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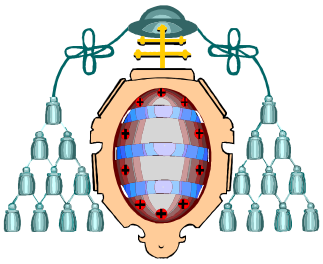

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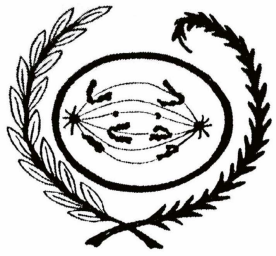

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D. Xosé Anxelu Gutiérrez Morán, Profesor/a del Master Biodiversidad Marina y Conservación de la Universidad de Oviedo,

CERTIFICA:

Que el Trabajo titulado: "**Coastal bacterioplankton in the Southern Bay of Biscay. Dynamics of low and high nucleic acid content bacteria in shallow and shelf waters"** presentado por D/Dña **Tamara Megan Huete Stauffer**, ha sido realizado bajo mi dirección y, considerando que reúne las condiciones necesarias, autorizo su presentación.

En Oviedo, a 5 de Julio de 2011

Vº Bº El Director del Master

El/La directora/a

José Luis Acuña

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Vº Bº El/La co-tutor/a

Heather Stoll

ABSTRACT

Heterotrophic bacteria in aquatic environments are universally distributed into two distinct flow cytometric populations based on their relative nucleic acid content: low (LNA) and high (HNA). The dynamics and possible regulation processes of both subpopulations were examined here by comparing weekly measurements over 2 years at a shallow coastal environment (L'Arbeyal beach) with monthly samples at three continental shelf stations off Xixón (southern Bay of Biscay). Similar seasonal variations of temperature and phytoplankton abundance (chlorophyll *a*) characterized inshore and offshore waters. With also similar total bacterial abundances (ranging from 2.15×10^5 to 3.72×10^6 cells ml^{-1}), the most remarkable difference found between shallow and shelf waters was the higher abundance and size of HNA bacteria at L'Arbeyal, likely related to its higher nutrient loading. Net growth rates estimated from coherent periods (6-26 weeks) of increase or decrease at L'Arbeyal ranged -0.017 to 0.036 day^{-1} for both subpopulations, proving that LNA cells were an active fraction of the bacterioplankton assemblage. LNA cells were strongly regulated by temperature (r^2=0.91 $p<0.01$), confirming previous suggestions about their independence from phytoplankton products. We also suggest that this subpopulation was subject to low grazing pressure year-round. In contrast, HNA cells were apparently more susceptible to nutrient availability and grazing mortality, especially at larger sizes. Our results provide further evidence to support fundamentally different ecological roles of the two cytometric subpopulations in aquatic environments.

INTRODUCTION AND AIMS

Marine heterotrophic bacterial communities are a key component of the picoplankton, the smallest size fraction composed of unicellular organisms between 0.2 and 2 μm of diameter (Sieburth et al. 1978), and are responsible for the bulk of nutrient and carbon recycling in pelagic ecosystems (Azam 1998, Fuhrman 1999, Ducklow 2000). There is wide interest in quantifying the contribution of marine bacteria to biogeochemical processes and in understanding their specific ecological roles (Cho and Azam 1990, Anderson and Ducklow 2001). The opening of what has been termed the "microbial black box" is, indeed, one of the most important issues in the agenda of modern investigation in marine microbial ecology (Azam and Malafatti 2007, Strom 2008). Progress in technology and molecular techniques has been essential in this process. In this regard, flow cytometry (FC) has become a powerful tool to explore marine bacterial communities (Gasol and del Giorgio 2000). Water samples can be processed fast for acquiring accurate counts, together with associated single-cell parameters (Sherr et al. 1999a, Gasol and del Giorgio 2000). Over a wide range of aquatic environments heterotrophic bacteria have been found to segregate in two different cytometric populations with characteristic relative nucleic acid content and size signatures (Li et al. 1995, Gasol et al. 1999, Troussellier et al. 1999), named low nucleic acid (LNA) and high nucleic acid (HNA) subpopulations. Despite their universal distribution in aquatic environments (Gasol and del Giorgio 2000, Bouvier et al. 2007), there is not an unanimous translation of these two flow cytometric populations into ecological, physiological or phylogenetic meaning, probably because LNA and HNA clusters are made up regionally of different bacteria at these three levels of organization (e.g. Zubkov et al. 2001, Caron 2005, Bouvier et al. 2007, Morán et al. 2011),.

During early stages of flow cytometric data gathering, HNA and LNA were gruesomely considered as the active and the inactive fractions of the same bacterial population (Jellet et al. 1996, Gasol et al. 1999, Lebaron et al. 2001). Indeed HNA cells were much more correlated to bacterial production as well as to bulk specific growth rates than LNA cells (Lebaron et al. 2001, Sevais et al. 2003, Morán et al. 2011). In contrast,

LNA bacteria were classified as dormant or dead cells, with such low physiological status so as to have no significant effect over the bulk assemblage (Gasol et al. 1999, Vaqué et al. 2001, Lebaron et al. 2002). This initial picture is rapidly evolving, and although there is still controversy, LNA bacteria have been reported to be able to show growth rates as high as their HNA counterparts (Zubkov et al. 2001, Longnecker et al. 2005, Nishimura et al. 2005,) and even to dominate in certain bacterial assemblages as in deep (Scharek and Latasa 2007) or oligotrophic waters (Zubkov et al. 2001,). LNA bacteria identification has been also predominantly associated to the SAR11 clade (Mary et al. 2006, Teira et al.2009, Schattenhofer et al. in press), suggesting a completely different phylogenetic composition of both subpopulations.

Total abundance of heterotrophic bacteria has been reported to vary little over a wide range of aquatic environments (Azam et al. 1983, Fogg 1995, Smith and del Giorgio 2003) from a few hundreds of thousands to a few million cells per ml (the average value commonly accepted is 10^6 cells ml $^{-1}$, Fogg 1995). Despite these rather constant values, natural bacterial populations show fluctuations in response to bottom-up (substrate availability) and top-down (grazing and viral pressure) control mechanisms, as well as with temperature (eg. Pernthaler et al. 1996, Gasol and Duarte 2000, Pomeroy and Wiebe 2001, Morán et al. 2010). In this regard, recent work has shown that the dynamics of the HNA and LNA subpopulations vary across environments and even shift seasonally within the same environment (Scharek and Latasa 2007, Ortega-Retuerta et al. 2008, Morán et al. 2010). Although controlled experiments are attempted at individually quantifying bottom-up controls such as the response to substrate additions (Wetz and Wheeler 2004, Bouvy et al. 2004) and top-down controls such as grazing rates (Iriarte et al. 2008, Longnecker et al. 2010), both bottom-up and top-down processes interact in the field as well as other environmental variables making it difficult to extrapolate results from laboratory experiments (Sanders et al. 1992, Sherr et al. 1999b, Vaqué et al. 2001, Gasol et al. 2002). At the loss of details on the relative importance of both types of control, net growth rates integrate specific growth and loss rates of each subpopulation and can further our understanding of the role of LNA and HNA bacteria in ecosystem functioning (Agawin and

Agustí 1997). Despite the lack of information on grazing rates, specific growth rates have been used preferentially to net growth rates in the literature since they provide a better assessment of the direct effect of bottom-up controls (Ducklow 2000) .

In the southern Bay of Biscay, the dynamics of the bacterial assemblage have been followed monthly since April 2002 at three continental shelf stations located offshore of Xixón (Asturias, Spain). The hydrographic conditions and seasonal patterns have been extensively studied in this typical temperate marine ecosystem alternating between stratification and mixing periods (Calvo-Díaz and Morán 2006, Morán et al 2007, Morán et al 2010, Franco-Vidal and Morán 2011). In addition to the shelf stations, we analyze here data from a shallow coastal site (L'Arbeyal beach) close to the sampled transect, which started being sampled for heterotrophic bacteria in May 2009. In this study we intend to analyze the bacterial dynamics of L'Arbeyal beach using the well-studied shelf stations as a reference. Due to the shallow nature of L'Arbeyal (<4 m), only surface samples from the shelf stations were used for comparison. Other than bacterial abundances and cell properties, environmental conditions [temperature, total and picoplanktonic (<2 µm) chlorophyll a and salinity] were explored as proxies for regulation factors of LNA and HNA subpopulations at the two sites. In a close up to L'Arbeyal bacterial dynamics we estimated *in situ* net growth rates in order to first contribute to characterize the bacterial assemblages of shallow coastal environments of the southern Bay of Biscay.

Aims

The specific aims of this study were: i) to compare the general bacterial dynamics and flow cytometric single-cell properties in the beach and the nearby shelf stations and ii) to estimate net growth rates of LNA and HNA bacteria in the beach and their linkage with environmental properties in order to further our knowledge of the ecological role of these two subpopulations.

MATERIAL AND METHODS

Study region and sample collection

L'Arbeyal beach is a shallow (ca. 4 m deep at high tide), coastal ecosystem affected by tidal mixing and located in an urban area close to an industrial harbour. Despite the absence of specific measurements it most likely lacks any limitation in nutrients through the water column all year round, as other very coastal systems (estuaries: Shiah et al. 1999; urban areas: Feuerpfeil et al. 2004; L'Arbeya beach: Nogueira pers. com). Between 2 and 28 nautical miles east lie the stations in the inshore-offshore transect off Xixón sampled within the RADIALES time-series project of the Spanish Institute of Oceanography (Fig. 1).

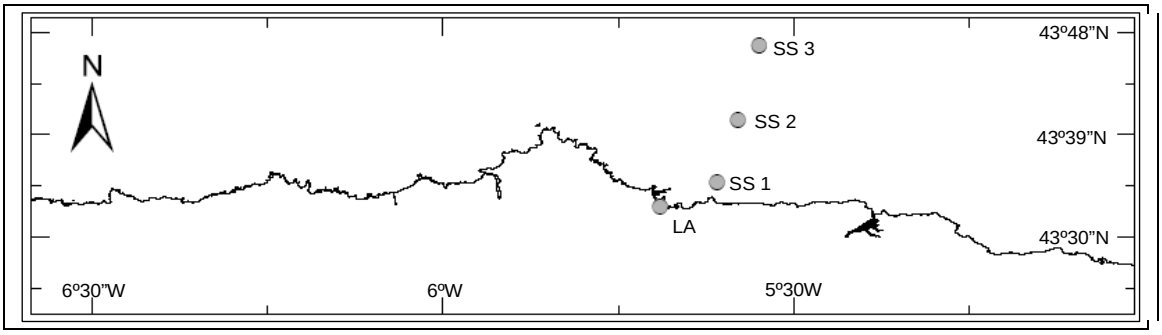

Fig. 1. Location in the Southern Bay of Biscay of the three RADIALES time-series stations (SS 1, SS 2 and SS 3) and the shallow coastal site of L'Arbeyal (LA).

Surface seawater samples were taken weekly from L'Arbeyal (LA) beach from the 18th May 2009 to 19th May 2011 and monthly from the three shelf stations (SS1, SS2 and SS3). Over the 2-year sampling period, the SS data did not show consistent differences along the inshore-offshore gradient in all the variables measured (see for instance temperature and chlorophyll *a* in Fig. 2). Therefore, unless otherwise indicated, data from the three stations will be considered jointly and referred to as SS.

