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Total and phytoplankton mediated bottom-up control of bacterioplankton change with temperature in NE Atlantic shelf waters

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ABSTRACT: The regulation of heterotrophic bacterial growth by resource supply (bottom-up control) was temperature-dependent in our analysis of data obtained during 2006 in the euphotic layer of the southern Bay of Biscay (NE Atlantic) continental shelf. The dataset was split into 2 subgroups using 16°C as the boundary between warm and cool waters based on differences in associated physico-chemical conditions, e.g. inorganic nutrient limitation at higher temperatures. The linear regressions between bacterial biomass (BB) and leucine incorporation rates (LIR) were significantly positive in both temperature regimes, thus indicating similar total bottom-up control, albeit with a slightly higher slope in warm waters (0.33 vs. 0.22). However, the relationship of LIR with phytoplankton biomass (chl *a*), which is an indicator of bottom-up control that is mediated by phytoplankton, was only significant in waters below 16°C. The analysis of bimonthly variations in the BB-LIR and LIR-chl *a* correlations indicated that the strength of total bottom-up control significantly increased while the role of phytoplankton in supplying DOM to bacteria diminished with mean temperatures over the 12 to 19°C range, suggesting a seasonal switch in the major source of substrates used by bacteria. We show that the abundance of cells with relatively high nucleic acid content (HNA), which are hypothesized to be the most active ones, was positively associated with bacterial production and specific growth rates in cool but not in warm conditions. These results suggest that HNA bacteria are good predictors of bulk activity and production in temperate ecosystems only when the community relies principally on phytoplankton substrates for growth and metabolism.

KEY WORDS: Bacterioplankton · Bottom-up control · Temperature · Bacterial biomass · Bacterial activity · Phytoplankton · Coastal waters

INTRODUCTION

Heterotrophic bacteria play a key role in aquatic ecosystems through their assimilation of dissolved organic matter (DOM) to sustain their metabolism (respiration) and build up new biomass (production). Bacterial production and biomass have been consistently shown to covary with the corresponding phytoplankton variables both within and across systems (Cole et al. 1988, Gasol & Duarte 2000, Kirchman et al. 2009), which has been interpreted as an indirect proof of regulation by substrates provided by planktonic primary producers. However, bacteria may also rely on DOM sources other than fresh production by phytoplankton; these may either be autochthonous or allochthonous, e.g. DOM produced after photochemical transformation of the ambient stocks of organic matter (Lindell et al. 1995) or terrestrial DOM in areas with important riverine inputs (Raymond & Bauer 2001, Anderson & Turley 2003). If bacteria are controlled by substrate supply but this bottom-up control is not directly exerted by phytoplankton, a lack of a significant relationship with algal variables would be expected (Findlay et al. 1991). Following Billen et al. (1990), a method of estimating the strength of bottom-up control on bacteria was proposed by Ducklow (1992) through an

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examination of the slopes and significance of Model I (ordinary least squares) linear regressions between log-log bacterial biomass and production. Bacterial production is thus taken as equivalent to the rate of substrate supply. This approach assumes roughly uniform growth rates within a given ecosystem. Briefly, significant slopes between 0.2 and 0.4 would indicate weak control, between 0.4 and 0.6 moderate control and >0.6 strong control (Ducklow 1992). Values from different marine regions suggest that the highest slopes are to be found in eutrophic regions, with low slopes or nonsignificant relationships between bacterial biomass and production being usually found in nutrient poor waters (Gasol et al. 2002).

Besides bottom-up control (Church 2008), top-down processes (bacterivory and viral lysis) and temperature also shape actual levels of bacterial biomass and production (Shiah & Ducklow 1994b, Jürgens & Massana 2008). Temperature has been frequently included among bottom-up controls (e.g. Hale et al. 2006, Kirchman et al. 2009), but since it is not a resource that can become limiting, we decided to consider it separately in this study. Therefore, bottom-up control here refers only to that exerted by DOM supply. The interplay between substrate supply and temperature gave rise to extended discussion of what controls bacterial growth in polar waters (Pomeroy & Deibel 1986, Pomeroy & Wiebe 2001, Yager et al. 2001, Kirchman et al. 2009). Different controlling factors frequently operate simultaneously on bacterial communities, rendering it unlikely to identify any single critical factor, although seasonal switches in the relative importance of temperature and bottom-up control have been documented (Shiah & Ducklow 1994b, Shiah et al. 2003). Nevertheless, in coastal areas that are deprived of important allochthonous sources, we would expect substrate supply to have a preponderant role year round as opposed to the greater effect of top-down processes in oligotrophic environments (Gasol et al. 2002). Here, we evaluate the importance of temperature in the bottom-up control of planktonic bacteria by analyzing the relationships between bacterial and phytoplankton properties under cool (<16°C) and warm (>16°C) conditions in samples taken over an annual cycle in a temperate shelf sea in the northeastern Atlantic Ocean. These 2 temperature regimes are consistently associated with distinct physico-chemical characteristics in this ecosystem: mixed, nutrient-sufficient conditions during the cool period and stratified, nutrient-depleted conditions during the warm period extending from summer well into fall (Fernández & Bode 1991, Calvo-Díaz & Morán 2006, Llope et al. 2007).

In marine ecosystems, 2 groups of planktonic bacteria with distinct relative nucleic acid contents (high, HNA, and low, LNA) (Li et al. 1995) have been reported. These groups are generally associated with different degrees of activity (Gasol et al. 1999, Lebaron et al. 2001, Longnecker et al. 2006) and occur universally, permitting us to also assess the response of LNA and HNA cells to bottom-up controls in the 2 temperature regimes and determine the predictability of bulk bacterial activity from variations in HNA cell abundance (Morán et al. 2007). Independent regulation of LNA and HNA groups is suggested in our analysis in accord with other authors (Bouvier et al. 2007). We finally conclude that variations in HNA bacterial abundance are a good proxy for total bacterial production and growth rates only when bottom-up control on bacterial communities is likely exerted by phytoplankton.

