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Variations in planktonic bacterial biomass and production, and phytoplankton blooms off A Coruña (NW Spain)*

JOAQUÍN VALENCIA[1,4], JULIO ABALDE[1], ANTONIO BODE[2], ANGELES CID[1], EMILIO FERNÁNDEZ[3], NICOLÁS GONZÁLEZ[2], JORGE LORENZO[2], EVA TEIRA[3] and MANUEL VARELA[2]

[1] Departamento de Biología Celular y Molecular, Universidad de A Coruña, Campus da Zapateira, s/n. 15080. A Coruña, Spain.
[2] Instituto Español de Oceanografía, Centro Oceanográfico de A Coruña, Apdo. 130, 15080 A Coruña Spain.
[3] Departamento de Biología Animal y Ecología, Universidad de Vigo, Campus Lagoas - Marcosende, s/n. 36200 Vigo, Spain.
[4] Present address: Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Muelle de Poniente s/n. Ap. 291, 07080 Palma de Mallorca, Spain. E-mail: joaquin.valencia@ba.ieo.es

SUMMARY: Bacterial abundance, biomass and heterotrophic activity, along with hydrographic and phytoplankton variables, were measured from February 1998 to September 1999 at two coastal stations off A Coruña (Galicia, NW Spain). Bacterial abundance varied from 0.1 to 1.8 x 10^6 cells ml^{-1} and water-column integrated bacterial production ranged from 34 to 1132 mg C m^{-2} d^{-1}. Two annual maxima of bacterial abundance and production were observed, in spring and summer, although relatively high abundance values remained until autumn. In autumn and winter production values were low with the exception of the results obtained in November 1998, when the highest values of bacterial production of the whole study were observed at both stations. Bacterial biomass constituted a significant, but low, fraction of particulate organic carbon (4-21 %) and was always lower than phytoplankton biomass (up to 40% of phytoplankton carbon). Also, bacterial production was generally < 30% of primary production, but in some situations (as in early spring or in autumn) bacterial production exceeded primary production, suggesting a delayed response of bacterial activity after a phytoplankton bloom.

Key words: bacterial production, bacterial abundance, phytoplankton biomass, primary production, phytoplankton blooms, NW Spain.

RESUMEN: VARIACIONES EN LA BIOMASA Y PRODUCCIÓN DE LAS BACTERIAS PLANCTÓNICAS, Y EN LAS PROLIFERACIONES DE FITOPLANCTON EN LA COSTA DE A CORUÑA (NO ESPAÑA). – Se midió la abundancia, biomasa y actividad heterotrófica bacteriana, junto con variables hidrográficas y fitoplanctónicas, entre febrero de 1998 y septiembre de 1999 en dos estaciones costeras de A Coruña (Galicia, NO España). La abundancia bacteriana varió entre 0.1 y 1.8 x 10^6 células ml^{-1} y la producción bacteriana integrada en la columna de agua tomó valores entre 34 y 1132 mg C m^{-2} d^{-1}. Se observaron dos máximos de abundancia y producción bacteriana en primavera y verano, aunque se mantuvieron valores relativamente altos de abundancia durante el otoño. En otoño e invierno los valores de producción fueron bajos con la excepción de los resultados obtenidos en noviembre de 1998, donde se observaron los mayores valores de producción de todo el estudio en ambas estaciones. La biomasa bacteriana constituyó una fracción significativa, aunque baja, del carbono orgánico particulado (4-21%) y fue siempre menor que la biomasa del fitoplancton (hasta un 40% del carbono fitoplanctónico). Del mismo modo, la producción bacteriana fue generalmente < 30% de la producción primaria, aunque en ciertas situaciones (como a principios de primavera o en otoño) la producción bacteriana excedió a la producción primaria, lo que sugiere un retraso en la respuesta de la actividad bacteriana a la proliferación del fitoplancton.

Palabras clave: producción bacteriana, abundancia bacteriana, biomasa de fitoplancton, producción primaria, proliferaciones de fitoplancton, NO España.

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INTRODUCTION

Planktonic bacteria are one of the main components of aquatic ecosystems, often constituting a significant fraction of total biomass and participating to a great extent in the flux of energy and elements like carbon. According to Cole *et al.* (1988), bacterial net production is equivalent on average to 30% of the water column primary production. The main sources of dissolved organic carbon for bacteria are the phytoplankton exudation and the release of dissolved substrates as a result of sloppy feeding of zooplankton (Azam *et al.,* 1983; Furhman, 1992). Therefore, most marine bacteria depend directly or indirectly on phytoplankton. In fact, it has been observed that the bacterial production is correlated with phytoplankton biomass and production (Cole *et al*., 1988; White *et al*., 1991). However, in coastal zones some of the organic matter processed by bacteria is from allochtonous origin (Opsahl and Benner, 1997). The availability of extra food sources often results in complex seasonal variation of coastal bacterioplankton, and some studies report the lack of covariation between bacteria and phytoplankton (Hoch and Kirchman, 1993). Nevertheless, other authors have found time-lags in the response of bacterioplankton to phytoplankton blooms (Ducklow, 1993; Li *et al.*, 1993).

The coast of A Coruña (Galicia, NW Spain) is a region of high primary production influenced by the seasonal upwelling of deep nutrient-rich waters (Varela, 1992; Bode and Varela, 1994; Casas, 1995). The upwelling occurs in the form of discrete events between March and October driven by northerly winds that cause the fertilisation of coastal and shelf waters with deep water nutrients (Fraga, 1981; Blanton *et al*., 1984). The dynamics of this ecosystem are based on the typical seasonal cycle of temperate seas defined by the alternance between periods of intense mixing of the water column, as in winter, and periods of water-column stratification, as in summer, although in this case the seasonal cycle is significantly modified by the upwelling (Varela, 1992; Casas, 1995). There are few data on seasonal variations of bacterioplankton off A Coruña (Varela *et al*., 1996; Barquero, 1999), although estimations based on data for other locations of Galicia suggest that most of phytoplankton biomass produced by the upwelling was consumed by bacteria (Bode and Varela, 1994).

The aim of this paper is to study the variability patterns in bacterial biomass and production in relation to phytoplankton productivity and proximity to the coast in a coastal area under the influence of upwelling. For this purpose, a procedure for measuring bacterial biomass and production based on some of the most widely used techniques (Porter and Feig, 1980; Kirchman, 1993) was adapted to the study area.

MATERIALS AND METHODS

Sampling

Two stations located near A Coruña (Galicia, NW Spain) were studied from February 1998 to September 1999 at approximately monthly intervals.

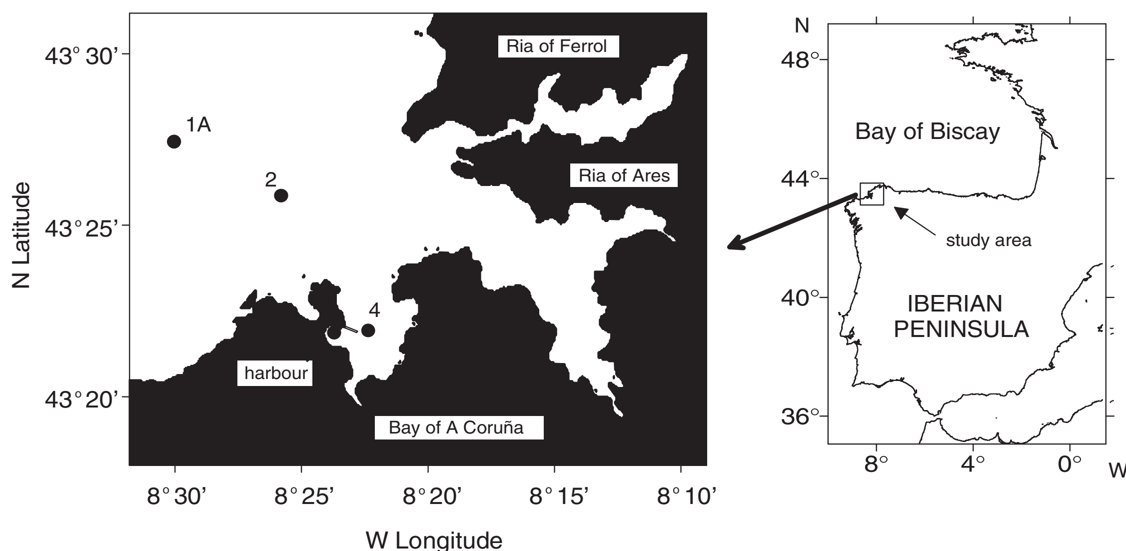

FIG. 1. – Map of the study area with location of sampling stations.