Samples from L'Arbeyal beach were collected with an acid-washed bucket directly from the beach pier at high tide (± 1 h), transferred to 125 ml dark bottles and processed immediately in the laboratory. Samples from shelf stations 1, 2 and 3 were collected on board of de RV "José de Rioja" from 2.5 l Nisking bottles mounted on a Rosette with an

attached Seabird 25 CTD (conductivity temperature and depth) probe. Surface water was then transferred to 500 ml dark bottles and kept on board until arrival to the laboratory (2-3 hours).

Bacterial abundance, single cell properties and biomass

Subsamples of 1.8-3.5 ml of unfiltered water were fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration), left 10 minutes in the dark and frozen at -80ºC until analysis. The abundance and single-cell properties of heterotrophic bacteria were obtained using a FACSCalibur benchtop flow cytometer (Becton-Dickinson) equipped with a laser beam emitting at 488 nm. Samples were acquired with Cell-Quest Pro (Becton-Dickinson) software and cytograms finally analyzed with Paint-A-Gate 3.0.2 (Beckton-Dickinson, 1999). Upon being thawed at room temperature, 400 µl aliquots were stained with 4 µl of the nucleic acid fluorochrome Syto 13 (Molecular Probes: Troussellier et al. 1999) at 2.5 µmol l^{-1} concentration and fluorescent beads of 1 µm diameter (Molecular Probes) were added as internal standard. Samples were run at low flow speed (10-20 µl min^{-1}). Calibration of bead concentrations and flow rates were performed always prior to analysis in order to check for possible day-to-day variations. Bead concentrations were checked to TruCount (Becton-Dickinson) beads of known concentration. Flow rate calibration was made by measuring the initial and final volumes of distilled water after 10 min flowing at low speed (Lebaron et al. 2001, Gasol and del Giorgio 2000). Bacterial abundance was calculated from flow rate and time of analysis since flow rate proved more constant than bead concentrations over time. (Gasol and del Giorgio 2000). The relative contribution of HNA cells to the total bacterial assemblage was expressed as the percentage of HNA cells [HNA cell abundance x 100 / (LNA and HNA cell abundance)]

Single-cell properties obtained include green fluorescence (FL1) and right angle light scatter or size scatter (SSC). These two variables allowed for the easy recognition in cytograms of the two clusters of cells (LNA and HNA) mostly based on FL1 differences. Relative values were then calculated by dividing raw fluorescence and side scatter data by the corresponding values of the fluorescent beads. Relative FL1 measurements did not

precise any further conversions while relative SSC was converted to cell diameter according to an empirical calibration described in detail in Calvo-Díaz and Morán (2006). Finally, assuming spherical form cell volume was converted into carbon units using Norland's (1993) conversion factor (C biomass = 120 x Vol $^{0.72}$). All three size variables (SSC, size-diameter and size-volume) are easily interconvertible, but for the sake of consistency by cell size we will always refer to cell volume (μm^3).

Envrionmental variables

Chlorophyll *a* concentrations were obtained from filtered water samples. Sequential filtration of 100 ml through 20, 2 and 0.2 µm pore size Nuclepore polycarbonate filters was made at SS while at LA, the method followed included two separate filtrations of 50 ml through 2 µm pore size polycarbonate filters and Whatman GF/F of 0.7 µm nominal pore size. We thus obtained total (the sum of the three fractions in SS and the amount retained onto GF/F filters in LA) and picoplanktonic (<2 µm) chorophyll *a* concentrations. All filters were frozen at -4ºC until analysis. For pigment extraction, filters were submerged in 6 ml of acetone at 90% concentration for 24 h in the dark at 4ºC and the fluorescence measured with a Perkin Elmer LB-50s spectrofluorometer calibrated with pure chlorophyll *a*.

Temperature was measured in L'Arbeyal with a *Temp6* digital thermometer (Eutech/Oakton Instruments) from May 2009 to December 2009 and additionally with a Seabird 19 CTD from this date on, which enabled us to include salinity measurements. On the shelf, temperature and salinity were measured with the Seabird 25 CTD probe.

Data analysis

Ordinary least square (Model I) linear regressions, t-tests and Pearson correlation matrices were performed using the statistical commercial packages of STATISTICA 7.1 (StatSoft 2005) and SPSS 15.0 (SPSS Inc. 2006). Figures were developed with ArcGis 9.2 (ESRI Inc. 2006) and Grapher 7 (Golden Software, 2007).

RESULTS

Bacterial abundance and environmental variables.

Temporal variations in temperature, chlorophyll *a*, total bacterial abundance and %HNA over the study period are shown in Fig. 2.

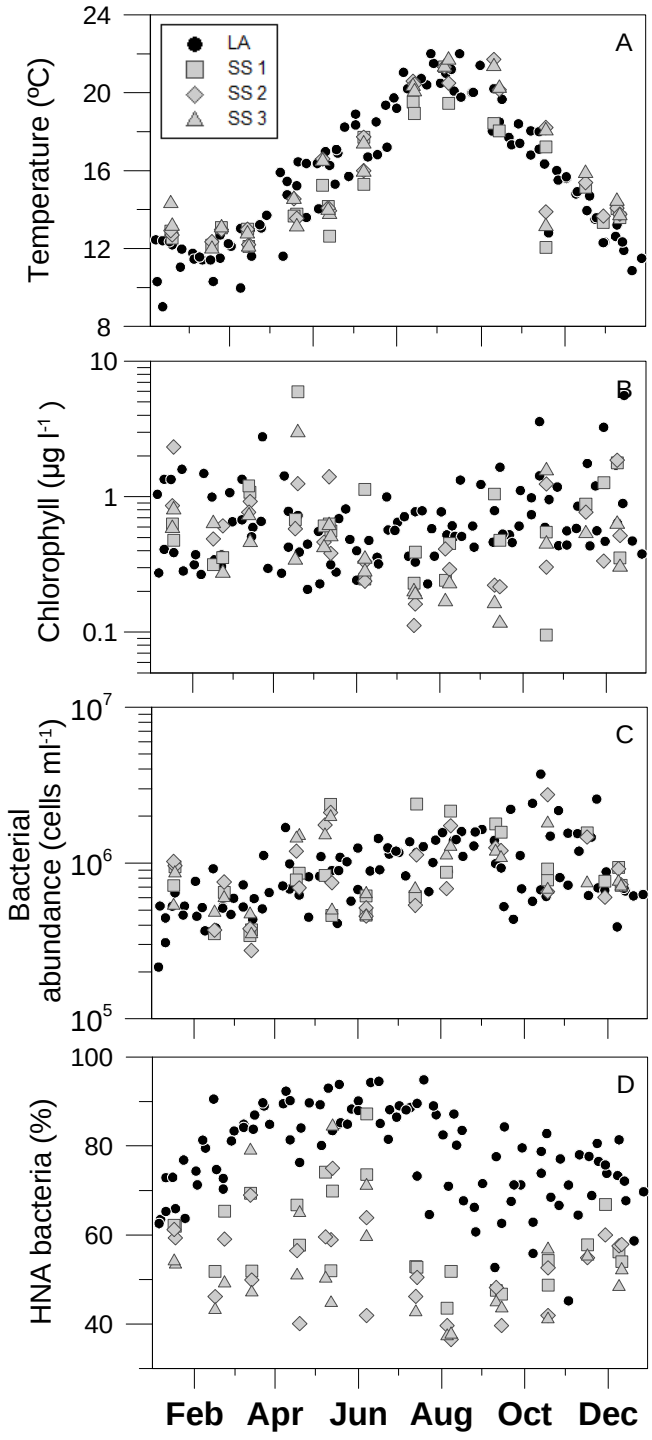

Fig. 2. Temporal variations at L'Arbeyal beach (black dots) and the continental shelf stations off Xixón (grey symbols) of temperature (A), total chlorophyll a (B), total bacterial abundance (C) and contribution of the HNA subpopulation to total cell numbers (D) with data pooled from the 2-year sampling period.

Surface temperature (Fig. 2A) had a similar behaviour in both areas. Although lower temperatures were observed during the coldest months in LA (9.2-22.2ºC) compared to SS (12.6-21.8ºC), seasonal changes match those previously described for a typical temperate ecosystem (e.g. Valdés and Lavín 2002, Calvo-Díaz and Morán 2006, Franco-Vidal and Morán 2011).

Surface salinity averages were very similar at SS (35.49 ± 0.03) and LA (35.14 ± 0.05) with typically lower values from October to March due to rain events and coastal discharge, and higher during the rest of the year except sporadically very low values at LA during summer (below 33).

Overall chlorophyll *a* concentrations were similar in both areas and ranged from 0.21 to 5.59 µg l^{-1} in LA and from 0.10 to 5.97 µg l^{-1} in SS. The seasonal pattern was more prominent in SS, with two maxima: in March-April and around October, corresponding to the stratification-mixing transition periods (Fig. 2B) (Franco-Vidal and Morán 2011).

Total abundance (TA) of heterotrophic bacteria in the region ranged from 2.15 x 10^5 to 3.72 x 10^6 cells ml^{-1} with similar values at the two sites (Mean ± SE: TA_{LA} 9.52 ± 0.54 x 10^5 cells ml^{-1}; TA_{SS} 9.72 ± 0.64 x 10^5 cells ml^{-1}). With all data pooled, TA increased progressively in LA from January to September-October and decreased in December, after showing considerable scatter in the autumn months (Fig. 2C). Two relative maxima where observed in SS, in April-May and September-October. In both LA and SS minimum values were coincident with the lowest temperatures, thus reflected in a positive correlation between TA and temperature, more marked in LA (Table 1).

	LA (n=100)				SS (n=73)			
	T (ºC)	Chl (µg l^{-1})	Chl<2 (µg l^{-1})	Salinity†	T (ºC)	Chl (µg l^{-1})	Chl<2(µg l^{-1})	Salinity
LNA (cells ml^{-1})	0.17	0.17	**0.24***	0.06	**0.34****	0.02	-0.01	0.04
HNA (cells ml^{-1})	**0.43****	0.19	**0.25***	0.09	0.09	0.17	0.17	-0.19
TOTAL (cells ml^{-1})	**0.39****	**0.20***	**0.28****	0.09	**0.24***	0.09	0.08	-0.07
%HNA	0.1 9	-0.09	-0.09	0.07	**-0.42****	0.16	0.10	**-0.31****

Table 1. Pearson's correlation coefficients between the environmental variables measured and total and relative bacterial abundances. Bold format was used to identify significant correlations (** p< 0,01; *p<0,05). Salinity measurements in L'Arbeyal included only 66 values († n=66).