MATERIALS AND METHODS

The study area is located in the central Cantabrian Sea (southern Bay of Biscay) continental shelf, for which heterotrophic bacterioplankton dynamics have been described (Barquero et al. 1998, Calvo-Díaz & Morán 2006, Morán & Calvo-Díaz 2009). Data analyzed here were obtained at 3 stations over the continental shelf (Stn 1: 43.58° N, 5.61° W; Stn 2: 43.67° N, 5.58° W; Stn 3: 43.78° N, 5.55° W) that were routinely sampled within the Spanish Institute of Oceanography (IEO) time-series programme RADIALES off Xixón. Environmental data including temperature, salinity, inorganic nutrient and chlorophyll *a* concentrations (chl *a*, µg l^{-1}) were taken monthly during 2006 at 8 depths (from the surface down to 100 m) and analyzed following conventional protocols (Calvo-Díaz & Morán 2006).

The abundance and biomass of LNA and HNA bacteria were estimated by flow cytometry as detailed elsewhere (Calvo-Díaz et al. 2004, Calvo-Díaz & Morán 2006). Cellular carbon content was calculated from biovolume, which was in turn estimated from right angle light scatter flow cytometry signals as described by Calvo-Díaz & Morán (2006). Total bacterial biomass (BB) was estimated as the sum of the biomass of the LNA and HNA groups. Bacterial activity was estimated from leucine incorporation rates (LIR, pmol Leu l^{-1} h^{-1}), and subsequently converted to bacterial production (BP) using empirically determined leucine to carbon conversion factors (Calvo-Díaz & Morán 2009). In addition to estimating community growth rates as BP:BB ratios, an index of individual cell activity (or growth rate) was calculated by dividing LIR by the total bacterial abundance (i.e. LNA+HNA cells). Although both indices were well correlated ($r = 0.80$, $p < 0.001$, $n = 99$), BP:BB ratios capture the seasonal variations in individual cell biomass and leucine to carbon conversion factors, while raw single-cell LIR data are also more easily compared with other studies.

Phytoplankton assemblages below the euphotic layer are unlikely to provide fresh photosynthate for bacterial growth. Hence, in order to avoid spurious correlations in the effect of phytoplankton on bottom-up control, we restricted the analysis of all bacterial and phytoplankton variables to data obtained within the euphotic layer (1% of surface incident irradiance), which ranged between 37 and 75 m for Stns 2 and 3 (Table 1). The euphotic layer depth was deeper than the bottom at Stn 1 year round.

To assess the degree of bottom-up control, log-log Model I linear regressions between bacterial biomass and activity, and between bacterial activity and chl *a* were calculated both for warm and cool conditions. We used LIR rather than BP because the former was considered a more direct, realistic measurement of substrate supply rate than a variable that takes into account how the incorporated leucine was actually transformed into biomass production (Calvo-Díaz & Morán 2009). We distinguished between total (BB-LIR) and phytoplankton mediated (LIR-chl *a*) bottom-up controls. To detect seasonal differences in these 2 modes of substrate control, Pearson correlation coefficients were calculated among BB, LIR and chl *a* for data pooled every 2 consecutive months (Jan–Feb, Mar–Apr, etc.). Pearson correlation analyses were also performed to determine the relationships among the abundance of LNA and HNA cells, chl *a*, and community activity and production rates. All statistical analyses were performed using STATISTICA software.

Autocorrelation may be a serious problem in statistical inference for the analyses described above (Pyper & Peterman 1998). Three possible contexts (spatial [horizontal], vertical and temporal) of autocorrelation were examined for our datasets. For every combination of stations and depths for BB, LIR and chl *a*, the autocorrelation function (up to 6 lags) failed to detect significant temporal autocorrelations. Spatial autocorrelation, however, was evident in both the horizontal and vertical scales. To account for it, we repeated the analyses after subtracting the mean annual values from individual values of BB, LIR and chl *a* at each station and depth, and the results showed the same patterns as obtained with the original dataset. Moreover, the regression and correlation analyses were also performed within stations with only surface data, thus removing all spatial autocorrelation, and yielding exactly the same conclusions, i.e. that BB-LIR and LIR-chl *a* relationships were consistently different in waters < and >16°C (see 'Results'). We concluded that the observed relationships were not caused by autocorrelation; therefore, we did not transform the original dataset (Pyper & Peterman 1998) for comparibility with previous work.

RESULTS

Fig. 1A shows the evolution of surface temperature at the 3 sampled stations during 2006. Temperatures >16°C corresponded with low inorganic nutrient con-

Table 1. Sampling date, euphotic layer depth (Z_{eu}, m) and mean euphotic layer values of temperature (°C), chl *a* (µg l^{-1}), bacterial abundance (cells ml^{-1}), the proportion of high nucleic acid cells (%, HNA), cellular carbon content (CCC, in fg C $cell^{-1}$) and leucine incorporation rates (LIR, pmol Leu l^{-1} h^{-1}) at the 3 stations