Station 2 (79 m) was situated over the shelf and station 4 (20 m) was located at the entrance of the Bay of A Coruña (Fig. 1). At both stations temperature, salinity, *in situ* fluorescence, irradiance, particulate organic carbon and chlorophyll *a* concentrations, primary production and bacterial abundance and production were measured. Vertical profiles of temperature, salinity, fluorescence and photosynthetically available radiation (PAR) were obtained with a CTD SBE-25 equipped with a LiCOR spherical sensor. Water samples were collected using 5 l Niskin bottles. Sampling depths were selected following the irradiance profile at 100, 50, 25, 10 and 1% of surface irradiance (PAR). At station 2, two additional samples were collected above and below 1% of surface irradiance. Furthermore, additional samples were collected at station 1A (100 m) and in the A Coruña harbour (Fig. 1) for some experiments complementary to the bacterial production technique.

Biomass and production of phytoplankton

Particulate organic carbon (POC) was analysed following the UNESCO procedure (1994), by the filtration of 500 or 1000 ml of seawater using Whatman GF/F glass-fibre filters. The filters were dried at 60ºC for 24 h and then analyzed using a CNH elemental analyser (Perkin Elmer). Chlorophyll *a* concentration was determined with a fluorimetric method (Yentsch and Menzel, 1963; Parsons *et al*., 1984; UNESCO, 1994) after filtration of up to 250 ml of water on Millipore Type F glass-fibre filters. Pigments were extracted with 90% acetone overnight (-10ºC). Chlorophyll *a* concentrations (Chl-*a*) were corrected for phaeopigments after acidification with 10% HCl. Biomass of phytoplankton was estimated from the chlorophyll *a* concentration using the conversion factor 50 g C (g Chl-a)$^{-1}$. Primary production rates (PP) were determined as described in Bode and Varela (1998). Two transparent bottles and one dark bottle from five depths within the euphotic zone (> 1% irradiance PAR) were inoculated with 4 µCi (148 KBq) $NaH^{14}CO_3$ and incubated in simulated *in situ* conditions for at least 2 hours. Light levels were simulated using neutral density screens and the incubator was refrigerated by running water. Incubations were terminated by filtration onto Millipore Type F filters. The filters were then treated with 10% HCl, scintillation cocktail (Ultima X-Gold, Packard) was added, and finally radioactivity was counted using a liquid scintillation counter (LKB Wallac).

Bacterial abundance, biomass, and production

Heterotrophic bacterial abundance was determined in samples preserved with glutaraldehyde (2% final concentration) using the method of Porter and Feig (1980). Ten millilitres of each sample was filtered onto 0.2 µm black, polycarbonate, Poretics membrane filters and stained with DAPI (4'-6-diamino-2-phenylindole, final concentration: 2.5 µg ml^{-1}) for 5 min. The filters were mounted with low-fluorescence oil on microscope slides and stored frozen. Heterotrophic bacteria were counted using ultraviolet light in an epifluorescence microscope (Olympus BH-2). Bacterial cellular carbon was estimated from biovolumes using the empirical equation of Norland *et al.* (1987):

$$C = 0.09\ BV^{0.9}$$

where C is the carbon content (pg C cell^{-1}) and BV is the bacterial biovolume (µm^3 cell^{-1}). Bacterial biovolumes were computed from measurements of bacterial dimensions using a graduated bar coupled to the microscope eyepiece. Cocci were considered as spheres and rods as cylinders.

Production of heterotrophic bacteria (BP) was estimated by ^{3}H-leucine incorporation, using the method described by Kirchman (1993), substituting the final filtration of bacteria by centrifugation (Smith and Azam, 1992). Four aliquots of water samples from each depth were inoculated with ^{3}H-leucine to a final concentration of 150 nM and incubated for 30 or 45 min in 1.5 ml Eppendorf vials. In addition, two 1ml controls killed with trichloroacetic acid (TCA, 5% w/v final concentration) were incubated for each depth. Incubations were terminated by the addition of 5% TCA to the incubation vials by repeated washing with 5% TCA and successive centrifugation (12000 rpm, 10 min). Scintillation cocktail (Ultima X-Gold, Packard) was added to the vials and radioactivity was measured in a liquid scintillation counter. The mean (± SD) coefficient of variation of the radioactivity measurements between the 4 replicates for each sample was 16% ± 10% (n = 235). The incubation was made at laboratory temperature but the measured rates were later corrected to *in situ* temperature. The recalculation was made using an empirically derived coefficient (Q_{10}) relating leucine incorporation to temperature increases of 10°C (e.g. Valiela, 1995):

$$Q_{10} = (r_1/r_2)^{(10/(t_1-t_2))}$$

where r_1 and r_2 are the rates determined at temperatures t_1 and t_2. The values of Q_{10} were calculated in two experiments incubating samples from the harbour station in a temperature range between –1.5 and 29.5ºC.

Linearity of leucine incorporation during the incubations was tested in time course experiments. Also, several experiments were run to ensure complete saturation of the incorporation by testing leucine concentrations between 2.5 and 320 nM for the incubation of samples from the station 1A and the harbour station. Incorporation of leucine versus concentration was adjusted to the Michaelis-Menten kinetic equation:

$$DPM = DPM_{max} [Leu] / (K_s + [Leu])$$

where *DPM* are the disintegrations per minute measured, DPM_{max} is the maximum DPM rate (equivalent to the maximum theorical rate of leucine incorporation), K_s is the semisaturation constant (nM Leu) and *[Leu]* is the final concentration of leucine in the incubation vials (nM Leu). The constants DPM_{max} and K_s were obtained by adjusting by linear regression to the equation:

$$[Leu] / DPM = a + b [Leu]$$

where $K_s = a/b$ and $DPM_{max} = 1/b$.

The conversion factors between leucine incorporation and cell growth or carbon production were determined experimentally by the procedures indicated in Kirchman and Ducklow (1993). For each experiment 200 ml of surface seawater from station 1A or the harbour station was added to 800 ml of autoclaved seawater and incubated at room temperature for 50 h. Every 3 or 4 h, aliquots of this culture were removed for the determination of bacterial abundance and leucine incorporation rates by the described procedures.

Daily rates were used for comparing bacterial production with primary production values integrated in the water column. For this purpose, it was assumed that bacterial production was constant during the day, as some studies suggest that daily variations in bacterial production in coastal zones are not very pronounced (e.g. Gasol *et al*., 1998). Daily primary production was computed from hourly rates multiplied by the number of sunlight hours, which were determined according to the model of Straskraba and Gnauck (1985).

Bacterial growth rates (μ) were computed as:

$$\mu = ln (1 + BP/BB)$$

and the corresponding duplication time (DT) as:

$$DT = ln2/\mu$$

Comparison of bacterial and phytoplankton biomass and production was made by integrating all biomass and production values in the whole water-column (surface to 79 m depth in the station 2 and surface to 20 m in station 4).

RESULTS

Methodological settings for bacterial production

No significant differences were found between the mean biovolumes of bacteria from stations, 2, 4 and the harbour station (Table 1, ANOVA $p > 0.05$). The mean biovolume obtained was 0.13 (± 0.14, SD) μm^3 $cell^{-1}$ (n = 408), with a resulting carbon content of 14.25 (± 14.84, SD) fg C $cell^{-1}$. However, given the relatively large variances observed for each station, we applied the respective mean carbon content of bacteria from stations 2 or 4 in all subsequent computations of biomass or production.