In contrast to total counts, there were marked differences between the abundances of the LNA and HNA groups in each, shown in Fig. 4 (L'Arbeyal HNA_{LA}: 7.39 ± 0.41 x 10^5 cells ml^{-1} and LNA_{LA}: 2.13 ± 0.19 x 10^5 cells ml^{-1}; Shelf stations pooled together HNA_{SS}: 5.16 ± 0.32 x 10^5 cells ml^{-1} and LNA_{SS} :4.57 ± 0.3 x 10^5 cells ml^{-1}). HNA bacteria were on average more abundant than LNA cells in both LA and SS, although the mean HNA/LNA abundance ratios differed substantially at the two sites (HNA/LNA_{LA} = 4.77; HNA/LNA_{ss} = 1.44). Consequently, mean %HNA (Fig. 4C) was significantly higher (t-test $p<0.001$, n_{SS} = 73; n_{LA} = 100) in LA ($\%HNA_{LA}$: 78.1 ± 1.0%; $\%HNA_{SS}$: 55.0 ± 1.2%) and virtually never did %HNA values at SS exceed those at LA year-round (Fig. 2D). At LA a steady increase was observed from January to April, with constantly high values (>80%) through July, followed by largely variable but lower values during the rest of the year. A bimodal distribution of %HNA was observed in SS, with maxima in spring and minima in August-September, with a slight increase in November. Fig. 2D shows also that the periods of relatively high data dispersion differed for the two areas, being earlier in the case of the SS. A tighter coupling between HNA and LNA abundances was observed in SS ($r = 0.80$; $p<0.01$, $n = 73$) than in LA ($r = 0.56$; $p<0.01$, $n = 100$)

With all data pooled, the LNA and HNA fractions responded differently to environmental variables in the two areas (Table 1). Although weak, we found a significant positive correlation with temperature of the abundance of HNA cells in LA while in SS only that of LNA cells was significantly correlated. Total and picoplanktonic (<2 µm) chlorophyll *a* concentrations showed weak correlations with bacterial abundances only in LA. Negative correlations between %HNA and both temperature and salinity were found at SS.

Flow cytometric properties

Nucleic acid content, estimated as the relative green fluorescence (FL1), was essentially the same in LA and SS for each bacterial subpopulations (HNA_{LA}: 2.42 ± 0.63 x 10^{-3}; HNA_{SS}: 2.15 ± 0.48 x 10^{-3}; LNA_{LA}: 5.11 ± 0.17 x 10^{-3}; LNA_{SS}: 5.08 ±0.09 x 10^{-3}), which showed also the same temporal pattern with maximum values in spring and minima through summer and autumn (Fig. 3 A and B).

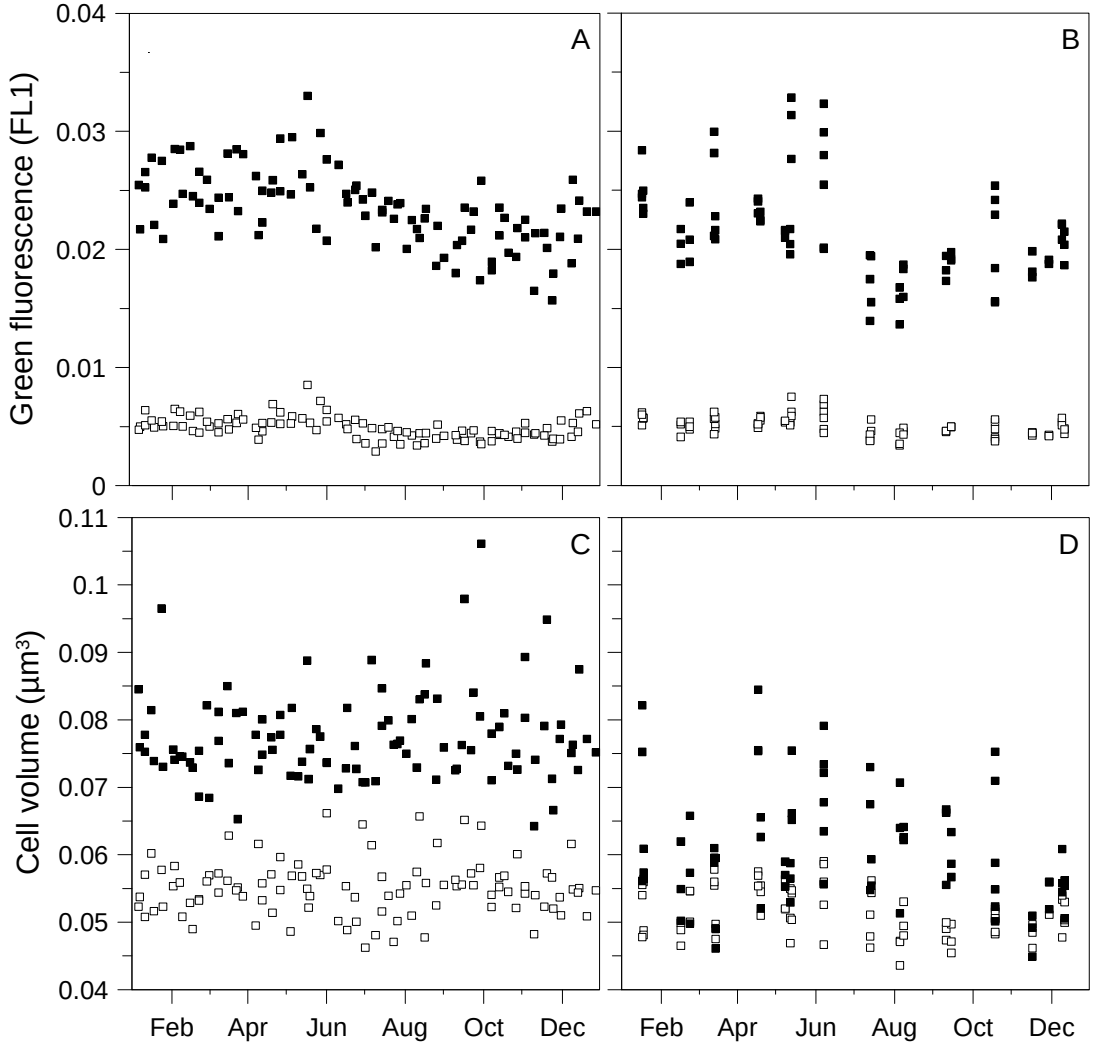

Fig. 3. Temporal variations of the flow cytometric properties green fluorescence (a surrogate of nucleic acid content) and cell volume calculated from SSC of the HNA (closed symbols) and LNA (open symbols) subpopulations at L'Arbeyal beach (A, C) and the shelf stations (B, D)

In contrast, cell volumes differed markedly in the two areas (Fig. 3C and D). HNA cells were larger at the beach than at the shelf stations (t-test, $p<0.001$, $n_{SS}=73$; $n_{LA}=100$) while LNA cells were more homogeneous (Fig. 4B; Mean cell volumes ± SE: HNA_{LA}: 0.077 ± 0.07 x 10^{-2} µm^3; HNA_{SS}: 0.061 ± 0.1 x 10^{-2} µm^3; LNA_{LA}: 0.055 ± 0.04 x 10^{-2} µm^3; LNA_{SS}: 0.051 ± 0.04 x 10^{-2} µm^3). Generally HNA cells were larger than LNA cells except occasionally during the winter and spring months in SS, where both tended to show sizes of 0.05-0.06 µm^3 (Fig. 3D).

Cell size and nucleic acid content were positively correlated both within fractions and between the HNA and LNA fractions except for LNA_{LA}, where size showed no relationship with FL1 values (Table 2), indicating that the larger the cell the greater the

nucleic acid content and that temporal changes in size or fluorescence were generally shared by the two subpopulations.

	LA (n=100)				SS (n=73)			
	Vol LNA (μm^3)	Vol HNA (μm^3)	FL1 LNA	FL1 HNA	Vol LNA (μm^3)	Vol HNA (μm^3)	FL1 LNA	FL1 HNA
Vol LNA (μm^3)	1	-	-	-	1	-	-	-
Vol HNA (μm^3)	**0.31****	1	-	-	**0.30****	1	-	-
FL1 LNA	0.01	0.06	1	-	**0.44****	**0.31****	1	-
FL1 HNA	0.022	**0.26***	**0.69****	1	**0.51****	**0.50****	**0.83****	1

Table 2. Pearson's correlation coefficients between the flow cytometric characteristics -relative green fluorescence (FL1) and cell volume (from side scatter)- of LNA and HNA subpopulations in L'Arbeyal and the shelf stations.

Bacterial biomass

Although mean annual values of total abundance were similar at the four sampled sites (Fig. 4A), an inshore-offshore pattern in bacterial biomass (overall range 3.99 - 71.32 µg C l^{-1}) became apparent (Fig. 4D), largely due to the greater abundance (Fig. 4A) and size (Fig. 4B) of HNA cells at L'Arbeyal.

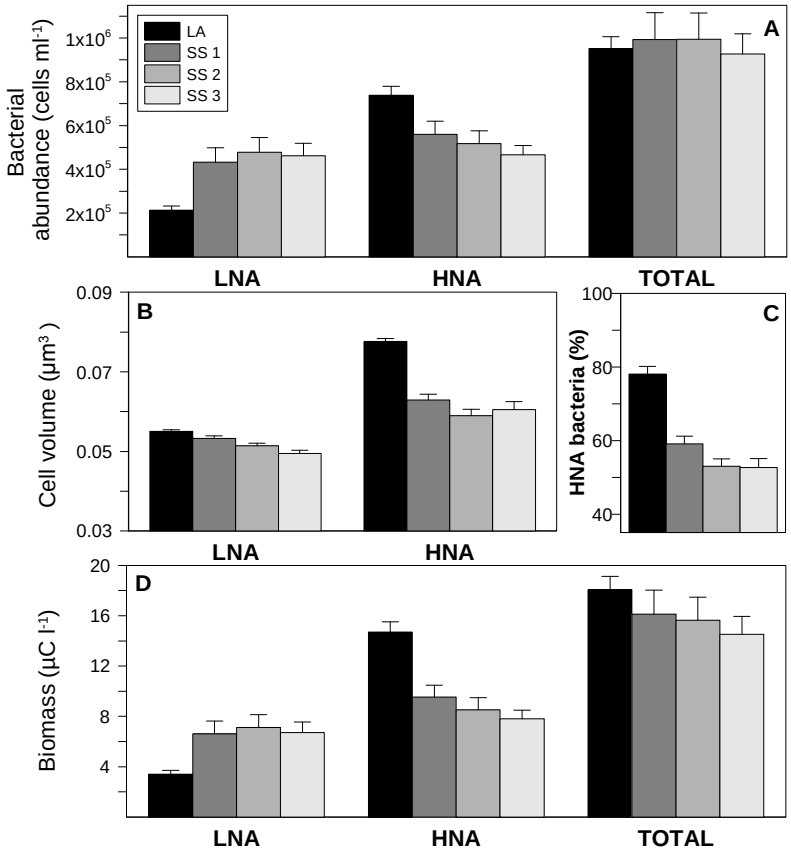

Fig. 4. Mean values of bacterial abundance (A), cell volume (B), % HNA bacteria (C) and biomass (D) at all four sites sampled (LA and the three shelf stations, SS1, SS2 and SS3).Error bars represent SE.

However, differences between LA and SS were not significant year round (t-test p = 0.438, n_{SS} = 73; n_{LA} = 100). Mean annual contributions of HNA cells to total abundance (Fig. 4C) were mirrored with only slight differences by biomass values. Thus, HNA cells contributed 81.5 ± 1.1% to total biomass in LA in contrast to the 57.6 ± 1.3% contribution in pooled SS.