Date	Stn	Z_{eu}	Temp.	Chl *a*	Bact. abund.	%HNA	CCC	LIR
Jan 12	1	>20	12.1	1.47	8.33×10^5	55	16.7	17.0
	2	58	12.9	0.68	7.65×10^5	51	17.0	9.2
	3	58	12.9	0.46	7.29×10^5	45	15.8	3.8
Feb 08	1	>20	12.3	0.44	3.34×10^5	50	15.1	8.2
	2	57	12.4	0.54	3.43×10^5	46	15.0	7.6
	3	57	12.4	0.47	3.83×10^5	48	15.5	4.1
Mar 16	1	>20	11.8	0.97	3.73×10^5	58	16.7	20.6
	2	43	11.8	1.79	5.40×10^5	58	15.7	28.0
	3	75	11.8	1.52	5.63×10^5	60	16.8	21.4
Apr 11	1	>20	12.6	0.21	4.99×10^5	81	16.1	26.4
	2	73	12.7	0.28	4.30×10^5	76	16.3	18.6
	3	55	12.6	0.63	6.67×10^5	76	16.1	26.2
May 11	1	>20	13.1	1.27	4.29×10^5	54	17.0	39.3
	2	51	13.3	0.85	7.33×10^5	59	16.2	22.1
	3	44	13.9	0.61	1.07×10^6	58	15.7	29.4
Jun 05	1	>20	13.5	3.12	9.17×10^5	73	15.4	57.3
	2	37	13.8	2.99	7.10×10^5	62	15.8	43.0
	3	39	15.3	1.07	7.26×10^5	53	15.7	33.7
Jul 11	1	>20	18.3	0.5	6.90×10^5	54	18.7	95.2
	2	62	16.1	0.34	7.26×10^5	45	16.7	23.3
	3	51	16.2	0.3	9.05×10^5	47	16.1	31.1
Aug 08	1	>20	17.3	1.07	2.11×10^6	59	16.3	114.0
	2	55	15.6	0.52	8.89×10^5	46	16.4	17.2
	3	59	15.2	0.52	1.10×10^6	44	15.8	23.1
Sep 07	1	>20	17.8	0.6	1.42×10^6	55	14.9	44.0
	2	62	16.9	0.37	8.50×10^5	50	14.9	15.9
	3	70	17.5	0.36	8.00×10^5	50	15.0	19.8
Oct 16	1	>20	19.1	0.6	9.23×10^5	52	14.6	30.1
	2	70	18.3	0.39	5.56×10^5	50	14.9	21.3
	3	70	18.6	0.39	6.88×10^5	50	15.2	28.5
Nov 09	1	>20	16.8	0.68	8.31×10^5	64	15.3	21.6
	2	50	16.9	0.87	7.89×10^5	61	14.8	12.4
	3	55	17	0.67	7.32×10^5	57	15.3	32.0
Dec 19	1	>20	15.1	0.87	1.13×10^6	64	16.9	55.3
	2	46	15.7	0.72	8.55×10^5	59	14.8	11.2
	3	59	15.7	0.81	9.03×10^5	57	15.2	8.3

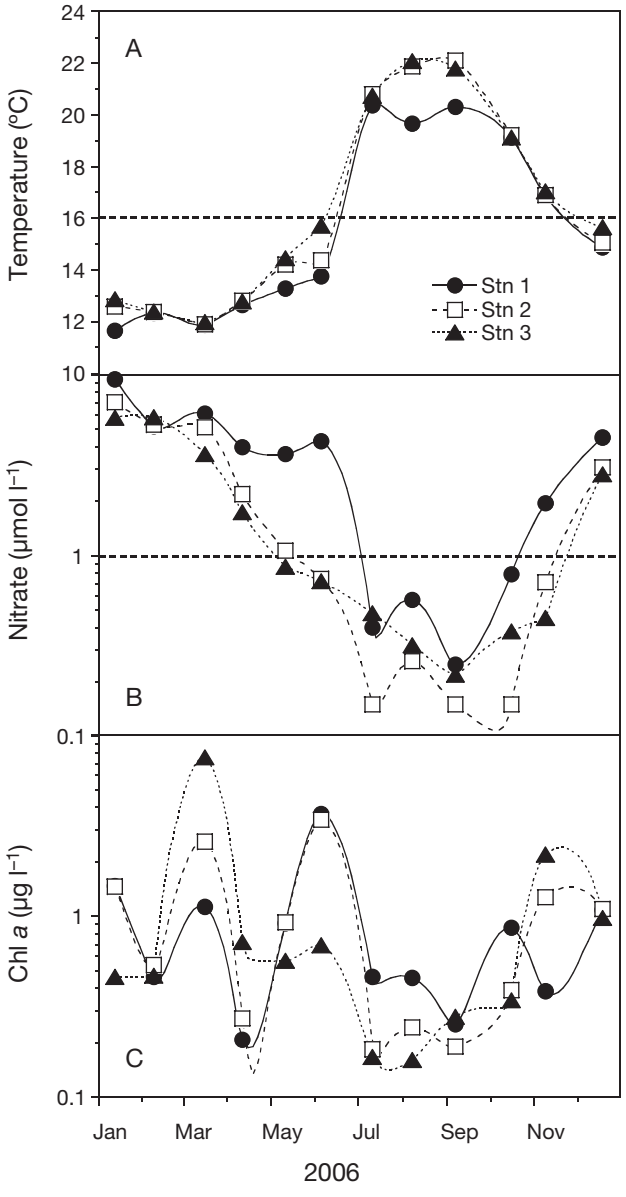

Fig. 1. Surface values of (A) temperature and concentrations of (B) nitrate and (C) chl *a* at the 3 sampling stations during 2006. Note the coincidence of limiting nitrate concentrations (<1 µmol l^{-1}) with temperatures >16°C (dashed lines)