In all saturation experiments complete saturation was not reached until leucine incorporation generally exceeded 100 nM (Fig. 2). However, high leucine concentrations (> 200 nM) produce an inhibition of incorporation. Because a saturating concentration of 100 nM is high compared with other studies (Kirchman, 1993; Wiebinga *et al*., 1997; Gasol *et al*., 1998) we explored the possibility of the existence of a first saturation at concentrations < 50 nM, while at higher concentrations inactive bacteria are stimulated (Fig. 2, Table 2). This stimulation would result in the existence of a new logarithmic growth phase, but the presence of this phase in our results is not apparent, since most experiments were best described by a single Michaelis-Menten curve (Fig. 2). In the absence

TABLE 1. – Bacterial biovolume in three different zones (mean and standard deviation, SD) and transformation to the carbon content using the ecuation of Norland *et al*. (1987).

Station	Biovolume (μm^3) Mean	Biovolume (μm^3) SD	n	Biomass (fg C $cell^{-1}$) Mean	Biomass (fg C $cell^{-1}$) SD
harbour	0.15	0.15	121	15.93	16.42
2	0.13	0.17	145	14.25	17.78
4	0.11	0.07	142	12.75	8.54
Mean:	0.13	0.14	408	14.25	14.84

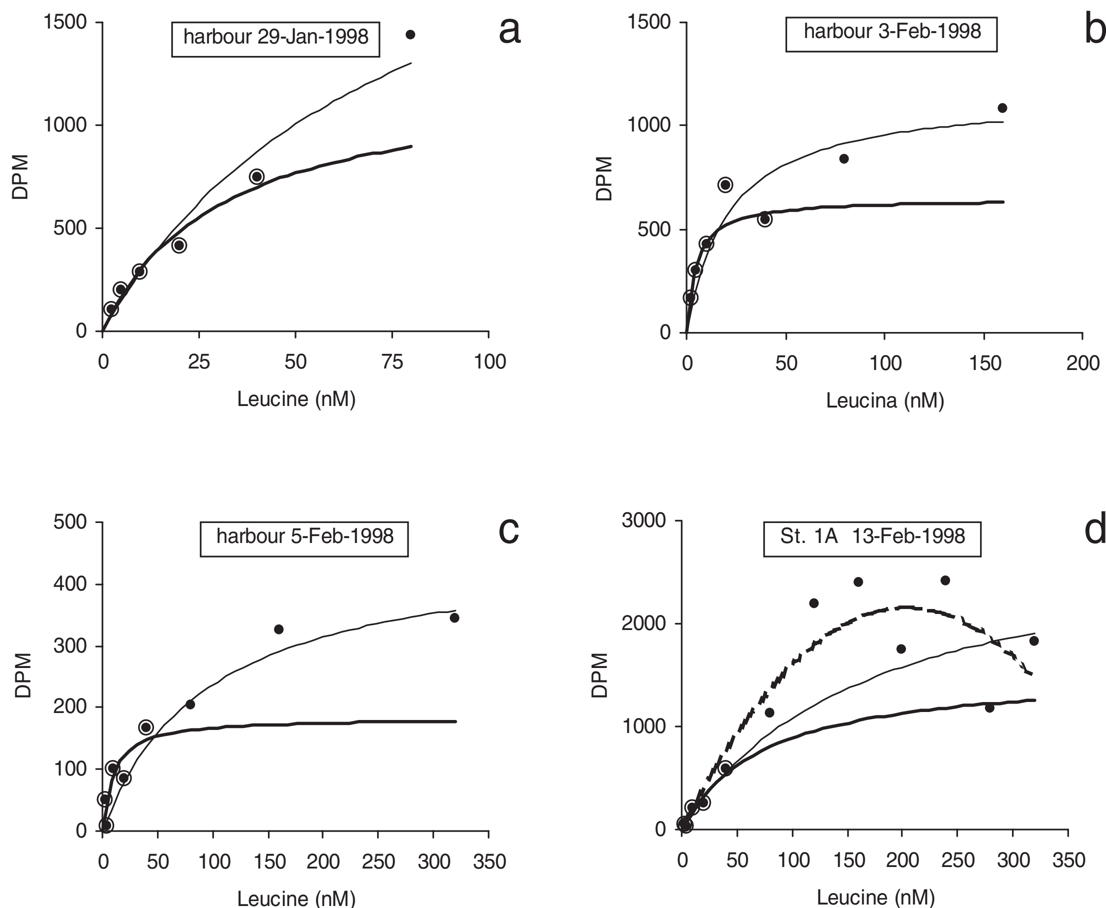

FIG. 2. – Incorporation of labeled leucine (as disintegrations per minute, DPM) at different initial concentrations of substrate. Continuous lines indicate curves fitted to Michaelis-Menten functions using either all data points (fine line) or only those points corresponding to leucine concentrations up to 50 nM (thick lines). The parameters of the Michaelis-Menten curves are in Table 2. Incubations were done with samples of the harbour (a, b and c) or station 1A (d). The dotted curve in d was fitted to all data points by using a polynomial function only for descriptive purposes.

of information about the actual concentration of leucine in the study area, and according to the experiments carried out, we used a concentration of 150 nM in all subsequent incubations. Nevertheless, from the values of the Michaelis-Menten saturation equation constants (Table 2) we estimated that the addition of leucine up to 150 nM would have multiplied the incorporation rates expected at 50 nM by 1.2 to 2.1 times.

In two incubation experiments made at different temperatures a positive correlation between temperature and bacterial production was found (overall r =

TABLE 2. – Values of the estimated Michaelis-Menten parameters for the four experiments done with increasing final concentrations of leucine. K_s: semisaturation constant (nM Leu), DPM_{max}: theorical maximum DPM rate, DPM_{150}: disintegrations per minute estimated from the Michaelis-Menten equation for a final concentration of 150 nM, DPM_{50}: disintegrations per minute estimated from the Michaelis-Menten ecuation for a final concentration of 50 nM, *factor*: relation between DPM_{150} and DPM_{50}, *SD*: standard deviation.

Station	Date	K_s	DPM_{max}	DPM_{150}	DPM_{50}	*factor*
harbour	29-Jan-1998	77.2	2555.1	1686.9	1004.4	1.7
harbour	3-Feb-1998	21.1	1156.7	1014.3	813.9	1.2
harbour	5-Feb-1998	96.5	465.5	283.3	158.9	1.8
1A	13-Feb-1998	171.3	2929.8	1367.7	661.9	2.1
					Mean:	1.7
					SD:	0.3

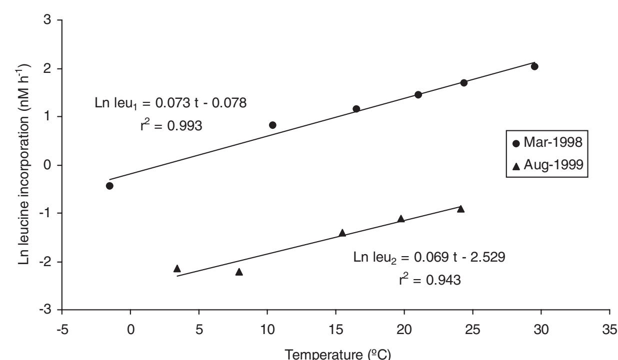

FIG. 3. – Incorporation of leucine at different incubation temperatures. Experiments were done with samples of the harbour on March 1998 and August 1999.

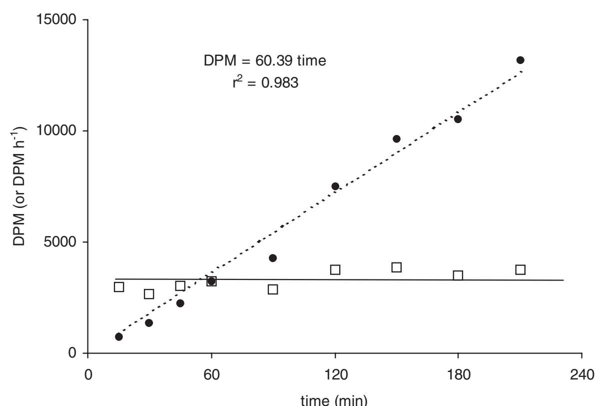

FIG. 4. – Time course leucine incorporation (as disintegrations per minute, DPM). The accumulation of labeled leucine was shown by the dotted line and the linear function, while the resulting leucine incorporation rate (DPM h^{-1}) was aproximately constant during the experiment (continuous line).