Net growth rates

Weekly sampling of bacterial abundance in LA allowed us to identify internally coherent periods of abundance increase or decrease for both subpopulations. Ordinary least squares linear regression between the natural logarithm of abundance and time in days was applied to each period and the slope was taken as the net growth rate estimate (day-1, d^{-1}). Each period included at least 6 successive dates (weeks) and all regressions were significant at p<0.05. Fig. 5 shows the abundance of HNA and LNA cells and the corresponding periods of net positive or negative growth (8 for HNA cells and 6 for LNA cells)

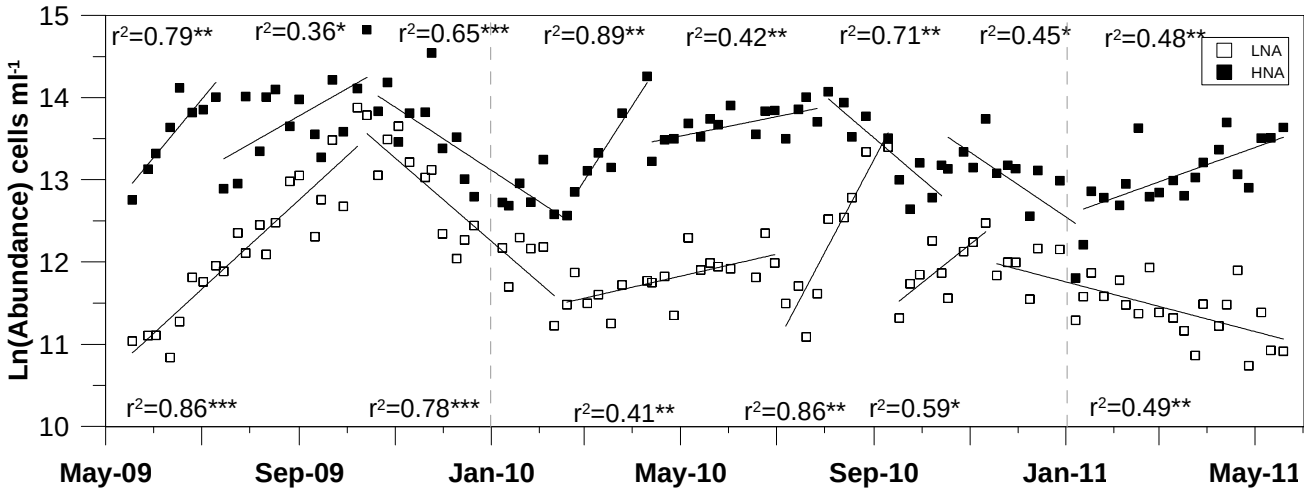

Fig. 5. Time-series of LNA and HNA bacterial abundances at L'Arbeyal showing the selected periods of net increase or decrease. Ordinary least squares linear regressions fitted to data are shown for each period. The coefficients of determination (r^2) are shown above (for HNA) or below (for LNA) each respective model (*p<0.05; **p<0.01;***p<0.001)

Ranges of variation in net growth rates (NGR) were very similar for both subpopulations (HNA: -0.016 to 0.030 d^{-1}; LNA: -0.017 to 0.036 d^{-1}). Taking only positive NGRs, the mean annual growth of LNA cells (0.018 ± 0.005 d^{-1}) was slightly higher than

corresponding HNA cells values (0.015 ± 0.003 d^{-1}). On a seasonal basis, NGRs of LNA and HNA bacteria followed different patterns (Fig. 6). LNA bacteria tended to show positive NGRs from April to November and negative values in winter, with maximum values in July-August. HNA bacteria positive NGRs were found from March to August with negative values for the rest of the year. Seasonal HNA cells maximum values were more variable than for LNA cells.

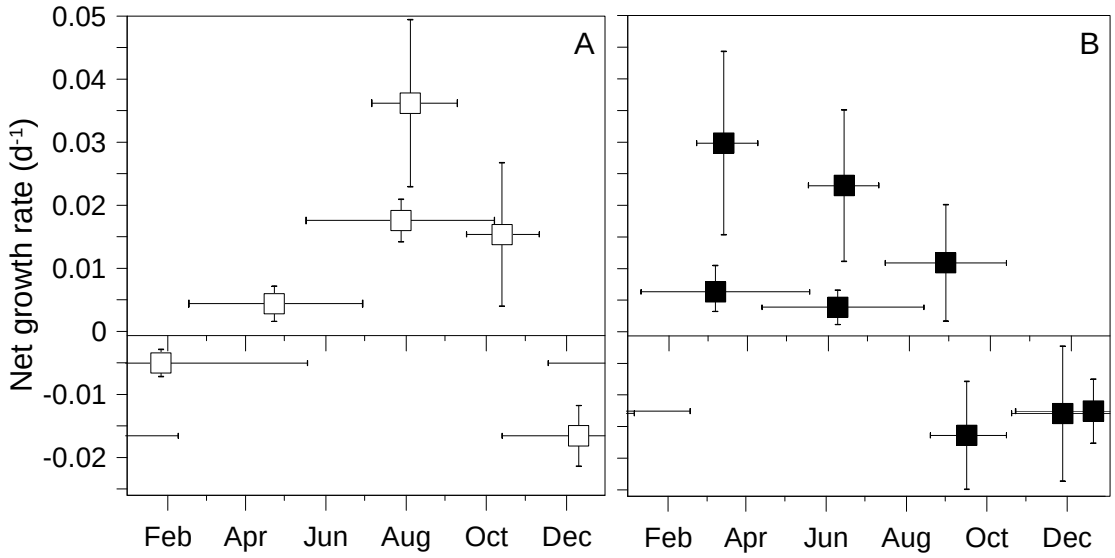

Fig. 6. Seasonal variations in net growth rates of LNA (A) and HNA (B) subpopulations with data pooled from the two years sampled. Horizontal bars represent the period's length while vertical bars represent the 95% confidence interval of the ordinary least squares linear regression slopes (see also Fig. 5 for details).

LNA cells NGRs were significantly and positively correlated with temperature (Fig. 7A), and negatively with total chlorophyll (Fig. 7C). In contrast, HNA cells NGRs showed no significant correlations with these variables (Fig. 7B and D).

Interestingly, we found different associations between cell size and NGRs for each subpopulation (Fig. 7E). Although non significant, there was a tendency in LNA cells to have bigger sizes at high growth rates, while HNA tended to have bigger sizes during the negative growth periods (Fig. 7E)

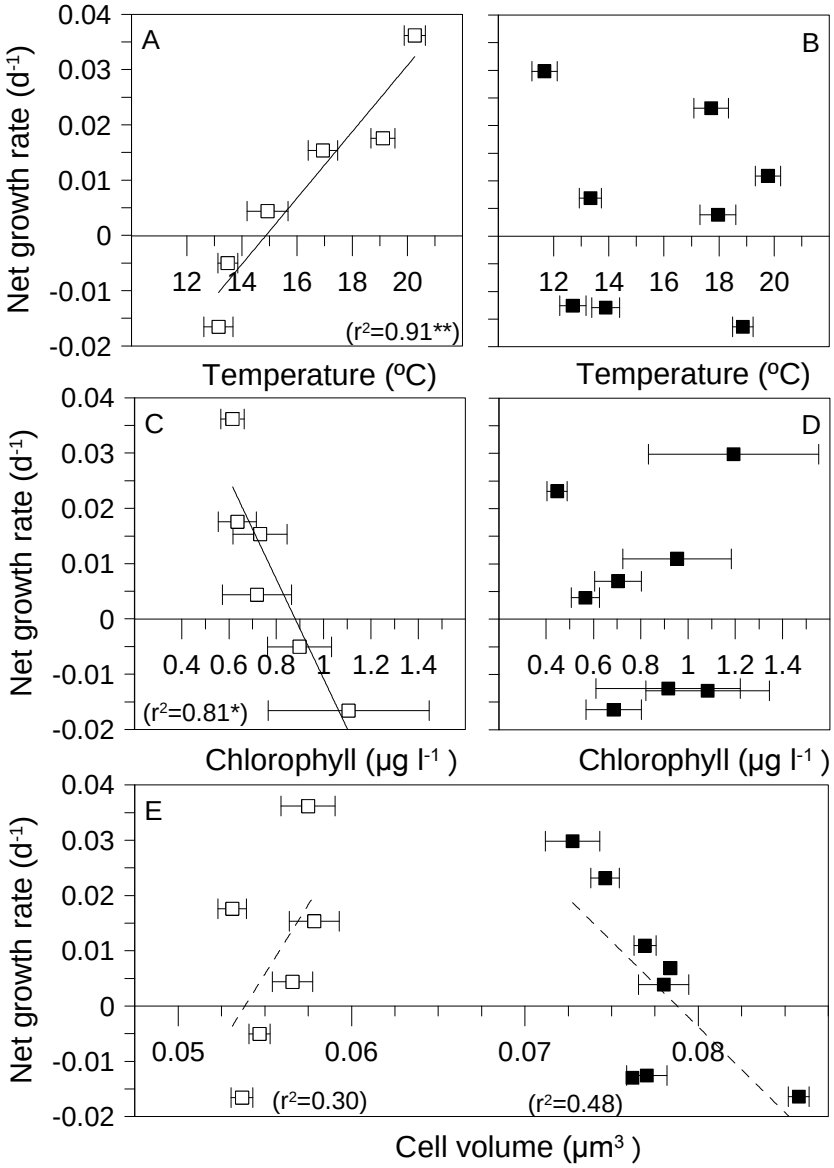

Fig. 7. Relationships of the net growth rates of LNA cells (open symbols) and HNA cells (closed symbols) with temperature (A, B), chlorophyll *a* (C, D) and cell volume (E). Ordinary least square linear regressions fitted are shown as solid lines when significant and as dashed lines when not. (r^2 values in braquets: *p<0.05; **p<0.01).

DISCUSSION

Bacterial abundance

The common view held in marine ecology that heterotrophic bacterial abundance and biomass remain quite uniform compared with other planktonic components (e.g. Vaqué 1996, Ducklow 1999), both with time and across a wide range of marine regions (Fogg 1995), was not contradicted by the beach and shelf stations data analyzed in this study (Fig 1, Fig. 4). In contrast, there were noteworthy differences between shallow and deeper waters off Xixón when the "microbial black box" was opened to consider the universally distributed clusters of LNA and HNA cells detected by flow cytometry.

Over the shelf, increases and decreases of HNA and LNA cells abundance during the studied period were strongly coupled ($r=0.80$, $p<0.01$, $n=73$). The abundance of both subpopulations followed a similar seasonal pattern, with their relative contribution remaining pretty stable and slightly higher for HNA cells (mean 55.0 %HNA). In general, these results, as well as the observed temperature variation and the bimodal chlorophyll *a* distribution match previous observations obtained within the RADIALES time-series monitoring programme of the SS bacterial assemblage (Calvo-Díaz and Morán 2006, Franco-Vidal and Morán 20011) and with other studies conducted nearby (Valdés and Lavín 2002, Valencia et al. 2003). However, the recurrent seasonal pattern of %HNA observed from 2002 until 2009 (Calvo-Díaz and Morán 2006, Franco-Vidal and Morán 2011, Morán pers. comm.) with maxima in April (>75%) and minima in August (<50%) was lost in 2010, precisely the central year of our sampling period. It partially recovered during 2011 (with a mean increase in %HNA from 54% in December 2010 to 72% in March 2011) which explains the high scatter seen of %HNA from January to April in Fig. 1D. Considering the 2010 exception, the repeatability of %HNA peak roughly associated with the phytoplankton spring bloom is quite relevant, and would agree with the predictable succession of distinct phylogenetic groups with environmental changes found elsewhere (Fuhrman et al. 2006). The reason why this pattern was lost during 2010 cannot be solved by the available dataset, but emphasizes the usefulness of sustained monitoring

programmes for understanding the functioning of pelagic ecosystems and their variability (Gasol 2007, Valdés et al. 2007)

In L'Arbeyal, the HNA_{LA} subpopulation was far more abundant, representing on average 78% of the total bacterial counts and 82% of the biomass. The active fraction of bacterioplankton communities has been reported to increase with the trophic state of the system (del Giorgio and Scarborough 1995, Longnecker et al. 2010) and this could explain the greater presence of HNA at the beach, since substrate limitation is most likely substantially lower in L'Arbeyal than on the shelf, in view of the inshore-offshore gradient in inorganic nutrients found in the central Cantabrian Sea off Cuideiru (Llope et al. 2007) and in the Xixón time-series (Nogueira pers. comm.). This is also supported by the fact that HNA_{LA} abundance was generally higher year round and reached maximum abundances in summer coincident with the warmest temperatures in contrast to the decrease in HNA_{SS} over the shelf during these same months.