centrations, as shown in Fig. 1B for surface concentrations of nitrate. Phosphate followed the same pattern and both nutrients strongly covaried year round within the euphotic layer ($r = 0.83$, $p << 0.001$, $n = 99$), with a NO_3 vs. PO_4 linear regression slope of 16.7. Surface values <1 µmol NO_3 l^{-1}, which is an indication of probable nutrient limitation, were always found at temperatures >16°C except for Stn 2 in June (at 14.4°C), and Stn 3 in May and June (at 14.5 and 15.7°C, respectively; Fig. 1). A strong negative correlation was found between temperature and nitrate concentration at the surface ($r = -0.90$, $p < 0.0001$, $n = 36$), which also held for the entire dataset ($r = -0.83$, $p < 0.0001$, $n = 100$). Although surface chl *a* was also correlated with temperature ($r = -0.59$, $p < 0.001$) and nitrate ($r = 0.50$, $p = 0.002$), Fig. 1C illustrates that chl *a* values were largely independent from one month to the next, precluding the existence of significant temporal autocorrelation in our dataset. Monthly variations in temperature, nutrients and phytoplankton and bacterioplankton standing stocks (Table 1) were highly consistent with previous reports (Calvo-Díaz & Morán 2006, Calvo-Díaz et al. 2008). Mean cellular carbon content of bacteria in the euphotic zone ranged between 14.6 and 18.7 fg C $cell^{-1}$ (Table 1).

As the first method of assessing control of bacteria by substrate supply, the linear regressions between bacterial biomass and activity in the 2 temperature regimes are shown in Fig. 2A. With pooled data, bacteria

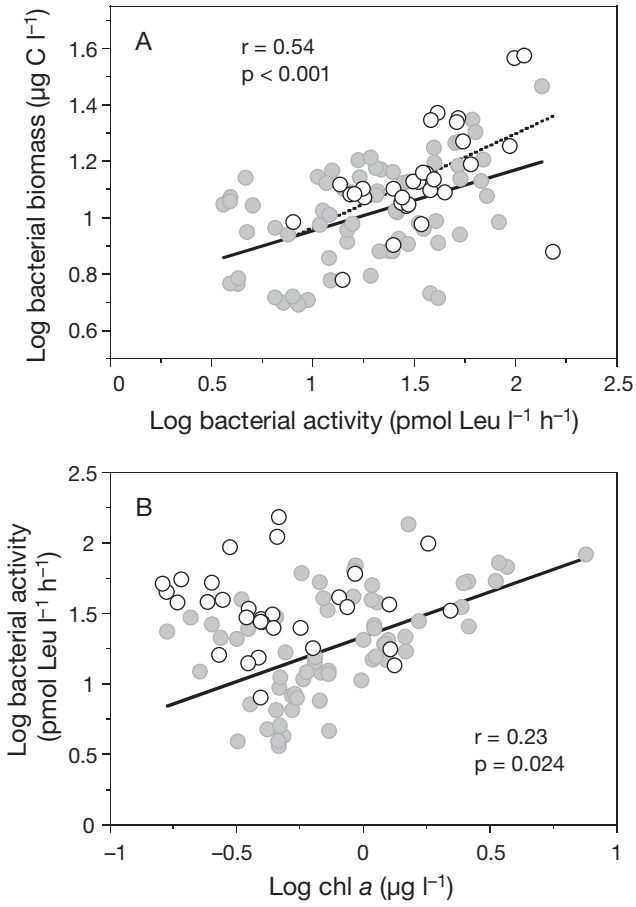

Fig. 2. Relationships between euphotic layer individual values of (A) bacterial biomass and activity, and (B) bacterial activity and phytoplankton biomass (chl *a*) during cool (<16°C, ●, solid lines) and warm (>16°C, ○, dotted line) conditions. Also indicated are the Pearson correlation coefficients for pooled (cool plus warm) data. Regression details are given in 'Results'

were weakly bottom-up controlled year round in the euphotic layer of the S Bay of Biscay continental shelf, with a slope of 0.27 ($r^2 = 0.29$, $p < 0.001$, $n = 99$). Linear regressions were log BB = 0.74 + 0.22 log LIR ($r^2 = 0.22$, $p < 0.001$, $n = 68$) for cool waters and log BB = 0.63 + 0.33 log LIR ($r^2 = 0.28$, $p < 0.002$, $n = 31$) for warm waters. Differences in the slopes and intercepts of both linear regressions were not so important as to render differences in the specific growth rates (BP:BB ratios) and cell-specific LIR between cool (mean ± SE: 0.05 ± 0.01 d^{-1} and $40 \times 10^{-21} \pm 4 \times 10^{-21}$ mol Leu $cell^{-1}$ h^{-1}, respectively) and warm waters (0.07 ± 0.01 d^{-1} and $51 \times 10^{-21} \pm 14 \times 10^{-21}$ mol Leu $cell^{-1}$ h^{-1}) significant.

Focusing on phytoplankton mediated bottom-up control, a significant correlation was found between bacterial activity and chl *a* for the whole dataset (Fig. 2B), although the percentage of total variance explained was very low (5%). However, a very different result from the BB vs. LIR analysis above was obtained when we analyzed cool and warm data separately. Bacterial activity was positively related to chl *a* in cool waters (log LIR = 1.34 + 0.64 log chl *a*, $r^2 = 0.28$, $p < 0.001$, $n = 68$) but no significant correlation was found in warm waters ($p = 0.75$).