0.984, $p < 0.01$, Fig. 3), but no significant differences were observed between the slopes of the regression line relating bacterial production and temperature computed separately for each experiment (t-Student Test, $p > 0.1$). From these experiments we obtained a mean Q_{10} value of 2.02 which was used to recalculate bacterial production rates at *in situ* temperature.

TABLE 3. – Empirical conversion factors between leucine and cell growth or carbon uptake. Cell to carbon uptake conversion was made using the estimated cell biomass for each station (Table 1). SD: standard deviation.

Date	Station	Conversion Factor [x 10^{17}cell (mol leu)$^{-1}$]
11-Feb-1998	harbour	4.44
6-Mar-1998	1 A	3.02
24-Mar-1998	harbour	2.37
23-Feb-1999	harbour	6.74
24-Mar-1999	1A	2.10
4-May-1999	harbour	2.83
	Mean:	3.58
	SD:	1.60

Time-course experiments revealed that the relation between leucine incorporation and incubation time was linear for at least 210 minutes (Fig. 4). Therefore, there was no difference between the results obtained with the two incubation times used (30 or 45 minutes).

Leucine incorporation was converted to carbon production using an empirical conversion factor derived from six experiments with a mean (± SD)

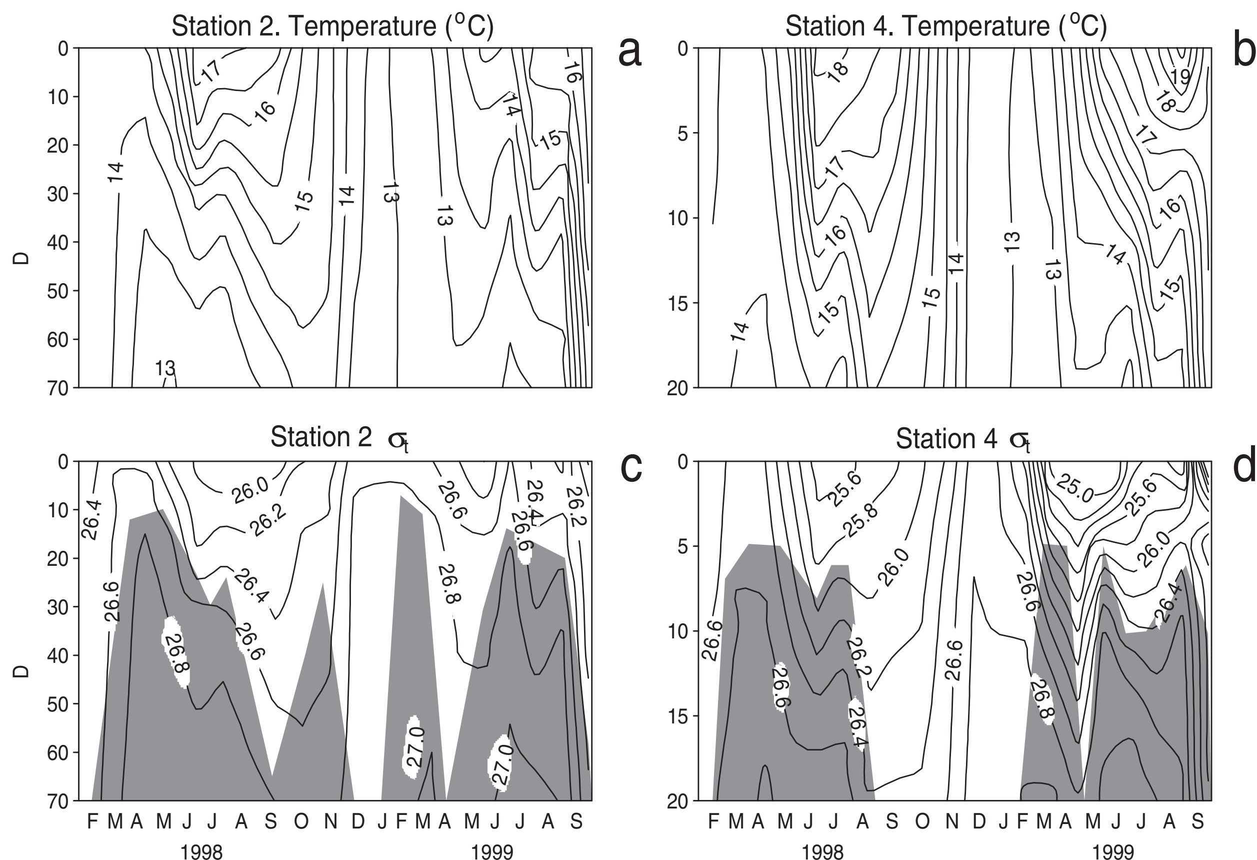

FIG. 5. – Distribution of temperature and σ_t during the study at stations 2 (a, c) and 4 (b, d). The shaded area indicates the layer below the upper mixing layer.

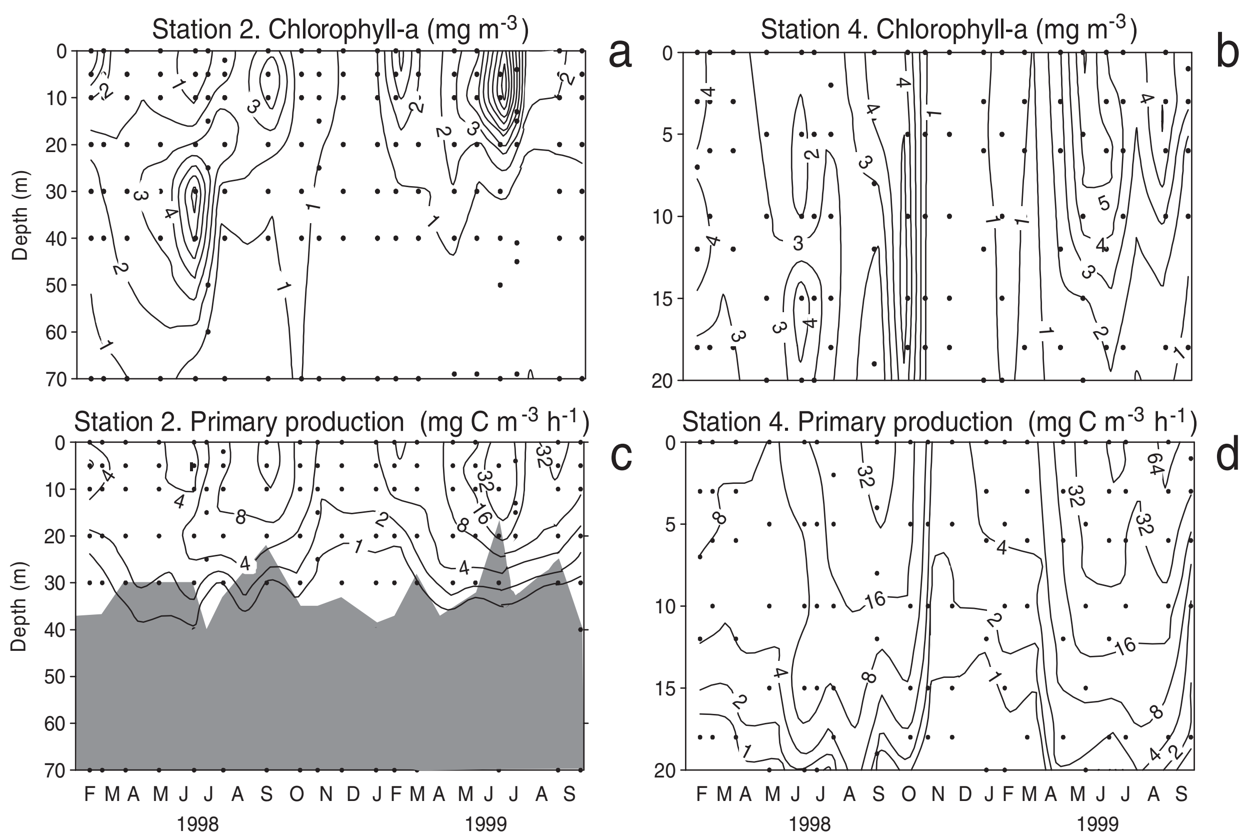

FIG. 6. – Distribution of chlorophyll-a concentrations and primary production during the study at stations 2 (a, c) and 4 (b, d). The shaded area indicates the layer below the euphotic zone (i.e. < 1 % of surface PAR irradiance)

value of 3.58 (± 1.6) x 10^{17} cell (mol leucine)$^{-1}$ (Table 3). Taking into account the mean biomass values computed (Table 1), the resulting conversion factors for stations 2 and 4 were 5.11 and 4.57 kg C (mol leucine)$^{-1}$ respectively.