In contrast, although mean LNA_{LA} abundances were much lower, LNA_{LA} cells showed higher variability (92% CV) than HNA_{LA} (55% CV) and at some periods, reached the same or higher abundances than HNA_{LA}, probably due to differential grazing or to different regulation of each subpopulation (discussed below) as inferred by the lower covariance of the abundances of HNA and LNA cells there ($r = 0.54$, $p<0.05$, $n = 100$) compared to the tight coupling detected on the shelf. Although two years are insufficient to draw sound conclusions about seasonality, it is interesting to note that overlaps in the abundance of LNA and HNA cells in L'Arbeyal were repeatedly found between September and October during 2010 and 2011 (Fig. 5).

Flow cytometric properties of LNA and HNA cells

Covariance in individual cell properties (FL1 as a proxy of DNA content and size obtained from SSC measurements) was observed for LNA_{SS}, HNA_{SS} and HNA_{LA} subpopulations. Both variables usually covary and fluorescence has even been used as a good proxy for size (Gasol and del Giorgio 2000). In the case of LNA_{LA}, size increased without a proportional increase in DNA content (Table 2). This would caution against using

SSC straightforward as size since it also integrates differences in shape and cell complexity or structure, as cytoplasmic granularity or protein content (Gasol and del Giorgio 2000) and since differences in the degree of FL1 and SSC coupling have been reported over different environments (Bouvier et al. 2007). We might speculate that one or more concurrent cell changes not involving DNA replication might occur at some life stages of LNA_{LA} cells thus explaining the uncoupling between FL1 and SSC, but why this would occur only to cells from L'Arbeyal remains elusive.

DNA content did not show variations between areas, and showed similar seasonal patterns and similar mean values for each subpopulation, with no overlap between them (Fig. 3A and B). This bimodal distribution was also related to different replicating stages (n versus 2n) within the same population (Bouvier et al. 2007), although the latest evidence available points towards differential bacterial composition with different genome sizes or number of copies in both the HNA and LNA subpopulations (Schattenhofer et al. in press). Particularly, the cultured representative of the SAR11 clade *Pelagibacter ubique* (Rappe et al. 2002), frequently only found and making the most of the LNA subpopulation (Mary et al. 2006), has the smallest genome among free living bacteria (Giovannoni et al. 2005)

Also relevant to this study are the differences in cell size between and within the HNA and LNA subpopulations from LA and SS. Mean sizes in SS for the HNA subpopulation were smaller than at LA (Fig. 4B) and can be related, as well as their higher abundances, to the trophic state of the system (del Giorgio and Scarborough 1995, Longnecker et al. 2010) since availability of nutrients and organic matter is thought to be high and virtually continuous in LA, as explained before. Conversely, the usually more active HNA cells (Gasol et al. 1999, Lebaron et al. 2001, 2002, Servais et al. 2003) would be prevented to increase in cell volume on the shelf stations during stratification due to their larger susceptibility compared to LNA cells to low nutrients supply (Nishimura et al. 2005, Cuevas et al. 2011). It may also be possible that HNA_{SS} and HNA_{LA} were composed of at least some different species in response to the prevailing environmental conditions in the two types of waters involving also conspicuous differences in size (Fig. 3C and D, Fig. 4B) LNA_{SS} and LNA_{LA} cells were much more homogeneous in size, supporting the suspicion of

a more homogeneous species composition, probably dominated by SAR11 cells (Mary et al. 2006, Teira et al. 2009, Schattenhofer et al. in press).

We found no seasonal patterns in cell size dynamics except for LNA in the shelf stations with maxima in spring and early summer and minima in September-October (Fig. 3B). This pattern had also been observed in the longer time-series at the SS, being significant only for the surface (0-10 m) waters (Morán and Huete-Stauffer, pers. comm.). Moreover, during these periods of size increase, LNA_{SS} cell size reached and even exceeded the corresponding values of the smallest $HNA_{SS,}$ also observed during spring months previously (Calvo-Díaz and Morán 2006). This pattern suggests that LNA cells on the shelf surface may be susceptible to some seasonal environmental forcing followed by size changes, for example the well known ecological inverse relationship between temperature and size (Atkinson et al. 2003).

LNA and HNA bacterial net growth rates

Taken together, bacterial net growth rates in L'Arbeyal were very low (systematically below the 0.1-1 d^{-1} interval proposed for marine bacteria, Kirchman et al. 1995), reflecting the overall effect of grazing on changes in abundance. Even the specific growth rate estimates from bacterial production to biomass ratios obtained at the surface of middle SS (SS 2) in 2006, much lower than direct estimates in predator-free incubators (Franco-Vidal and Morán 2011), were on average 7 to 23 times higher than our NGRs values. NGRs calculated by Wetz and Wheeler (2004) were higher as well, although performed during a simulated phytoplnktonic bloom. Not withstanding the different approaches, the net growth rates reported here highlight differences between the two bacterial subpopulations.

In L'Arbeyal LNA cells reached and even exceeded the net growth rates of HNA cells (Fig. 6), strongly contradicting their earlier consideration as inactive or dead cells (Gasol et al. 1999, Lebaron et al. 2001) since absolute increases in abundance (Fig. 5) can only be due to cell division. The different response of each subpopulation to environmental variables such as temperature and chlorophyll a may indicate different regulation

processes for each subpopulation, which in turn might be associated to a different phylogenetic composition, as already suggested. As seen in Fig. 7, neither temperature nor chlorophyll *a* had significant effects over HNA_{LA} NGRs. In contrast, with the limitations of a much constrained set of ancillary measurements, the principal variable regulating LNA_{LA} growth seems to be temperature. Although both HNA and LNA growth rates were low at the end and beginning of the year, there is clearly a seasonal pattern in the LNA subpopulation net growth. Also, increasing or decreasing periods were more stable, longer and easier to identify for the LNA subpopulation, suggesting that regulation of LNA is subjected to fewer control processes than HNA bacteria.

The observed high positive coupling between LNA_{LA} and temperature (Fig. 7A) could be related to the physiological activation of the LNA_{LA} subpopulation above the 15ºC threshold (Fig. 7A). Interestingly, compared with HNA bacteria and other single-cell probes, the specific growth rates of LNA bacteria also showed the strongest response to temperature in a temperate estuary (Morán et al. 2011). This response was detected, as well, offshore as a continuous increase in LNA abundance with temperature (Table 1 and Morán et al 2010), probably escaping grazing or other mortality processes with low but rather constant metabolic rates, as opposed to HNA cells (Longnecker et al. 2010).

The independency of HNA_{LA} NGRs and the strong inverse correlation of LNA_{LA} NGRs to chlorophyll a must not be taken literally. Instead, it can be interpreted either as phytoplankton not being limiting for HNA_{LA} and LNA_{LA} bacterial growth in LA or that non phytoplanktonic DOC sources were also available, consistent with the weak correlations found. Positive association of bulk specific growth rates and bacterial production to chlorophyll *a* has been reported over a wide range of ecosystems (Cole et al. 1988, Gasol and Duarte 2000, Barbosa et al 2001, Scharek and Latasa 2007, Ortega-Retuerta 2008) and in previous studies in the nearby shelf stations (Franco-Vidal and Morán 2011) and it is one general paradigm that bacteria rely on phytoplanktonic substrates (Gasol and Duarte 2000). However, indirect support for the consideration of L'Arbeyal as a meso- to eutrophic site (Llope et al. 2007) can obscure any direct relationship (Findlay et al. 1991)

Over the continental shelf, the abundance of the LNA_{SS} subpopulation showed a positive correlation with temperature and none with chlorophyll *a* (Table 1), as also found at the beach site, suggesting that on the shelf, temperature could also act as the prevailing factor for LNA cell growth and that LNA cells would most closely follow a seasonal pattern. Moreover, in previous work in SS, LNA_{SS} abundance was shown to increase at temperatures above 16ºC with no coupled increase in HNA_{SS} abundance, likely associated to the lack of enough substrates for HNA growth during the stratification period (Morán et al. 2010).

Regarding cell size, although not significant, we found an opposite effect on the NGRs of the two bacterial subpopulations. This may be relevant for the structure of the bacterial assemblage since size is a key factor determining top-down regulation by grazing (González et al. 1990). At higher growth rates, HNA_{LA} bacteria tended to show smaller sizes (Fig. 7E). This finding, together with the significant (although low) negative correlation between HNA_{LA} abundance and size ($r=-0.20$; $p<0.05$; $n=100$) is consistent with the size-selective predation theory (Gonzalez et al. 1990, Longnecker et al. 2010, Jürgens and Güde 1994), roughly stating that the more active and bigger cells are preferentially preyed upon with a subsequent decrease in the mean size of the remaining cells. LNA_{LA} bacteria showed the opposite trend; at higher growth rates the mean size of the cells was higher (Fig. 7E). According to this theory, LNA_{LA} cells may escape systematically high predation pressures probably due to the small size they reach even at maximum values. This would be consistent with the LNA subpopulation developing seasonal patterns more closely related to temperature without abrupt changes in abundance from grazing (Fig. 5).

In response to the widespread earlier consideration of the HNA fraction as the truly active cells, there has been recent controversy regarding the physiological status and role of LNA within the bacterial community. Through phylogenetic analyses clear differences between both subpopulations have been found with *Roseobacte*r, *Bacteriodetes*, *Gammaproteobacteria* and *Cytophaga-Flavobacterium* as the dominant species of the more active and diverse HNA subpopulation (Zubkov et al. 2001, Alonso-Sáez et al. 2007, Shattenhofer et al. in press) and the *Alphaproteobacteria* SAR11 as the almost exclusive

representatives of the LNA subpopulation (Mary et al. 2006, Teira et al. 2009, Schattenhofer et al. in press). In contrast, Servais et al. (2003) found the same dominant phylotypes among both subpopulations with higher resolution methods and associated these completely different results to the natural heterogeneity of bacterial assemblages and the environments in which they thrive. Particularly, the species composition and their relative contribution may vary in relation to the system's trophic state and the interaction of other abiotic factors such as temperature and salinity (Alonso-Sáez et al. 2007). HNA and LNA cells may not have the same ecological meaning in different systems but in any case, clearly the general classification of active versus inactive cells is too rough so as to provide useful categories (Morán et al. 2010). A growing body of literature neglects the general consideration of LNA as inactive or dead cells (Zubkov et al. 2001, Longnecker et al. 2005, Jochem et al. 2004) and, as also demonstrated here, this bacterial fraction was able to show net growth rates as high as HNA cells.

