primary production would have contributed a higher percentage of BCD in spring than in late summer.

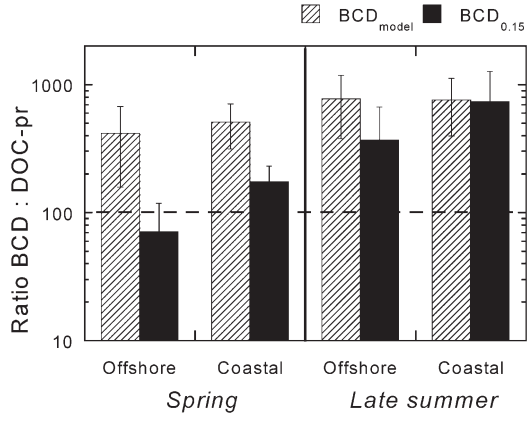

Fig. 9. Ratio of BCD to DOC-pr, expressed as a percentage, for the 2 sampling periods and groups of stations (offshore and coastal). The dashed line represents equal values of BCD and DOC-pr. Error bars represent the standard error of the mean (SE)

DISCUSSION

Partitioning of phytoplanktonic organic carbon production

Two sources of variability in nutrient supply for phytoplankton were compared in this study regarding their effect on the partitioning of primary production and the contribution of algal DOC to bacterial carbon requirements: (1) the different hydrographic conditions found in spring and late summer, which included upwelling and downwelling pulses (Fig. 2); and (2) the coastal-offshore gradient, with nutrient concentrations generally increasing inwards (Table 1).

The general observation of a higher biomass and production of phytoplankton in late summer than in spring was likely linked to the contrasting general hydrographic conditions of upwelling and downwelling, respectively. Assessments of seasonal variability in PER are not abundant, but similar mean PER values (5% in spring, 9% in late summer) were reported by Lignell (1990) for the coastal Baltic Sea. However, in the Baltic, higher PER and lower nutrient concentrations were found in summer rather than in spring (Larsson & Hagström 1982, Lignell 1990). Our results were well in accordance with the typical 5 to 10% range in PER described for coastal waters (Mague et al. 1980, Nagata 2000). Late summer PER values were very similar to those reported by Teira et al. (2001a) for the same region under a similar upwelling scenario, but downwelling results differed notably between both studies. These authors found a photic-layer integrated mean PER of 33% in autumn with a clear northward current at the shelf-break, which produced downwelling hydrographic conditions similar to those of our spring cruise. Although PER usually increased with depth in the survey of Teira et al. (2001a), their surface values were still generally higher than 10%. Without considering the different methodology

used in both studies (i.e. GF/F vs membrane filters, end-point vs kinetic experiments), which could result in even greater differences between their PER values and ours, the contribution of picoplankton to biomass and production was much greater in their samples (up to 68 and 87%, respectively). As will be discussed below, this observation may have increased PER notably. However, both sets of data had virtually identical log-log regression slopes (0.38 and 0.37) between DOC-pr and POC-pr, strongly suggesting that the inverse relationship between PER and productivity is the usual situation in the NW Iberian coastal region, as has been frequently reported for other individual systems (Baines & Pace 1991).

Although the distribution of algal biomass followed the commonly observed ocean-wards decrease (Fig. 3) concurrent with an increase in the abundance of *Prochlorococcus* (Table 1) as in previous reports (Partensky et al. 1999a,b), no clear coastal-offshore pattern was found for the partitioning of primary production. That short-term hydrodynamical changes rather than the coastal-offshore gradient exerted a major effect on organic carbon partitioning, was confirmed by the variability observed at Stns D_1 and Sl in the upwelling-relaxation-upwelling sequence (Fig. 4). These results could indirectly support the effect of nutrient conditions on PER since comparatively more photosynthate was released as DOC under the relatively nutrient-deficient conditions of upwelling relaxation (Fig. 4). However, increases in PER and DOC^B-pr might also be due to changes in phytoplankton composition since the relaxation of upwelling coincided with increases in the relative contribution of small cells to total chl *a* (from 18 to 23% at Stn D_1 and from 10 to 19% at Stn Sl). Similar results were found by Lancelot (1983) in the North Sea, who also stressed the importance of algal taxonomic composition in attempts to correlate nutrient concentrations and PER. The changes shown in Fig. 4 confirm the important short-term variability of the primary production partitioning in highly hydrodynamic marine areas either through nutrient-mediated changes in the cellular biosynthetic capability (Wood & Van Valen 1990) or changes in phytoplankton composition (Morán & Estrada 2001).