Remarkable seasonal patterns emerged when we plotted together the correlation coefficients of BB-LIR and LIR-chl *a* calculated on a 2 mo basis (Fig. 3A). Total bottom-up control (i.e. BB-LIR correlation) was maximal from summer through autumn, while phytoplankton mediated bottom-up control (i.e. LIR-chl *a* correlation) peaked in winter and spring and was absent during the summer months. This contrasting behaviour is better shown when the corresponding r values are plotted against a seasonally varying property in the euphotic layer like temperature. Fig 3B shows opposing signs of variations over the ~12 to 19°C range of mean euphotic layer temperatures: strongly significantly increasing for BB-LIR ($r = 0.98$, $p = 0.001$, $n = 6$) and decreasing for LIR-chl *a* ($r = -0.92$, $p = 0.011$, $n = 6$).

HNA cells were slightly more abundant year round in the euphotic layer than LNA cells (mean 56 ± 2% SE), with annual maxima being found in April (~80%) and minima (~45%) being found in July–August (Table 1), as previously reported (Calvo-Díaz & Morán 2006). Fig. 4 shows the relationships of the abundance of the 2 bacterial groups with temperature in the 2 regimes. While both HNA and LNA cell abundances were positively correlated with temperature in cool waters ($r = 0.55$ and 0.57, respectively, $p < 0.001$), only LNA bacteria increased in numbers with further warming ($r = 0.42$, $p = 0.02$; Fig. 4A,B). Consequently, the percentage of HNA cells became strongly negatively correlated with temperature in waters >16°C ($r = -0.75$, $p < 0.001$) but no relationship was found below

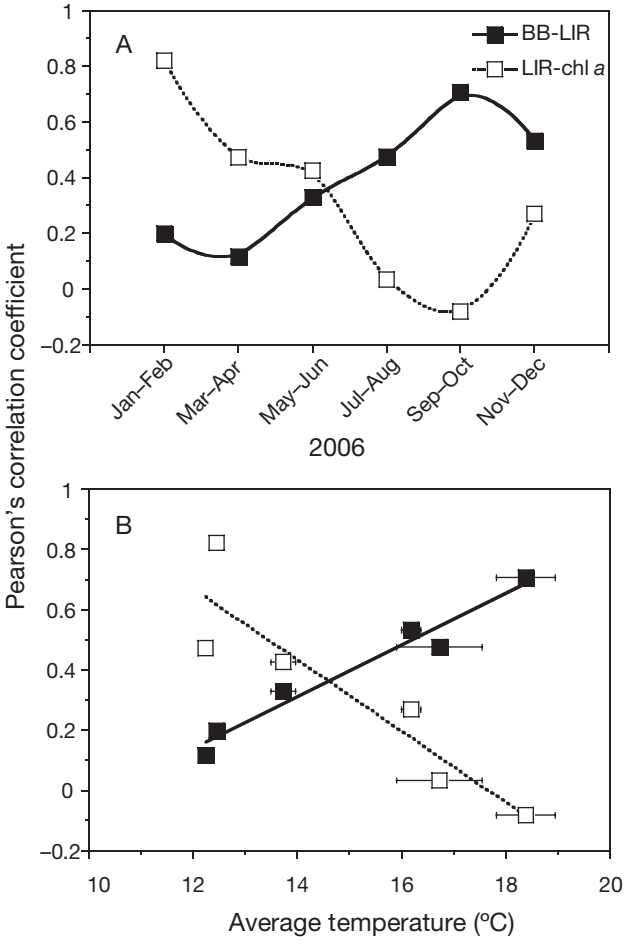

Fig. 3. (A) Monthly variations in the Pearson's correlation coefficients (r) of log BB-log LIR and log LIR-log chl *a* for euphotic layer data pooled every 2 mo. BB: bacterial biomass; LIR: leucine incorporation rates. (B) Relationship between the r in (A) and mean euphotic layer temperature (±SE). See 'Results' for details

that temperature (Fig. 4C). In contrast, LIR was significantly correlated with temperature in both temperature regimes ($r = 0.27$, $p = 0.02$ and $r = 0.37$, $p = 0.04$, respectively), with a maximum at ~20°C, despite considerable scatter in the data (Fig. 4D).

The abundance of HNA bacteria was significantly and positively correlated with LIR and BP in cool waters ($r = 0.57$ and 0.50, respectively) but not in warm waters. The correlation between LNA cell abundance and LIR was only significant in warm waters (Table 2). The proportion of HNA cells (%HNA) was significantly and positively correlated with all 4 bulk activity variables assessed (LIR, BP, BP:BB and single-cell LIR) in waters <16°C, with correlation coefficients ranging from 0.35 to 0.53, but bore no significant correlation with any of them in the warm regime. Finally, HNA cell abundance was significantly correlated with chl *a*