In our particular case, we observed both coupled and uncoupled phases of HNA_{LA} and LNA_{LA} net growth (Fig. 4). This would argue towards a completely different regulation of both subpopulations on an annual basis and that there may be periods with similar responses to factors not assessed in this study. However, the fact that there was also virtually no size or fluorescence overlap between the two factions in LA over time (Fig. 3A and C) makes it hard to imagine a continuum of metabolic stages within the same phylogenetic groups (Bouvier et al. 2007). In the case of the LA bacterial community, it seems more likely that each subpopulation was comprised by distinct phylogenetic groups with generally separate dynamics.

Bottom-up vs. top-down control of bacterial standing stocks

Bearing in mind that net growth rates are the balance between bottom-up (substrate) and top-down (mortality) controls, we can approach the effect of the former processes based on the response to chlorophyll *a* and temperature. From our results, bottom-up control was weak in both LA and SS, in contrast with a previous study showing a higher apparent bacterial dependence on phytoplankton in winter-spring than in summer at

shelf station 2 (Morán et al 2010). The absence of positive relationships between bacterial NGRs and chlorophyll a (negative correlation in the case of LNA cells) would argue against direct trophic coupling between phytoplankton and bacteria in L'Arbeyal, or alternatively, plentiful algal substrates year-round. Our results also suggest that bacterial bottom-up regulating processes related to stratification-mixing periods were stronger over the outer shelf than at L'Arbeyal beach

Covariations of bacterial production and biomass with chlorophyll *a* have been reported for a wide range of ecosystems and incubation experiments (Bird and Kalff 1984, Cole et al. 1988). Although this coupling has been related particularly to the HNA subpopulation (Gasol and Duarte 2000, Wetz and Wheeler 2004, Morán et al. 2007), similar results for LNA bacteria in oligotrophic and deep environments are not rare (Ortega-Retuerta et al. 2008, Shareck and Latasa 2007). Although it seems that HNA bacteria rely more on phytoplankton substrate availability than LNA cells (Morán et al. 2011) probably due to their higher activity and substrate needs (Cuevas et al. 2011), we could not demonstrate it unquestionably in this study, other than reporting the finding that LNA cells were apparently more independent on phytoplankton substrates than HNA cells (Fig. 7C and D). Our lack of measurements of inorganic and organic nutrient concentrations, preclude us to conclude whether inshore and offshore continental shelf bacterial communities off Xixón rely on sources of DOC other than freshly produced by phytoplankton (including inland inputs or the result of particulate organic matter solubilization) or whether the true relationships of bacteria and phytoplankton were obscured by a low temporal resolution or by grazing processes.

Mean cell volume of bacteria are partly be the result of protistan grazing but may be also related to other processes such as internal cell cycles (del Giorgio and Gasol 2000, Bouvier et al. 2007). There is little to be said about top-down control processes without being probably too speculative but predation pressure may be responsible for part of the observed changes in abundance (Fig. 5) as well as for the weak or non-significant correlations with chlorophyll.

Clearly our data are insufficient to fully explain the complex interactions of bacterial assemblages with other environmental variables but the estimated net growth rates confirm distinct responses of the HNA_{LA} and LNA_{LA} subpopulations to temperature and phytoplankton that could be made extensible to the entire continental shelf in accordance to previous studies, demonstrating an essentially different behaviour of the universally distributed flow cytometric subgroups.

CONCLUSIONS

In conclusion, bacterial flow cytometric subpopulations differed in their dynamics in shallow and deeper waters over the central Cantabrian sea continental shelf. In general, we observed that the LNA subpopulation was a physiologically active fraction showing temporal changes in abundance and size that grew at even higher net rates than HNA cells. Our results support the view that LNA and HNA are phylogenetically distinct fractions regulated by different factors; particularly, temperature was the most relevant factor controlling the LNA subpopulation while HNA seemed to be more susceptible to predation due to their larger sizes. We have not found direct evidence of HNA being tightly coupled to phytoplanktonic biomass, but the higher abundances and sizes observed in/at L'Arbeyal compared with the shelf stations suggest that substrate limitation was probably negligible in the beach shallow waters. Finally, we suggest that the HNA cluster from the more nutrient enriched beach site site may differ in at least some important species from the HNA cluster of the shelf stations reflected in different %HNA dynamics and size distributions, while LNA cells were probably more homogeneous over the two study areas.

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ANNEX I. Data I

Environmental variables and total and relative abundances of bacterial subpopulations.

St	DATE	JDAY	TºC	T CTD	Sal	Chl	Chl <2	L Ab	H Ab	T Ab	%HNA
LA	18/05/2009	138	15,30	-	-	0,28	0,12	6,22E+04	3,47E+05	4,09E+05	84,79
LA	28/05/2009	148	15,70	-	-	0,48	0,17	6,66E+04	5,02E+05	5,69E+05	88,30
LA	02/06/2009	153	18,90	-	-	0,40	0,20	6,67E+04	6,09E+05	6,75E+05	90,12
LA	11/06/2009	162	16,70	-	-	0,48	0,19	5,09E+04	8,37E+05	8,88E+05	94,27
LA	17/06/2009	168	18,50	-	-	0,36	0,18	7,88E+04	1,36E+06	1,43E+06	94,51
LA	25/06/2009	176	17,20	-	-	0,57	0,33	1,35E+05	1,00E+06	1,14E+06	88,14
LA	02/07/2009	183	19,20	-	-	0,65	0,33	1,28E+05	1,04E+06	1,17E+06	89,03
LA	10/07/2009	191	20,20	-	-	0,36	0,27	1,56E+05	1,21E+06	1,37E+06	88,62
LA	15/07/2009	196	20,60	-	-	0,33	0,19	1,45E+05	3,98E+05	5,43E+05	73,24
LA	24/07/2009	205	20,40	-	-	0,23	0,15	2,32E+05	4,23E+05	6,54E+05	64,59
LA	29/07/2009	210	21,50	-	-	0,36	0,18	1,82E+05	1,22E+06	1,40E+06	87,02
LA	07/08/2009	219	21,00	-	-	0,52	0,23	2,56E+05	6,26E+05	8,81E+05	70,97
LA	11/08/2009	223	21,20	-	-	0,61	0,46	1,78E+05	1,21E+06	1,39E+06	87,18
LA	17/08/2009	229	22,00	-	-	1,33	0,87	2,62E+05	1,33E+06	1,59E+06	83,51
LA	26/08/2009	238	20,00	-	-	0,61	0,37	4,34E+05	8,51E+05	1,29E+06	66,21
LA	01/09/2009	244	21,40	-	-	1,23	0,63	4,67E+05	1,17E+06	1,64E+06	71,55
LA	11/09/2009	254	20,20	-	-	0,79	-	2,22E+05	7,68E+05	9,90E+05	77,59
LA	15/09/2009	258	18,50	-	-	1,65	0,36	3,47E+05	5,81E+05	9,29E+05	62,61
LA	22/09/2009	265	17,70	-	-	0,52	0,23	7,17E+05	1,49E+06	2,21E+06	67,55
LA	29/09/2009	272	18,40	-	-	0,61	0,31	3,21E+05	7,96E+05	1,12E+06	71,27
LA	08/10/2009	281	16,80	-	-	0,98	0,61	1,06E+06	1,35E+06	2,41E+06	55,85
LA	14/10/2009	287	17,10	-	-	3,58	2,81	9,71E+05	2,74E+06	3,72E+06	73,86
LA	21/10/2009	294	12,80	-	-	0,95	0,48	4,68E+05	1,02E+06	1,49E+06	68,48
LA	27/10/2009	300	16,00	-	-	1,18	0,53	7,23E+05	1,45E+06	2,17E+06	66,66
LA	03/11/2009	307	15,60	-	-	0,56	0,25	8,51E+05	7,02E+05	1,55E+06	45,21
LA	10/11/2009	314	14,80	-	-	0,58	0,28	5,49E+05	9,96E+05	1,54E+06	64,47
LA	20/11/2009	324	14,70	-	-	0,43	0,18	4,56E+05	1,01E+06	1,46E+06	68,87
LA	24/11/2009	328	13,50	-	-	1,20	0,42	4,99E+05	2,07E+06	2,57E+06	80,58
LA	01/12/2009	335	12,50	12,33	34,88	0,47	0,20	2,29E+05	6,47E+05	8,76E+05	73,84
LA	10/12/2009	344	13,50	13,21	34,81	0,64	0,49	1,70E+05	7,42E+05	9,12E+05	81,39
LA	15/12/2009	349	11,90	11,90	-	5,59	4,55	2,13E+05	4,47E+05	6,60E+05	67,71
LA	21/12/2009	355	10,80	10,87	35,18	0,47	0,31	2,54E+05	3,61E+05	6,14E+05	58,68
LA	08/01/2010	8	10,30	10,30	-	0,27	0,16	1,93E+05	3,36E+05	5,29E+05	63,50
LA	12/01/2010	12	9,20	9,01	34,77	0,41	0,27	1,20E+05	3,23E+05	4,44E+05	72,88
LA	19/01/2010	19	12,30	12,19	34,47	0,39	0,07	2,19E+05	4,24E+05	6,43E+05	65,91
LA	26/01/2010	26	12,10	11,97	34,53	0,28	-	1,92E+05	3,37E+05	5,28E+05	63,72
LA	03/02/2010	34	11,20	11,75	35,13	0,31	0,17	1,96E+05	5,67E+05	7,63E+05	74,34
LA	10/02/2010	41	11,60	11,41	34,58	1,49	0,61	7,51E+04	2,91E+05	3,66E+05	79,51
LA	18/02/2010	49	10,40	10,31	35,56	0,34	0,16	9,68E+04	2,86E+05	3,83E+05	74,72
LA	23/02/2010	54	11,50	11,50	-	0,37	0,22	1,43E+05	3,82E+05	5,25E+05	72,72
LA	03/03/2010	62	12,20	12,12	35,33	0,65	0,41	9,86E+04	4,94E+05	5,93E+05	83,37
LA	10/03/2010	69	10,20	9,97	35,39	1,35	0,47	1,09E+05	6,15E+05	7,24E+05	84,88
LA	18/03/2010	77	11,80	11,61	35,40	0,59	0,31	7,70E+04	5,14E+05	5,91E+05	86,96
LA	25/03/2010	84	13,60	13,05	35,21	2,77	0,84	1,23E+05	9,95E+05	1,12E+06	88,98
LA	10/04/2010	100	13,10	11,61	35,40	1,42	0,63	1,30E+05	1,56E+06	1,69E+06	92,30
LA	13/04/2010	103	15,70	15,44	35,61	0,42	0,24	1,27E+05	5,54E+05	6,80E+05	81,38
LA	21/04/2010	111	16,80	16,45	35,51	0,39	0,20	1,37E+05	7,20E+05	8,56E+05	84,04
LA	27/04/2010	117	13,80	13,60	35,35	0,45	0,26	8,51E+04	7,30E+05	8,15E+05	89,56
LA	06/05/2010	126	14,00	14,04	35,21	0,23	0,09	2,18E+05	8,79E+05	1,10E+06	80,10
LA	14/05/2010	134	16,50	16,27	35,34	0,31	0,14	1,48E+05	7,45E+05	8,93E+05	83,45
LA	20/05/2010	140	17,40	16,89	35,48	0,69	0,12	1,61E+05	9,26E+05	1,09E+06	85,21
LA	25/05/2010	145	18,50	18,23	35,36	0,81	0,44	1,54E+05	8,64E+05	1,02E+06	84,88
LA	02/06/2010	153	18,70	18,34	35,64	0,24	0,12	1,50E+05	1,09E+06	1,24E+06	87,95
LA	18/06/2010	169	17,00	16,82	32,75	0,32	0,15	1,35E+05	7,69E+05	9,04E+05	85,05
LA	24/06/2010	175	19,80	19,36	33,91	1,00	0,35	2,31E+05	1,02E+06	1,25E+06	81,51
LA	30/06/2010	181	19,90	19,73	35,13	0,56	0,23	1,61E+05	1,03E+06	1,19E+06	86,48
LA	07/07/2010	188	21,40	21,04	35,05	0,71	0,43	9,83E+04	7,28E+05	8,26E+05	88,10
LA	15/07/2010	196	19,40	19,40	-	0,77	0,34	1,22E+05	1,04E+06	1,17E+06	89,56
LA	20/07/2010	201	21,00	20,73	35,36	0,79	0,31	6,55E+04	1,21E+06	1,27E+06	94,85
LA	27/07/2010	208	22,20	22,00	35,01	0,58	0,38	1,11E+05	8,96E+05	1,01E+06	88,99
LA	03/08/2010	215	20,90	20,49	35,52	0,77	0,40	2,74E+05	1,29E+06	1,57E+06	82,49
LA	13/08/2010	225	20,10	20,08	35,27	0,51	0,24	2,80E+05	1,13E+06	1,41E+06	80,18