Notwithstanding this short-term variability, the gathered data set allowed us to throw some light on general questions concerning algal DOC release. The absence or the weak correlation between DOC-pr and chl *a* has been interpreted as contradicting Bjørnsen's (1987) hypothesis of passive diffusion as the main mechanism of algal DOC production (Baines & Pace 1991, Morán et al. 2001). Passive diffusion should be related to the surface:volume ratio and, as this ratio increases in small cells, a surrogate of total cell size such as total biomass or chl *a* should somehow reflect the variability in DOC-pr. It may seem hazardous to infer a cellular mechanism based on correlation analysis, but the finding that only POC-pr and TOC-pr (but not chl *a*) explained part of the overall variance in DOC-pr (Fig. 5) indicates that the fraction of organic carbon released extracellularly depended largely on the concurrent photosynthetic rate. Our measured PER values suggest that the appearance of labeled DOC in the experiments, whatever the exact mechanism of algal DOC release, was very likely due to the normal activity of phytoplanktonic cells in contrast to the interpretation of Teira et al. (2001a,b). These authors suggested that their up to 3-fold higher PER values could not only be caused by algal physiological processes, but likely included grazing by microzooplankton (Nagata 2000).

Total biomass (as chl *a*) may not be a good predictor of dissolved primary production rates, but the size-composition of phytoplankton appears to play an important role (Fig. 6), as previously hypothesized by some authors (e.g. Kiørboe 1993) and recently recognized by Teira et al. (2001a,b). These authors (2001a) found a significant relationship between PER and the percentage of picoplanktonic chl *a* for a range of 3 to 68% of chl $a < 2$ µm in the same area. Albeit our experiments were limited to a range more typical of meso- to eutrophic conditions (only 2 to 27% of chl $a < 3$ µm), as much as 89% of the variance in DOC_B-pr could be explained by the percentage of picoplanktonic (i.e. <3 µm) chl *a* (Fig. 6). Autotrophic picoplankton in our samples was generally dominated by *Synechococcus* cyanobacteria (Table 1). Based on the significant correlation between their abundance and DOC^B-pr, it could be speculated that *Synechococcus* was the organism responsible for the increase in relative dissolved primary production in waters with a significant contribution of picophytoplankton. PER values greater than the commonly accepted upper limit of 15 to 20% (Baines & Pace 1991, Nagata 2000) can be expected in oligotrophic waters (Teira et al. 2001b), where picoplankton dominates both biomass and production (Li et al. 1992, Buck et al. 1996, Bell & Kalff 2001). The size-distribution of primary producers and/or the abundance of cyanobacteria might prove to be a useful tool to estimate DOC production rates over these vast regions of the world ocean.

Bacterial abundance and production

Bacterial abundance was comparable to that of previous studies in the region (Hanson et al. 1986, Tenore et al. 1995, Zdanowski & Figueiras 1997, Barbosa et al. 2001), with values generally higher than 106 cells ml^{-1}. A higher abundance of heterotrophic bacteria was found in late summer, as in previous surveys in the Ría

de Vigo (Zdanowski & Figueiras 1997), probably as a combined result of higher temperatures and higher levels of accumulated DOC (Álvarez-Salgado et al. 1999). The absence of a significant relationship between biomass and production for the whole area would indicate that bacterial biomass was limited by predation rather than organic carbon supply (Billen et al. 1990, Ducklow & Carlson1992).

Although our results of biomass and production (Table 2) were higher than those reported in Barbosa et al. (2001), especially in late summer, they still are in the lower range of values reported for other upwelling systems (e.g. Kirchman et al. 1995, Pomroy & Joint 1999). Since both our results and those of Barbosa et al. (2001) were based on direct estimations of bacterial biovolume and empirical Leu to carbon conversion factors, they indicate that the contribution of bacteria to carbon processing in upwelling affected marine regions can be substantially lower than previously reported.