Environmental variability

Upwelling conditions were detected in May and July 1998, and in June and September 1999 (Figs. 5a and 5b). The upwelling events were traced at both stations, although more clearly at station 2. The downwelling situations were observed at the end of September in both years, and they were characterised by a deepening of the mixing layer (Figs. 5c and 5d) caused by the accumulation of surface water towards the coast.

There were interannual differences in the timing of the stratification and mixing periods. In 1998, the stratification period at station 2 began in April and continued until November (Fig. 5a). In 1999, the stratification began in February and remained until September, and even when no thermal stratification was observed in January and April of this year there was a thin layer of haline stratification near the surface. Nevertheless, in both years the maximum depth of the mixing layer during the stratification period (58 and 65 m respectively) was observed in September during downwelling conditions.

Similar changes were observed at the coastal station. In 1998 the pycnocline of this station was formed in March and could be recognised until September 1998 (Figs. 5b and 5c). The maximum depth of the mixing layer during the stratification period was observed in June. In 1999 the stratification period began in February and remained until September. As at station 2, there was no thermal stratification in April 1999, but there was haline stratification. (Fig. 5d). The upper mixing layer reached a maximum of 10 m depth in June, July and at the end of September.

Phytoplankton biomass and production

The main phytoplankton blooms occurred between February and September (Fig. 6). The highest values of biomass and production coincided with or occurred immediately after the upwelling pulses. Biomass maxima in the water column were

TABLE 4. – Surface-to-bottom integrated values of phytoplankton biomass (PB), primary production (PP), bacterial biomass (BB), bacterial production (BP) and concentration of particulate organic carbon (POC). Biomass and POC values are given in mg C m^{-2}, while production values are given in mg C m^{-2} d^{-1}. SD: standard deviation, n: number of cases.

	Station 2					Station 4				
Date	PB	PP	BB	BP	POC	PB	PP	BB	BP	POC
Feb-1998	5895	895	998	382	4666	3741	1061	295	—	2719
Mar-1998	3282	86	1299	721	6449	2059	252	333	571	3046
Apr-1998	8427	1292	987	887	—	3945	1606	242	723	3262
May-1998	7016	875	773	632	6402	2893	1046	253	247	3795
Jun-1998	12234	1269	507	296	6900	3196	2387	171	306	3132
Jul-1998	5037	1937	609	443	4646	2861	2835	206	149	2900
Aug-1998	5182	2240	1058	502	8214	3043	2830	239	347	5070
Sep-1998	6270	3453	677	79	8441	2427	3682	175	43	4725
Oct-1998	5040	1092	764	167	8107	4699	1983	180	55	2927
Nov-1998	2185	462	415	1010	6512	540	257	—	190	2583
Dec-1998	2237	440	421	56	7800	717	272	116	86	3207
Jan-1999	2089	464	497	54	4725	702	365	142	88	3456
Feb-1999	5513	1100	594	54	5271	1599	556	121	63	1848
Mar-1999	1532	378	544	102	—-	522	332	111	69	—
Apr-1999	6377	3572	392	262	6519	1293	1766	123	110	1657
May-1999	4703	4147	433	454	4963	2943	6423	127	1132	2857
Jun-1999	13496	12543	634	212	7220	4201	6952	272	96	4844
Jul-1999	2657	2367	478	89	5729	1905	2944	275	34	2446
Aug-1999	2925	3543	443	229	6067	3241	6420	186	209	2322
Sep-1999	3405	1699	—	478	6607	1490	1771	—	253	2523
Mean :	5275	2193	659	355	6402	2401	2287	198	251	3122
SD :	3202	2723	259	285	1239	1277	2126	69	281	936
n :	20	20	19	20	18	20	20	18	19	19

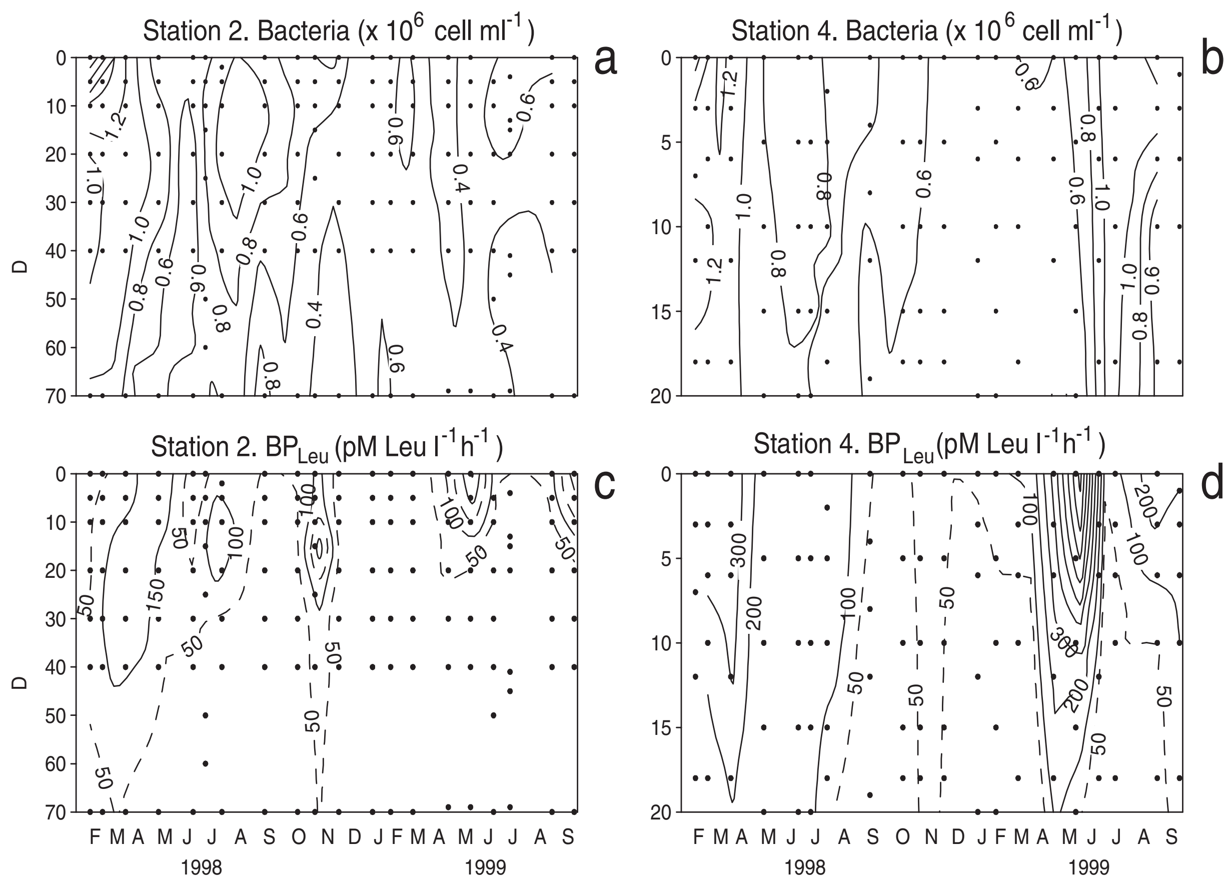

FIG. 7. – Distribution of bacterial abundance and leucine incorporation during the study at stations 2 (a, c) and 4 (b, d).

located near the surface or in a subsurface layer (e.g. at 30 m depth at station 2 in June 1998), whereas the peaks of photosynthetic production were generally situated near the surface (i.e. in the upper 10 m). Minimum values were obtained during winter mixing conditions (between November and January), when biomass and production values were low and without fluctuations. During downwelling situations the values of biomass and production were relatively high, but lower than those observed during upwelling conditions. Integrated phytoplankton biomass ranged from 1532 to 13496 mg C m^{-2} at station 2 and from 522 to 4699 mg C m^{-2} at station 4 (Table 4). Correspondingly, primary production ranged from 86 to 12543 mg C m^{-2} d^{-1} at station 2, and from 252 to 6952 mg C m^{-2} d^{-1} at station 4 (Table 4).