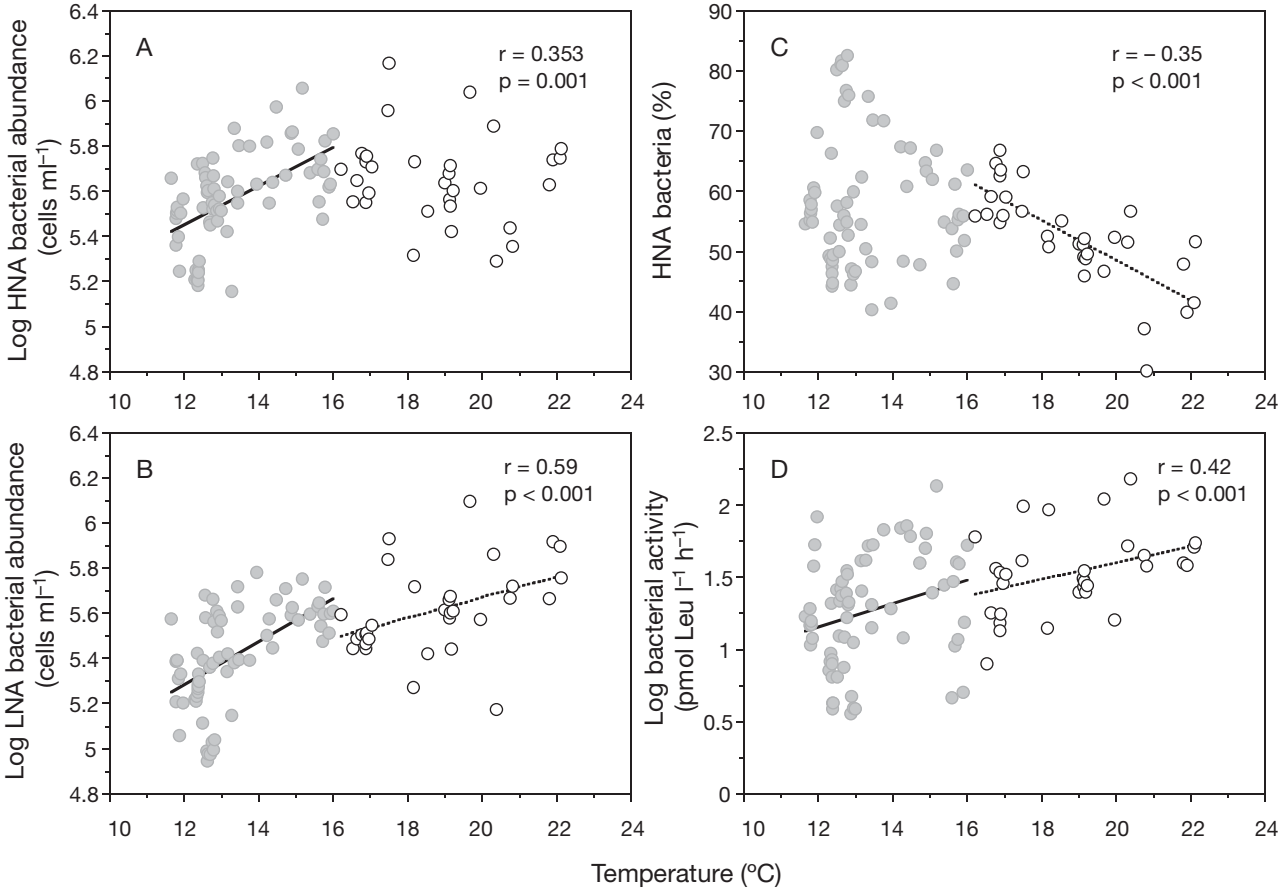

Fig. 4. Relationships between cool (●) and warm (O) individual temperatures within the euphotic layer and the (A) abundance of HNA and (B) LNA bacteria, (C) the contribution of HNA bacteria to total numbers, and (D) bacterial activity. Fitted lines represent significant Pearson correlations given in 'Results'; r and p are for the pooled (cool plus warm) dataset. HNA: high nucleic acid, LNA: low nucleic acid

Table 2. Pearson correlation coefficients (r) between the absolute abundance of total, HNA and LNA bacteria (cells ml^{-1}) and the relative abundance of HNA cells (%HNA), and chl *a* (µg l^{-1}) and bulk activity variables in cool and warm waters. HNA: high nucleic acid, LNA: low nucleic acid; LIR: leucine incorporation rates (pmol Leu l^{-1} h^{-1}); BP: bacterial production (µg C l^{-1} d^{-1}); BP:BB: growth rates (d^{-1}; BB: bacterial biomass); single-cell LIR: cell-specific LIR (×10^{-21} mol Leu cell^{-1} h^{-1}). Significant correlations ($p < 0.05$) are in **bold**

		Chl *a*	LIR	BP	BP:BB	Single-cell LIR
<16°C	log HNA	**0.29**	**0.57**	**0.50**	−0.10	0.15
n = 68	log LNA	**0.24**	0.03	−0.04	**−0.43**	**−0.36**
	log total	**0.29**	**0.40**	**0.32**	**−0.28**	**−0.28**
	%HNA	0.06	**0.53**	**0.55**	**0.35**	**0.53**
>16°C	log HNA	**0.39**	0.30	0.23	**−0.54**	**−0.31**
n = 31	log LNA	−0.16	**0.46**	0.19	**−0.59**	−0.14
	log total	0.13	**0.40**	0.21	**−0.62**	−0.25
	%HNA	**0.73**	−0.22	0.04	0.08	−0.22

in both temperature regimes, while the correlation of LNA bacterial abundance with chl *a* was only significant in cool waters below 16°C (Table 2).

DISCUSSION

The assessment of the role of resource supply in 2 temperature regimes (cool and warm) allowed us to suggest a broad seasonal difference in the prevailing source of DOM for bacterial growth and metabolism in the southern Bay of Biscay continental shelf. The use of temperature to split the dataset was chosen based on its close match with nutrient-limited and nutrient-sufficient conditions, which are generally associated with stratified and well-mixed water columns, respectively (Fig. 1). We were confident that the 16°C isotherm represented an ecological boundary in this ecosystem since the linear regression between temperature and NO_3 concentration for pooled data from the euphotic layer predicted a temperature of 16.1°C for the limiting concentration

of 1 µmol l^{-1} of nitrate. A change in some bacterial properties was observed around this temperature, as also found in other temperate waters, albeit at different temperatures (e.g. 12°C in Hoch & Kirchman 1993 and 20°C in Shiah & Ducklow 1994a). Disentangling the complex interactions of temperature and inorganic and organic resource availability was not the object of the present study. By organizing our data around temperature rather than inorganic nutrient or chl *a* concentrations, which were also related (Fig. 1), we provide a common, independent framework for future comparisons with other temperate regions.