Relationships between phytoplankton and heterotrophic bacteria

Relationships between bacterial production and algal biomass, like the one shown in Fig. 7, have been frequently perceived as a direct and immediate dependence of bacteria on phytoplankton activity (Cole et al. 1988, Ducklow & Carlsson 1992). Hence, along with previous reports (Hanson et al. 1986, Barbosa et al. 2001), a tight algal-bacterial coupling could be postulated for the NW Iberian margin. On the other hand, the low ratio of bacterial to primary production (BHP:PP) in our experiments (4 ± 5%), far from the typical 30 to 50% (Cole et al. 1988, Ducklow & Carlson 1992), would be indicative of uncoupling between the 2 groups (Bird & Karl 1999). The BHP:PP ratio, however, presents some caveats when used for the purpose of characterizing the amount of organic carbon channeled through heterotrophic bacterioplankton. The elusive question of coupling between phytoplankton and bacterioplankton can be better addressed when the following is taken into consideration. First, primary production measurements should always include the dissolved fraction. Second, BCD should be preferred to BHP since the variability in the amount of carbon respired can be large. Third, the scales of integration are critical if time lags occur between maxima of phytoplankton and bacterial activity.

We directly measured algal supply of DOC in our experiments and estimated BCD with both a constant ($BCD_{0.15}$) and a variable BGE (BCD_{model}). On average, we would have needed a BGE as high and unrealistic (del Giorgio & Cole 1998) as 99% for DOC-pr to be enough to meet BCD in the region. A possible drawback of the use of del Giorgio & Cole's model for estimating BCD is that probably many of the BHP values included in it, used the theoretical value of Simon & Azam (1989) of 3.1 kg C mol Leu^{-1} rather than empirical Leu to C conversion factors. Our conversion factors were always lower than the theoretical one with the exception of that found at Stn R (3.6 kg C mol Leu^{-1}). Notwithstanding these considerations, the 2 estimates of BCD very likely constrained the maximum amount of carbon that could be processed by bacteria. Our results show that no significant relationship existed between DOC-pr and BCD whatever the estimate used (Fig. 8), strongly suggesting uncoupling between both planktonic groups at the examined short time scales. Yet, an apparent phytoplankton-bacterioplankton uncoupling would also be observed if algal and bacterial respective peaks of activity are lagged, such as the 30 h found by Barbosa et al. (2001) during a Lagrangian drift experiment in the region.

The different ratios of bacterial to phytoplanktonic biomass in coastal and offshore stations agreed with reports for the same (Barbosa et al. 2001) and other regions (Pedrós-Alió et al. 1999). However, no definite conclusions can be made on a different trophic relationship between both planktonic groups in the 2 areas. A trend for DOC-pr to approach BCD in offshore waters in both periods was suggested only with a fixed BGE of 15% (Fig. 9), but not with variable BGE estimates from the model. At the innermost Stn R, the one receiving the greatest external input of DOC (Doval et al. 1997), a high excess of BCD over DOC-pr was observed in both cruises. Away from the influence of allochthonous inputs, we have shown that BCD could be entirely met by DOC-pr in open ocean Antarctic waters (Morán et al. 2001, Morán & Estrada 2002). In the NW Iberian coastal region, algal DOC would potentially contribute more to BCD in spring than in late summer (Fig. 9), when bulk DOC concentrations tend to be higher (Álvarez-Salgado et al. 1999).

The high surface phytoplankton growth rates during both cruises (1 division d^{-1}, F. G. Figueiras & L. M. Lorenzo pers. comm.) plus the short incubations of small volume samples made the contribution of cellular lysis (Gobler et al. 1997, Agustí et al. 1998) or sloppy feeding and excretion by grazers (Lampert 1978, Jumars et al. 1989) to our measured DOC-pr unlikely. At best, phytoplankton DOC-pr would amount to one-third of the gross ecosystem DOC production given for similar times of the year in Barbosa et al. (2001). Autochthonous DOC is produced by algal release, but also by the above-mentioned processes. According to Nagata (2000), DOC release by mesozooplankton would be half of the dissolved primary production rates presented here, whereas protozoan grazers could produce twice as much DOC than algae (Hasegawa et al. 2000, Nagata 2000). Allochthonous

sources, such as riverine runoff or sewage, DOC previously accumulated *in situ* after past upwelling episodes (Álvarez-Salgado et al. 2001) and/or export from the estuary to the shelf (Doval 1997, Álvarez-Salgado et al. 2001) may also have supplemented algal production of labile carbon in order to meet the needs of heterotrophic bacteria in these coastal waters.

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