Bacterial biomass and production

Bacterial abundance varied between 0.1 and 1.8 x 10^6 cells ml^{-1}, with a very similar pattern in both stations (Fig. 7). Two annual maxima were observed, in spring and summer, and values remained relatively high until autumn. The vertical distribution in the water column showed peaks generally located above the pycnocline. In fact, the pycnocline was a discontinuity in the abundance profiles, below which the distribution of bacterial abundance was fairly homogenous. However, in some cases the peaks of bacterial biomass were located near the bottom (e.g. February and April 1999 at station 2), probably due to the resuspension of sediments in periods of high vertical mixing. Water-column integrated bacterial biomass ranged from 392 to 1299 mg C m^{-2} at station 2 and from 111 to 333 mg C m^{-2} at station 4 (Table 4).

Seasonal changes in bacterial production (Figs. 7c and 7d) followed those of abundance and were very similar at both stations. Two annual maxima were found, one in mid- spring and the other in summer, the latter lower than the former. In autumn and winter production values were low with the exception of the results obtained in November, when the highest values of production of the whole study were observed at both stations. However, low bacterial production values were also found in some summer samples, such as those of July 1999.

Water-column integrated values varied between 54 and 1010 mg C m^{-2} d^{-1} at station 2 and between 34 and 1132 mg C m^{-2} d^{-1} at station 4 (Table 4), which represents a wider range of variation than those found for biomass.

Relationships between bacteria and phytoplankton

As expected, there was a significant, positive correlation between bacterial production and bacterial biomass (Table 5), although it only accounted for < 12% of the variance in production. In turn, biomass and production of bacteria were significantly correlated with most environmental and phytoplankton variables (Table 5). Negative correlation values between bacterial production and depth, salinity and σ_t were the consequence of the location of maximum production values near the surface, and their occurrence mostly in spring and summer. Bacterial biomass was negatively correlated with depth, but not with salinity or σ_t. However, both bacterial variables were positively correlated with temperature, POC, chlorophyll and primary production.

The deeper water-column at station 2 than at station 4 can be related to some of the differences found in sestonic and bacterial variables between the two stations (Table 6). For instance, water-column integrated values of PB, POC and BB at station 2 were significantly higher than those at station 4. However, when volumetric data were considered, the values of PB and POC (but not BB) at station 4 were significantly higher than those at station 2. Such differences were maintained in the ratios between BB and PB or POC, which reached signifi-

TABLE 5. – Values of Pearson correlation coefficients between bacterial variables and some environmental variables calculated from the volumetric values. Significance levels are indicated by asterisks: (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, n.s.: non significant differences ($p > 0.05$). The number of data used in each correlation is indicated between brackets. S: salinity, t: temperature, σ_t: density, POC: particulate organic carbon, Chl-a: chlorophyll-a, PP: primary production, BB: bacterial biomass, BP: bacterial production.

	BB	BP
Depth	-0.23 *** (n = 223)	-0.23 *** (n = 232)
S	n.s. (n = 223)	-0.34 *** (n = 232)
t	0.31 *** (n = 223)	0.30 *** (n = 232)
σ_t	-0.22 ** (n = 223)	-0.45 *** (n = 232)
POC	0.31 *** (n = 203)	0.34 *** (n = 213)
Chl-a	0.27 *** (n = 222)	0.29 *** (n = 231)
PP	0.15 * (n = 185)	0.29 *** (n = 194)
BB	—	0.18 ** (n = 215)
BP	0.34 *** (n=207)	—

TABLE 6. – Results of Mann-Whitney tests of the differences between mean values at stations 2 and 4 using either water-colum integrated or volumetric data of phytoplankton biomass (PB), particulate carbon (POC), primary production (PP), bacterial biomass (BB), bacterial production (BP), bacterial growth rate (μ), and the ratios BP/PB, BP/PP and BB/POC. Significance levels are indicated by asterisks: (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$, n.s.: non significant differences ($p > 0.05$), n: number of cases. The ranking of significant mean values is indicated in brackets where applicable.

Variable	n	Integrated values Significance	n	Volumetric values Significance
PB	40	*** (St 2 > St 4)	239	*** (St 2 < St 4)
POC	37	*** (St 2 > St 4)	220	*** (St 2 < St 4)
PP	40	n.s.	200	* (St 2 < St 4)
BB	37	*** (St 2 > St 4)	223	n.s.
BP	39	n.s.	232	*** (St 2 < St 4)
BB/PB	37	* (St 2 > St 4)	222	*** (St 2 > St 4)
BB/POC	34	* (St 2 > St 4)	203	*** (St 2 > St 4)
BP/PP	39	n.s.	200	n.s.
μ	36	* (St 2 < St 4)	232	*** (St 2 < St 4)

cantly higher values at station 2 than at station 4, either using integrated or volumetric data. Such differences indicate that, despite the differences in depth between stations, average phytoplankton and seston concentrations were higher near the coast, while bacterial biomass was more constant. Furthermore, the values of both PP and BP were not significantly different between stations for water-column integrated data, but volumetric values at station 4 were significantly higher than those at station 2, indicating a relative enhancement of both phytoplankton and bacterial production rates near the coast. Also, station 4 displayed significantly higher bacterial growth rates (water-column mean ± SD = 0.71 ± 0.52 d^{-1}, n = 17) than station 2 (0.40 ± 0.28 d^{-1}, n = 19), which are equivalent to average biomass