Table 1 (Part 1 of 3). Environmental variables and total and relative abundances of bacterial subpopulations. St: Station; JDAY: Julian day; TºC: temperature (ºC); Sal:salinity; Chl: Total chlorophyll *a* (µg ml^{-1}); Chl<2: picoplanktonic chlorophyll *a* (µg ml^{-1});L Ab: LNA bacteria abundance; H Ab: HNA bacteria abundance; T Ab: Total bacterial abundance; %HNA: %HNA bacteria.

St	DATE	JDAY	TºC	T CTD	Sal	Chl	Chl <2	L Ab	H Ab	T Ab	%HNA
LA	18/08/2010	230	20,10	19,77	35,60	0,51	0,30	3,56E+05	7,46E+05	1,10E+06	67,70
LA	27/08/2010	239	20,30	20,01	35,48	0,42	0,23	6,22E+05	9,60E+05	1,58E+06	60,71
LA	10/09/2010	253	17,90	18,03	35,60	0,46	0,24	6,59E+05	7,35E+05	1,39E+06	52,71
LA	17/09/2010	260	19,80	19,66	35,57	0,53	0,15	8,24E+04	4,42E+05	5,25E+05	84,29
LA	24/09/2010	267	17,40	17,32	35,55	0,46	0,19	1,25E+05	3,10E+05	4,35E+05	71,27
LA	30/09/2010	273	17,40	17,40	-	1,11	0,46	1,40E+05	5,43E+05	6,83E+05	79,56
LA	08/10/2010	281	18,40	18,04	35,51	0,74	0,29	2,11E+05	3,57E+05	5,67E+05	62,87
LA	14/10/2010	287	18,10	18,00	35,24	1,43	0,66	1,43E+05	5,29E+05	6,71E+05	78,77
LA	18/10/2010	291	17,00	16,34	35,20	0,59	0,32	1,05E+05	5,05E+05	6,10E+05	82,79
LA	28/10/2010	301	15,60	15,51	35,63	0,44	0,26	1,85E+05	6,21E+05	8,06E+05	77,08
LA	03/11/2010	307	16,30	15,68	35,36	0,44	0,15	2,07E+05	5,13E+05	7,20E+05	71,22
LA	11/11/2010	315	15,00	14,92	34,84	0,85	0,37	2,61E+05	9,28E+05	1,19E+06	78,03
LA	18/11/2010	322	14,20	13,94	34,97	1,76	0,74	1,38E+05	4,80E+05	6,18E+05	77,66
LA	25/11/2010	329	13,50	13,58	33,53	0,56	0,25	1,62E+05	5,29E+05	6,92E+05	76,52
LA	30/11/2010	334	12,30	12,30	-	3,25	1,38	1,62E+05	5,06E+05	6,68E+05	75,73
LA	09/12/2010	343	12,50	12,62	34,59	1,71	0,73	1,04E+05	2,85E+05	3,89E+05	73,352
LA	14/12/2010	348	12,80	12,34	35,24	0,89	0,24	1,92E+05	4,96E+05	6,88E+05	72,10
LA	28/12/2010	362	11,60	11,49	35,09	0,38	0,18	1,90E+05	4,37E+05	6,27E+05	69,72
LA	07/01/2011	7	12,60	12,45	35,09	1,04	0,13	8,03E+04	1,34E+05	2,15E+05	62,58
LA	12/01/2011	12	12,60	12,40	35,09	1,35	0,41	1,07E+05	2,01E+05	3,08E+05	65,29
LA	17/01/2011	17	12,60	12,35	35,07	1,34	0,51	1,43E+05	3,85E+05	5,28E+05	72,95
LA	25/01/2011	25	11,10	11,04	35,41	1,59	0,07	1,07E+05	3,56E+05	4,63E+05	76,84
LA	04/02/2011	35	11,60	11,45	34,29	0,37	0,22	1,31E+05	3,24E+05	4,55E+05	71,26
LA	08/02/2011	39	11,60	11,56	34,71	0,27	0,06	9,67E+04	4,21E+05	5,17E+05	81,31
LA	16/02/2011	47	11,70	11,41	34,88	0,99	0,32	8,69E+04	8,31E+05	9,18E+05	90,54
LA	23/02/2011	54	12,80	12,69	35,22	0,31	0,08	1,52E+05	3,60E+05	5,12E+05	70,32
LA	01/03/2011	60	12,30	12,25	35,03	1,07	0,25	8,81E+04	3,79E+05	4,67E+05	81,12
LA	10/03/2011	69	13,20	13,03	35,49	0,67	0,26	8,25E+04	4,39E+05	5,22E+05	84,18
LA	17/03/2011	76	13,10	12,95	35,43	0,51	0,19	7,06E+04	3,64E+05	4,35E+05	83,76
LA	24/03/2011	83	13,40	13,22	35,41	0,66	0,14	5,22E+04	4,55E+05	5,08E+05	89,71
LA	29/03/2011	88	14,20	13,70	35,55	0,30	0,15	9,77E+04	5,47E+05	6,45E+05	84,85
LA	08/04/2011	98	16,00	15,90	35,35	0,27	0,14	7,48E+04	6,38E+05	7,12E+05	89,50
LA	13/04/2011	103	15,10	14,76	35,45	0,78	0,21	9,69E+04	8,90E+05	9,87E+05	90,18
LA	20/04/2011	110	15,40	15,22	35,64	0,73	0,27	1,47E+05	4,74E+05	6,21E+05	76,29
LA	27/04/2011	117	16,60	16,36	35,62	0,21	0,02	4,62E+04	4,02E+05	4,48E+05	89,70
LA	05/05/2011	125	16,50	16,36	35,62	0,55	0,17	8,82E+04	7,34E+05	8,22E+05	89,28
LA	11/05/2011	131	17,00	16,97	34,50	0,69	0,129	5,56E+04	7,37E+05	7,92E+05	92,98
LA	19/05/2011	139	17,10	17,08	35,57	0,36	0,210	5,51E+04	8,38E+05	8,93E+05	93,83
SS1	14/05/2009	134	-	12,63	35,55	0,558	0,102	1,39E+05	3,22E+05	4,60E+05	69,90
SS1	08/06/2009	159	-	15,29	35,37	1,131	0,105	7,88E+04	5,38E+05	6,17E+05	87,23
SS1	14/07/2009	195	-	19,51	35,47	0,230	0,058	2,87E+05	3,22E+05	6,09E+05	52,88
SS1	06/08/2009	218	-	21,33	35,65	0,242	0,097	4,94E+05	3,81E+05	8,74E+05	43,56
SS1	11/09/2009	254	-	18,43	35,50	1,049	0,530	9,31E+05	8,45E+05	1,78E+06	47,57
SS1	19/10/2009	292	-	12,06	35,66	0,095	0,041	3,99E+05	3,79E+05	7,78E+05	48,75
SS1	17/11/2009	321	-	15,15	35,27	0,882	0,270	6,58E+05	9,02E+05	1,56E+06	57,81
SS1	10/12/2009	344	-	14,04	35,10	1,784	0,726	4,10E+05	5,26E+05	9,36E+05	56,21
SS1	19/01/2010	19	-	12,53	34,78	0,478	0,280	3,59E+05	5,85E+05	9,44E+05	61,95
SS1	17/02/2010	48	-	12,17	35,60	0,315	0,105	1,70E+05	1,83E+05	3,53E+05	51,83
SS1	16/03/2010	75	-	12,10	35,67	1,076	0,107	1,79E+05	1,93E+05	3,72E+05	51,89
SS1	20/04/2010	110	-	13,76	35,67	5,969	0,325	3,63E+05	4,95E+05	8,59E+05	57,68
SS1	13/05/2010	133	-	14,16	35,06	0,630	0,074	1,14E+06	1,24E+06	2,38E+06	51,96
SS1	08/06/2010	159	-	17,70	35,53	0,247	0,095	1,22E+05	3,41E+05	4,63E+05	73,58
SS1	15/07/2010	196	-	18,94	35,41	0,391	0,125	1,12E+06	1,26E+06	2,38E+06	52,80
SS1	09/08/2010	221	-	19,44	35,54	0,452	0,155	1,04E+06	1,12E+06	2,16E+06	51,84
SS1	15/09/2010	258	-	18,04	35,62	0,478	0,022	8,38E+05	7,36E+05	1,57E+06	46,76
SS1	19/10/2010	292	-	17,23	35,67	0,547	0,322	4,19E+05	4,96E+05	9,15E+05	54,25
SS1	30/11/2010	334	-	13,34	34,69	1,269	0,337	2,54E+05	5,12E+05	7,65E+05	66,85
SS1	12/12/2010	346	-	13,58	35,59	0,354	0,138	3,30E+05	3,88E+05	7,18E+05	54,03
SS1	18/01/2011	18	-	12,89	35,00	0,632	0,078	2,71E+05	4,46E+05	7,17E+05	62,19
SS1	24/02/2011	55	-	13,07	35,38	0,356	0,043	2,25E+05	4,24E+05	6,49E+05	65,34
SS1	15/03/2011	74	-	12,99	35,44	1,207	0,235	1,05E+05	2,37E+05	3,42E+05	69,35
SS1	18/04/2011	108	-	13,66	35,78	0,678	0,386	2,58E+05	5,18E+05	7,76E+05	66,74

Table 1 (Part 2 of 3). Environmental variables and total and relative abundances of bacterial subpopulations. St: Station; JDAY: Julian day; TºC: temperature (ºC); Sal:salinity; Chl: Total chlorophyll *a* (µg ml^{-1}); Chl<2: picoplanktonic chlorophyll *a* (µg ml^{-1});L Ab: LNA bacteria abundance; H Ab: HNA bacteria abundance; T Ab: Total bacterial abundance; %HNA: %HNA bacteria.