Total and phytoplankton mediated bottom-up control

We used 2 empirical approaches from the literature to estimate the degree of bottom-up control of bacterial assemblages. The first one, considered here as total or absolute bottom-up control, relates the response of bacterial abundance or biomass to the rate of bacterial production or uptake of labelled substrates (Billen et al. 1990, Ducklow 1992). Under a steady-state assumption, the latter variables are proportional to the rate of substrate supply. A log-log plot of both sets of variables must be significant with a linear regression slope >0.20 to allow conclusion that bacteria are bottom-up controlled (Ducklow 1992). This approach has been applied in diverse ecosystems (Shiah & Ducklow 1995, Dufour & Torréton 1996, Gasol et al. 2002, Hale et al. 2006, Garneau et al. 2008). Significant relationships were found here in both temperature regimes, indicating that the ecosystem responded to higher substrate supply by accumulating bacterial biomass. The need for relatively uniform growth rates as an implicit assumption of this method was warranted by the fact that no significant differences were found in BP:BB ratios or in cell-specific LIR between cool and warm waters. A recent meta-analysis has demonstrated that the increase in bacterial growth rates with temperature is mostly restricted to extremely low temperatures (–1.8 to 2°C; Kirchman et al. 2009), which is likely linked with substrate limitation at higher temperatures (López-Urrutia & Morán 2007). Total bottom-up control using the aforementioned analysis would integrate the supply of both organic and inorganic substrates needed for bacterial growth, without any specific differentiation among possible sources.

Frequently viewed as mutually exclusive controls (Pace & Cole 1994), top-down processes may also shape bacterial communities in the presence of significant control by substrate supply (Strom 2008).The fact that total bottom-up control was weak on an annual basis (Fig. 2A) would indicate a strong role of top-down processes in this ecosystem, according to the original formulation of Billen et al. (1990) and Ducklow (1992). However, we cannot attribute all unexplained variance to control by predators in the absence of direct measurements. Other possible factors such as temperature likely play a role in the variations of the BB-LIR relationships. Temperature control indeed appeared to be important during winter months in this dataset as implied by the analysis of the slopes between LIR and temperature (A. Calvo-Díaz & X. A. G. Morán unpubl. results); this is similar to the observations in Chesapeake Bay of Shiah & Ducklow (1994b). We therefore cannot discount a significant influence of either protozoan or viral mortality on bacterial standing stocks, especially in summer (Solic & Krstulovic 1994, Solic et al. 1998). Yet, bacteria seem to be slightly more bottom-up controlled in warm conditions, coincident with low inorganic nutrient inputs (Fig. 1) and in agreement with other seasonal studies (Hoch & Kirchman 1993, Shiah & Ducklow 1994a, Shiah et al. 2003).

The other empirical method of assessing bottom-up control of bacterial communities considers the influence of phytoplankton biomass or production on corresponding bacterial variables (e.g. Cole et al. 1988, White et al. 1991). This control would be a specific case within the more general or total bottom-up control estimated from the BB-LIR relationships discussed in the previous paragraph. The good relationship between phytoplankton and bacterioplankton variables when data from diverse ecosystems are gathered stands out as 'one of the few undisputed patterns in aquatic microbial ecology' (Gasol & Duarte 2000, p. 101). The rationale for this covariation is that bacteria are dependent on a direct flux of DOM from phytoplankton for growth. Although chl *a* may be a rough estimate of dissolved primary production (Pace & Cole 1996, Morán & Estrada 2002), it is a reasonable proxy at relatively large scales of variability in phytoplanktonic biomass and production such as in annual studies (Marañón et al. 2004). The low percentage of variance that was explained in the pooled data set was due to clearly different relationships in cool and warm waters (Fig. 2B). It is worth noting that chl *a* values were similarly variable in both situations, with coefficients of variation of 106 and 83%, respectively.

Although total bottom-up control was not substantially different between cool and warm waters (Fig. 2A), bacterial activity (Fig. 2B), specific growth rates (BP:BB ratios) and cell-specific LIR (data not shown) behaved fundamentally differently in response to phytoplankton in the 2 temperature regimes. Our interpretation is that bacteria were dependent on the substrates directly supplied by phytoplankton in cool waters but not in warm waters. The explanation is that nutrient-limited phytoplankton in waters >16°C

(Fig. 1) were not able to provide enough substrates for meeting bacterial requirements; or alternatively, phytoplankton-produced DOM was of low quality (Obernosterer & Herndl 1995) so as to preclude a significant covariation between phytoplankton biomass and bacterial activity. We suggest that bacteria depend on DOM year round but this DOM must be of different origins: freshly derived from phytoplankton during cool, nutrient-sufficient months (winter–spring), and previously accumulated or allochthonously derived during warm, nutrient-limited months (summer–autumn), as suggested by Serret et al. (1999) in their seasonal study of community metabolism in the same region. A switch in the principal carbon source for bacteria from phytoplankton-produced to accumulated semilabile DOM as the growth period progressed was also suggested for the Ross Sea (Ducklow 2003). The remarkably opposed variation of the bimonthly indices of total (BB-LIR correlation) and phytoplankton (LIR-chl *a* correlation) bottom-up control with temperature (Fig. 3) gives further support to the hypothesis of a seasonal switch in the major sources of bacterial substrate in this ecosystem. Since temperature strongly covaried with inorganic nutrient concentrations (Fig. 1), similar relationships as that shown in Fig. 3B were obtained with mean nitrate concentrations.