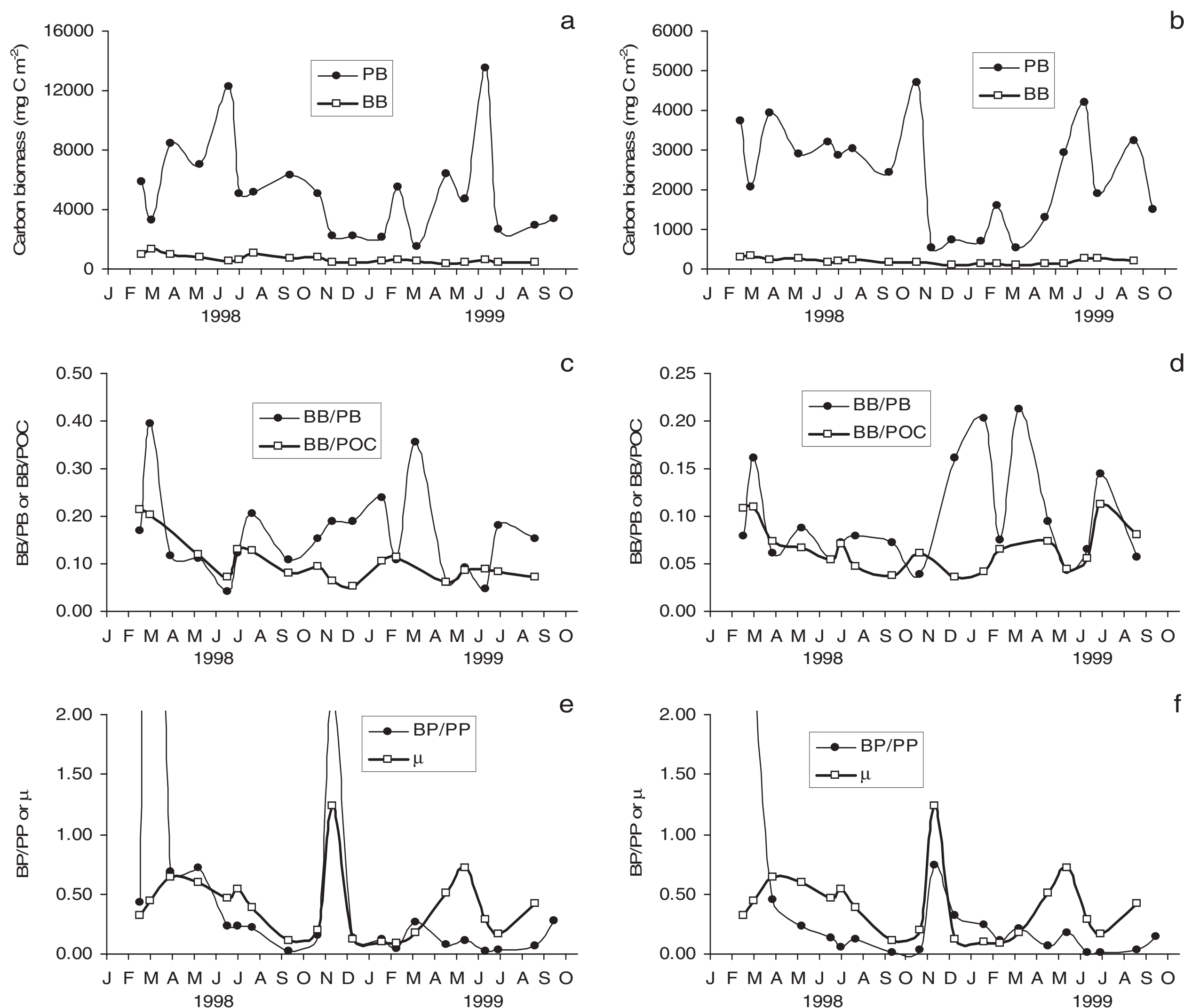

FIG. 8. – Seasonal variation of surface-to-bottom integrated PB abd BB, along with ratios BB/PB, BB/POC and BP/PP, and bacterial growth rates (μ) for station 2 (a, c, e) and station 4 (b, d, f).

duplication times of 0.98 and 1.73 days respectively. Interestingly, the BP increase was, on average, proportional to that of PP, since there were no significant differences between stations in the BP/PP ratio either using integrated or volumetric data (Table 6).

Water-column integrated bacterial biomass was always lower than phytoplankton biomass. At station 2, BB/PB ratio ranged from 4 to 40%, while at station 4 it ranged from 4 to 21% (Fig. 8). As occurred with BB/PB, BB/POC ratio values displayed a wider range at station 2 (5 to 21%) than at station 4 (4 to 11%). The variability in the ratios between bacterial and phytoplankton or seston carbon stocks and between production of bacteria and phytoplankton was related to the appearance of the main blooms of phytoplankton (Fig. 8). The occurrence of these blooms, marked by high increases in water-column integrated phytoplankton biomass, could not be traced as correspondingly high increases in BB at any of the studied stations. Also, relative increases in average BB/POC or BB/PB ratios were displaced in time between the main peaks of phytoplankton, and a similar pattern was found in bacterial growth rates and BP/PP ratios. However, there were occasional cases in which the peaks in PB were coincident with peaks in bacterial growth rates, as in April 1998 at both stations and in August 1999 at station 4 (Fig. 8). It must be noted that the values of BP/PP exceeding 100% observed in March 1998 at both stations coincided with the lowest value of primary production, while the high value in November 1998 was caused by an increase in bacterial production.

DISCUSSION

Estimation of bacterial production

The main methodological aspects that affect the estimation of bacterial production using the method of leucine incorporation are the utilisation of a saturating leucine concentration and the conversion from leucine incorporation to cells and carbon units (Kirchman, 1993). Large additions of leucine are used to avoid the utilisation of unlabeled leucine and for repressing *de novo* synthesis of intracellular leucine (Kirchman, 1993). There are not many publications with experimental determinations of the saturating concentrations in natural populations of marine bacteria. Therefore, while Kirchman (1993) recommends a final concentration of 10 nM, Wiebinga *et al*. (1997) in bacteria from the Somalia Current and Gasol *et al*. (1998) in the Mediterranean found a saturating concentration of 20 nM. These values contrast with the results obtained in the saturation experiments of this study (where the leucine incorporation saturates at > 100 nM), which could led to an overestimation of BP because of an artificial stimulation of the bacteria. However, recent studies on the Galician shelf showed saturating leucine concentrations of 160 nM, which are equivalent to those employed in this study (Barbosa *et al*., 2001). Also, Van Looij and Rieman (1993) concluded that it was necessary to add up to 230 nM of leucine to minimise isotope dilution in bacteria from an eutrophic fjord. Moreover, there are studies showing that the addition of leucine at concentrations > 100 nM does not artificially stimulate bacterial production during short incubations (Kirchman *et al*., 1985; Chin-Leo and Kirchman, 1988; Simon and Azam, 1989; Van Looij and Riemann, 1993).

The second important aspect affecting the estimation of bacterial production (and also bacterial biomass) is the carbon content per cell. The mean carbon content obtained in this study (14.25 fg C $cell^{-1}$ at station 2 and 12.75 fg C $cell^{-1}$ at station 4) are equivalent to those found in other studies, such as those of in bacteria from the Galician margin (6.1-12.6, Barbosa *et al.*, 2001), from the Bay of Biscay (11.35 fg C $cell^{-1}$, Iriberri *et al*., 1990), from the Nordic fjords (from 7 to 19 fg C $cell^{-1}$, Fagerbakke *et al*., 1996) and even from oceanic zones (12.4 fg C $cell^{-1}$, Fukuda *et al*., 1998). Nevertheless, our results are lower than those of the majority of the studies about marine bacterioplankton (20 fg C $cell^{-1}$, Lee and Fuhrman, 1987), as well as those in recent determinations in coastal and oceanic bacteria from other places (30.2 fg C $cell^{-1}$, Fukuda *et al*., 1998; 17 fg C $cell^{-1}$, Bode *et al*., 2001), which suggest that bacterial biomass estimations of this study are conservative.

In contrast, the conversion factor between leucine and carbon production used in this study is higher than those employed in other studies (Table 7), including the theoretical range of 1.5-3.1 Kg C mol^{-1} suggested by Simon and Azam (1989). For instance, had we applied the theoretical conversion factor, the values of bacterial production of stations 2 and 4 would have been 0.67 and 0.61 times lower respectively. However, the mean leucine-to-cell conversion factor employed in our computations was the average of 6 different experiments made at different sampling times and covering extreme situa-

TABLE 7. – Conversion factors between leucine incorporation (mol), increase of the cells number and carbon production in different studies. The range or the mean value is indicated.

Conversion factor in cell units (10^{17} cells mol^{-1})	Conversion factor in carbon units (Kg C mol^{-1})	Reference	Observations
	1.5-3.1	Simon and Azam (1989)	Theoretical
0.7[a]		Hoch and Kirchman (1993)	Estuary
0.6-1.7		Li *et al.*(1993)	Open ocean
0.2-3.0		Carlson *et al.* (1994)	Open ocean
0.2-3.3		Kirchman (1992)	Open ocean
	0.3-2.1	Gasol *et al.* (1998)	Coast and open ocean
0.7-2.5	0.4-4.3	Bode *et al.* (2001)	Open ocean
	0.2-0.8	Barbosa *et al*. (2001)	Shelf waters
	2.3[a]	Biddanda *et al.* (1994)	Coast
2.1-6.7	4.6-5.1	This study	Coast

[a] mean value.

tions within the study area. Moreover, the absolute values of bacterial carbon production obtained are not particularly high when compared with other studies in marine ecosystems (Cole *et al.*, 1988; White *et al*., 1991). Therefore, a general overestimation of BP because of the use of high conversion rates in our study is not likely.

Spatial variations

The differences in bacterial growth rate between the two stations, caused mainly by the relatively higher values of BP at the coastal station compared to those observed on the shelf, suggest the existence of different bacterial populations living in each environment or, alternatively, the existence of an environmental difference at the coastal station enhancing bacterial growth. In support of the first hypothesis we noted that, though mean size was not significantly different between stations, bacteria from station 2 displayed a high variability in individual size, which suggests the existence of a highly diverse population. On the other hand, high BP near the coast may be the consequence of high concentrations of organic susbtrates which would be readily used by bacteria. Although measurements of dissolved organic carbon concentrations or production rates were not available for the coastal station during the study, we found significantly higher POC and PB at the coastal station than at the shelf station. Such a difference could also be paralleled by dissolved organic carbon, as found at other locations of Galicia (Doval *et al.*, 1997). Other studies have also noted the high availability of dissolved organic carbon for bacterial growth near the coast compared to offshore environments (Ducklow and Carlson, 1992; Ducklow, 2000).

Temporal variations

The variations in oceanographic conditions and phytoplankton reported in this study agree with those described for this ecosystem (Valdés *et al*., 1991; Casas, 1995; Bode *et al*. 1996; Varela *et al*. 1996; Casas *et al*., 1997, 1999; Bode and Varela, 1998; Barquero, 1999), although the maximum value of primary production found during the upwelling of June 1999 (12543 mg C m^{-2} h^{-1}) is the highest ever recorded in this ecosystem.

Bacterial abundance values in this study are within the range found before at station 2 between January 1995 and December 1997 (0.6-30.6 x 10^5 cell ml^{-1}, Barquero, 1999), as well as within the range of the published values for other coastal areas in northern Spain (Hanson *et al*., 1986; Unanue *et al*., 1992; Tenore *et al*., 1995; Barquero *et al*., 1998; Morán *et al*., 1999; Barbosa *et al*., 2001; Varela *et al*., in press), with the exception of abundance values of highly eutrophic systems such as the Ría de Vigo (4.9-62.0 x 10^5 cell ml^{-1}, Zdawnoski and Figueiras, 1997). The relatively low variation in bacterial abundance when compared with other variables (e.g. bacterial production, phytoplankton production and biomass) was also noted in other studies (Carlson *et al*., 1996; Wiebinga *et al*., 1997)

In relation to bacterial production, there are no previous measurements on the coast of A Coruña, although some studies made some estimates of bacterial production from biomass and conversion factors from the literature (e.g. Bode and Varela, 1994; Barquero, 1999). In this study, the minimum value of bacterial production (4.8 x 10^{-2} mg C m^{-3} d^{-1}) coincides with the minimum found by Tenore *et al.* (1995) in samples from the Galician shelf, while the maximum production (145.2 mg C m^{-3} d^{-1}) is higher

than most values reported (1.0-3.8 mg C m^{-3} d^{-1}, Hanson *et al*., 1986; 13.9-48.6 mg C m^{-3} d^{-1}, average of cold and warm season respectively, Unanue *et al*., 1992; 0.1-28.7 mg C m^{-3} d^{-1}, Tenore *et al*., 1995; 0.9-48.4 mg C m^{-3} d^{-1}, Barquero *et al*., 1998; 0.13-3.52 mg C m^{-3} d^{-1}, Barbosa *et al*., 2001), but is similar to values recently measured in the nearby Ría de Ferrol (0.3-162.6 mg C m^{-3} d^{-1}, Varela *et al.*, in press). Cole *et al*. (1988) found a mean value (± SD) for the bacterial production of marine and freshwaters planktonic ecosystems of 26 ± 33 mg C m^{-3} d^{-1}, while White *et al*. (1991) cited a mean value for marine ecosystems of 18 mg C m^{-3} d^{-1}. Considering that the mean value (± SD) of this study is 12 ± 17 mg C m^{-3} d^{-1}, we can conclude that the measured values of bacterial production were low in comparison with most aquatic ecosystems. However, these comparisons must be interpreted in a rather general way given the different bacterial biovolumes, radiolabeled substrates and concentrations used to estimate incorporation of carbon by bacteria in different studies.

When considering factors controlling bacterial production, some studies in upwelling areas found a negative relationship between upwelling pulses and bacterial production (Sorokin and Kogelschatz, 1979; Sorokin, 1981; Hanson *et al.*, 1986; Bak and Nieuwland, 1993; Tenore *et al*., 1995; Barquero *et al*., 1998), concluding that the latter decays during the initial stages of the upwelling process but increases as the upwelling relaxes. However, in this study there was no clear relation between upwelling pulses and bacterial production, probably because the sampling frequency employed was not very suitable for the study of this relationship. Also, temperature was generally an important factor influencing bacterial production in most ecosystems (White *et al*., 1991; Ducklow and Shiah, 1993) but in our results it only explained less than 9% of the variance in bacterial production.

Bacteria and phytoplankton

Bacterial biomass was always lower than phytoplankton biomass, as expected in a eutrophic area (Gasol *et al*., 1997). Ducklow and Carlson (1992) affirm that the BB/PB ratio of coastal zones is generally < 0.2, which is consistent with our results for the coastal station, but not for station 2, where values of this ratio were frequently > 0.2. The seasonal variation of the ratio BB/PB reflects mostly seasonal changes in phytoplanktonic biomass, so this ratio is high when the phytoplankton biomass is low and conversely, as bacterial biomass was relatively less variable. Our results also agree with published regressions between bacterial abundance and chlorophyll-a, which had slope values < 1 (Ducklow and Carlson, 1992; Gasol *et al*., 1997; Gasol and Duarte, 2000, and references therein), indicating that the relative contribution of bacteria to total planktonic biomass decreases when autotrophic biomass increases. The BB/POC ratio values found in this study were higher than those found by Varela *et al*. (1988) in a late spring bloom in the Galician coast and lower than those of a spring bloom in the North Atlantic (Ducklow *et al*., 1993). Nevertheless, the value of BB/POC estimated in the Galician margin (Barbosa *et al*., 2001) and at neritic stations in the Bay of Biscay during summer (Barquero *et al*., 1998) are close to the range found in this study.

According to Cole *et al.* (1988) the mean value for the integrated BP/PP is 0.3 for different aquatic ecosystems, although recently Ducklow (2000) suggested that the average value would rather be between 0.1 and 0.2. Barbosa *et al*. (2001) reported variations of BP/PP ratio near 0.7 during upwelling episodes on the south Galician shelf. During our study most values of the BP/PP ratio were < 0.3, but in some situations (as in early spring or in autumn) this ratio can be >1. Such extremely high values can be interpreted as the delayed response of bacterial activity after a phytoplankton bloom. In this regard, there are reports of BP/PP ratio values of between 0.5 and 1 after phytoplankton blooms (Lucas *et al*., 1986; McManus and Peterson, 1988; Ducklow, 1993), suggesting that these situations were non-steady, transient periods of biomass decomposition (Wiebinga *et al*., 1997). In contrast, during periods of low primary production bacterial activity would be closely coupled to the release of phytoplanktonic dissolved organic matter. Although in our results bacterial production was significantly correlated with phytoplankton biomass and production, the influence of these variables on bacterial production within the studied ecosystem was notably lower than in cross-system analysis (Cole *et al*., 1988).

In conclusion, planktonic bacteria constitute a significant, but low, fraction of POC in the pelagic ecosystem of A Coruña, characterised in general by high biomass of phytoplankton. Despite a relatively constant biomass, bacterial production can reach values far exceeding concurrently measured primary production rates. Such excesses in bacterial production are likely to occur after phytoplankton blooms. The higher bacterial growth rates at the coastal site

than at the shelf station were probably the consequence of differences in the bacterial communities living in each environment, but also of the high concentration of organic substrates near the coast.

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