An alternative explanation for these findings is that, rather than being a phytoplankton mediated effect, inorganic nutrient limitation directly impacted bacteria during summer (Joint et al. 2002, Mills et al. 2008).This would strengthen total bottom-up control as implied by the seasonal changes in Fig. 3A. When bacteria are relieved of nutrient limitation and become more nutritious for grazers, they would be more heavily grazed (del Giorgio et al. 1996), thus decreasing the strength of total bottom-up control in parallel with a higher dependence of their activity on high quality substrates provided by phytoplankton.

The role of bacteria with low and high nucleic acid content

It is not fully clear what the relative nucleic acid content, LNA or HNA, means in terms of bacterial activity (Gasol et al. 1999, Lebaron et al. 2001, Zubkov et al. 2001, Lebaron et al. 2002, Longnecker et al. 2005, Ortega-Retuerta et al. 2008). However, both the abundance and proportion of HNA cells have been found to correlate positively with bulk activity much more frequently than those of LNA cells (Bouvier et al. 2007, Morán et al. 2007). With an overall increase in bacterial activity from ~5 to >100 pmol Leu l^{-1} h^{-1} along the 12 to 22°C temperature range (Fig. 4D), responses of HNA and LNA cell abundance to temperature clearly differed between cool and warm waters (Fig. 4A,B). The opposite variation of %HNA and leucine incorporation rates with temperature ($r = -0.35$ and 0.42, respectively, with all data pooled) would discourage the ready use of the latter index to predict bacterial activity in our ecosystem, although noticeable differences were seen by separately analyzing the relationships in cool and warm waters.

With a geographically more extensive dataset obtained in the winter–spring transition along the NW and N Iberian coast, Morán et al. (2007) hypothesized that HNA bacterial abundance was a plausibly good predictor of bulk bacterial activity and growth rates in coastal waters only when bacteria were bottom-up controlled, using the same 2 approaches shown in this study. Here, we provide further insight into how the type of bottom-up control may modulate the predictive ability of both the total and relative abundance of the HNA group of cells (Table 2). Thus, %HNA could be used as a proxy for all 4 estimates of bulk bacterial activity (with up to 30% of the variance being explained) but only during the cool period (Table 2), usually extending from November through June in our study region (Fig. 1). In their analysis of growth rates at the surface and deep chlorophyll maximum of NW Mediterranean waters, Scharek & Latasa (2007) also suggested that HNA bacteria were more dependent on phytoplankton substrates than LNA bacteria. Consistent with this idea, absolute HNA bacterial abundance was significantly correlated with chl *a* in both temperature regimes, yielding similar correlation coefficients, while that of LNA bacteria was lower in cool waters and not significant during the warm period (Table 2). In our previous analysis, both the total bottom-up control and that attributed to phytoplankton generally coincided spatially (see Fig. 5 in Morán et al. 2007). Interestingly enough, when this was not the case and the regression of BB vs. LIR was not significant but that of LIR vs. chl *a* was, HNA was still a good predictor of leucine incorporation rates, which is exactly the result demonstrated here with a new dataset. In contrast with Morán et al.'s (2007) study on geographical variations during the winter–spring transition, we have shown here that BB-LIR and LIR-chl *a* relationships may behave differently and indeed showed a remarkable opposite response to temperature (Fig. 3). Based on these results, we conclude that substrate supply must come mainly from phytoplankton for the correlations between HNA bacterial abundance and bulk activity properties to be significant (Table 2), and specifically for %HNA to be useful as an activity index for bacterial assemblages (Gasol et al. 1999). Thus, the strongly consistent increase in %HNA values (from ~50 to 80%) from winter to early spring (Calvo-Díaz & Morán 2006) would reflect a direct dependence of HNA cells on dis-

solved primary production. Conversely, the significant increase in %HNA with depth in summer stratified waters and the opposite variation in LIR (Morán & Calvo-Díaz 2009) would be indicative of bacterial communities relying on sources of DOM other than phytoplankton.

Adding to the debate on which of the 2 groups of cells drives community performance, Scharek & Latasa (2007) recently found that LNA bacteria showed higher growth rates than HNA bacteria at the deep chlorophyll maximum in the NW Mediterranean. Direct (Zubkov et al. 2001, Jochem et al. 2004, Longnecker et al. 2005) and indirect (Sherr et al. 2006, Ortega-Retuerta et al. 2008) evidence that LNA bacteria may be occasionally as active or even more active than HNA cells is supported by the fact that this was the group that covaried with LIR during warm conditions (Table 2). In open ocean subtropical waters, LNA bacteria have been identified with the SAR11 group (Mary et al. 2006), which is responsible for a major share of heterotrophic metabolism in nutrient-poor environments (Morris et al. 2002). The significant increase in cell-specific leucine incorporation rates with increase in mean individual biomass of both LNA and HNA cells (data not shown, see Table 1 for mean community values) is in agreement with 'the larger the bacterial size, the higher the activity' hypothesis (Stevenson 1978, Gasol et al. 1995). It would also suggest that there is more structure in bacterial communities than is implied by the simple LNA-HNA dichotomy, and that size must also be taken into account rather than relying on absolute or relative abundances of any of the 2 groups (Morán et al. 2007).

In conclusion, weak total bottom-up control of bacterial assemblages in the southern Bay of Biscay was maintained in cool and warm waters, with the suggested role of phytoplankton in providing substrates for bacteria only being detectable at temperatures below 16°C. Temperature had contrary effects on total and phytoplankton-mediated bottom-up controls. The 2 temperature regimes were characterized by different relationships of HNA and LNA bacterial abundance with bulk bacterial activity, and our results suggest that HNA bacteria were more strongly dependent on phytoplankton DOM for growth and metabolism than LNA cells. The proportion of HNA cells proved to be a good indicator of bulk community performance only during the cool period, when bottom-up control by phytoplankton likely prevailed.

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