St	DATE	JDAY	TºC	T CTD	Sal	Chl	Chl <2	L Ab	H Ab	T Ab	%HNA
SS1	09/05/2011	129	-	15,24	35,65	0,608	0,172	2,15E+05	6,16E+05	8,32E+05	74,13
SS2	14/05/2009	134	-	13,93	35,24	0,381	0,173	1,87E+05	5,60E+05	7,47E+05	75,0
SS2	08/06/2009	159	-	15,99	35,44	0,332	0,042	1,86E+05	3,29E+05	5,16E+05	63,9
SS2	14/07/2009	195	-	20,59	35,57	0,112	0,009	2,88E+05	2,47E+05	5,35E+05	46,2
SS2	06/08/2009	218	-	21,32	35,65	0,411	0,232	4,13E+05	2,72E+05	6,85E+05	39,7
SS2	11/09/2009	254	-	21,71	35,38	0,222	0,104	6,49E+05	6,04E+05	1,25E+06	48,2
SS2	19/10/2009	292	-	13,88	35,62	1,243	0,075	1,59E+06	1,15E+06	2,75E+06	42,0
SS2	17/11/2009	321	-	15,38	35,44	0,768	0,297	6,60E+05	8,04E+05	1,46E+06	54,9
SS2	10/12/2009	344	-	14,08	35,01	1,865	0,747	3,88E+05	5,26E+05	9,15E+05	57,5
SS2	19/01/2010	19	-	12,69	34,75	2,334	0,955	3,89E+05	5,68E+05	9,57E+05	59,4
SS2	17/02/2010	48	-	12,35	35,53	0,488	0,191	2,00E+05	1,71E+05	3,70E+05	46,1
SS2	16/03/2010	75	-	12,20	35,69	0,922	0,285	1,38E+05	1,37E+05	2,74E+05	49,9
SS2	20/04/2010	110	-	13,54	35,63	1,248	0,272	4,14E+05	2,77E+05	6,91E+05	40,1
SS2	13/05/2010	133	-	14,08	35,14	1,402	0,469	8,66E+05	1,24E+06	2,11E+06	58,9
SS2	08/06/2010	159	-	17,72	35,58	0,238	0,120	2,64E+05	1,91E+05	4,55E+05	41,9
SS2	15/07/2010	196	-	20,44	35,33	0,161	0,066	5,59E+05	5,69E+05	1,13E+06	50,5
SS2	09/08/2010	221	-	20,49	35,41	0,290	0,089	1,10E+06	6,32E+05	1,73E+06	36,5
SS2	15/09/2010	258	-	20,15	35,70	0,216	0,112	7,26E+05	4,77E+05	1,20E+06	39,7
SS2	19/09/2010	262	-	18,21	35,76	0,302	0,167	3,10E+05	3,44E+05	6,55E+05	52,6
SS2	30/11/2010	334	-	13,66	35,09	0,335	0,024	2,41E+05	3,61E+05	6,02E+05	60,0
SS2	12/12/2010	346	-	13,76	35,60	0,516	0,221	3,06E+05	4,21E+05	7,27E+05	57,9
SS2	18/01/2011	18	-	12,9243	34,8422	0,858	0,388	3,96E+05	6,27E+05	1,02E+06	61,3
SS2	24/02/2011	55	-	12,9726	35,5007	0,611	0,115	3,08E+05	4,45E+05	7,53E+05	59,1
SS2	15/03/2011	74	-	12,9817	35,5021	0,769	0,106	1,18E+05	2,61E+05	3,79E+05	69,0
SS2	18/04/2011	108	-	14,5496	35,6521	0,580	0,191	5,19E+05	6,73E+05	1,19E+06	56,5
SS2	09/05/2011	129	-	16,60	35,72	0,462	0,084	7,11E+05	1,05E+06	1,76E+06	59,5
SS3	14/05/2009	134	-	13,86	35,25	0,525	0,205	7,71E+04	4,19E+05	4,96E+05	84,5
SS3	08/06/2009	159	-	16,04	35,46	0,359	0,102	2,56E+05	3,78E+05	6,34E+05	59,6
SS3	14/07/2009	195	-	20,44	35,58	0,207	0,091	3,91E+05	2,91E+05	6,82E+05	42,7
SS3	06/08/2009	218	-	21,42	35,64	0,174	0,046	7,02E+05	4,17E+05	1,12E+06	37,3
SS3	11/09/2009	254	-	21,45	35,32	0,169	0,075	6,52E+05	5,31E+05	1,18E+06	44,9
SS3	19/10/2009	292	-	13,24	35,63	1,615	0,321	1,06E+06	7,38E+05	1,80E+06	41,1
SS3	17/11/2009	321	-	15,97	35,64	0,554	0,358	3,32E+05	4,10E+05	7,42E+05	55,2
SS3	10/12/2009	344	-	14,54	35,65	0,648	0,394	3,91E+05	3,66E+05	7,58E+05	48,3
SS3	19/01/2010	19	-	13,26	35,39	0,830	0,509	4,01E+05	4,59E+05	8,60E+05	53,4
SS3	17/02/2010	48	-	12,08	35,48	0,657	0,233	2,74E+05	2,08E+05	4,82E+05	43,2
SS3	16/03/2010	75	-	12,20	35,70	0,477	0,156	1,84E+05	1,64E+05	3,49E+05	47,1
SS3	20/04/2010	110	-	13,22	35,71	3,083	0,716	5,25E+05	9,73E+05	1,50E+06	65,0
SS3	13/05/2010	133	-	14,07	35,77	0,634	0,244	1,09E+06	8,83E+05	1,97E+06	44,7
SS3	08/06/2010	159	-	17,48	35,48	0,290	0,102	1,32E+05	3,24E+05	4,57E+05	71,1
SS3	15/07/2010	196	-	20,16	35,33	0,196	0,067	-	-	-	-
SS3	09/08/2010	221	-	21,79	35,57	0,237	0,099	7,94E+05	4,80E+05	1,27E+06	37,7
SS3	15/09/2010	258	-	20,34	35,71	0,120	0,044	6,08E+05	4,70E+05	1,08E+06	43,6
SS3	19/10/2010	292	-	18,15	35,75	0,464	0,265	2,92E+05	3,83E+05	6,75E+05	56,8
SS3	30/11/2010	334	-	-	-	-	-	-	-	-	-
SS3	12/12/2010	346	-	13,79	35,60	0,312	0,158	3,40E+05	3,70E+05	7,09E+05	52,1
SS3	18/01/2011	18	-	14,41	35,90	0,608	0,288	2,44E+05	2,88E+05	5,32E+05	54,1
SS3	24/02/2011	55	-	13,17	35,84	0,283	0,102	3,04E+05	2,94E+05	5,98E+05	49,2
SS3	15/03/2011	74	-	12,86	35,64	0,752	0,086	9,82E+04	3,70E+05	4,68E+05	79,0
SS3	18/04/2011	108	-	14,64	35,69	0,353	0,142	7,05E+05	7,33E+05	1,44E+06	51,0
SS3	09/05/2011	129	-	16,59	35,78	0,436	0,062	7,52E+05	7,58E+05	1,51E+06	50,2

Table 1 (Part 3 of 3). Environmental variables and total and relative abundances of bacterial subpopulations. St: Station; JDAY: Julian day; TºC: temperature (ºC); Sal:salinity; Chl: Total chlorophyll *a* (µg ml^{-1}); Chl<2: picoplanktonic chlorophyll *a* (µg ml^{-1});L Ab: LNA bacteria abundance; H Ab: HNA bacteria abundance; T Ab: Total bacterial abundance; %HNA: %HNA bacteria.

ANNEX II. Data II

Flow cytometric single-cell properties and biomass of bacterial subpopulations

St	DATE	L SSC	H SSC	L FL1	H FL1	L Diam	H Diam	L Vol	H Vol	L Biom	H Biom	T Biom
LA	18/05/2009	0,052	0,091	0,009	0,033	0,472	0,554	0,055	0,089	0,98	7,64	8,62
LA	28/05/2009	0,054	0,077	0,007	0,030	0,477	0,529	0,057	0,078	1,08	10,06	11,14
LA	02/06/2009	0,055	0,072	0,006	0,028	0,480	0,520	0,058	0,074	1,09	11,76	12,85
LA	11/06/2009	0,047	0,068	0,006	0,027	0,458	0,511	0,050	0,070	0,75	15,58	16,33
LA	17/06/2009	0,052	0,071	0,005	0,025	0,473	0,518	0,055	0,073	1,25	26,00	27,25
LA	25/06/2009	0,047	0,071	0,004	0,025	0,457	0,518	0,050	0,073	1,99	19,23	21,22
LA	02/07/2009	0,044	0,069	0,004	0,023	0,445	0,513	0,046	0,071	1,79	19,53	21,31
LA	10/07/2009	0,045	0,069	0,003	0,020	0,451	0,514	0,048	0,071	2,23	22,83	25,06
LA	15/07/2009	0,049	0,085	0,004	0,023	0,462	0,545	0,052	0,085	2,19	8,47	10,66
LA	24/07/2009	0,044	0,075	0,004	0,023	0,448	0,526	0,047	0,076	3,27	8,38	11,65
LA	29/07/2009	0,051	0,076	0,004	0,024	0,470	0,528	0,054	0,077	2,84	24,29	27,12
LA	07/08/2009	0,048	0,080	0,004	0,022	0,460	0,535	0,051	0,080	3,82	12,82	16,65
LA	11/08/2009	0,055	0,071	0,003	0,022	0,479	0,518	0,057	0,073	2,90	23,29	26,19
LA	17/08/2009	0,045	0,084	0,004	0,023	0,450	0,543	0,048	0,084	3,75	28,10	31,85
LA	26/08/2009	0,050	0,069	0,004	0,019	0,465	0,514	0,053	0,071	6,62	16,05	22,68
LA	01/09/2009	0,053	0,075	0,004	0,019	0,473	0,525	0,056	0,076	7,41	23,19	30,60
LA	11/09/2009	0,053	0,071	0,004	0,020	0,473	0,518	0,055	0,073	3,51	14,73	18,24
LA	15/09/2009	0,053	0,075	0,005	0,021	0,473	0,526	0,056	0,076	5,51	11,52	17,03
LA	22/09/2009	0,054	0,074	0,004	0,022	0,478	0,524	0,057	0,076	11,62	29,37	40,99
LA	29/09/2009	0,055	0,080	0,004	0,017	0,480	0,536	0,058	0,081	5,25	16,37	21,61
LA	08/10/2009	0,049	0,069	0,005	0,018	0,464	0,514	0,052	0,071	16,19	25,39	41,58
LA	14/10/2009	0,054	0,078	0,004	0,021	0,477	0,532	0,057	0,079	15,62	55,69	71,32
LA	21/10/2009	0,052	0,072	0,004	0,020	0,470	0,519	0,055	0,073	7,33	19,58	26,91
LA	27/10/2009	0,049	0,074	0,005	0,019	0,463	0,523	0,052	0,075	10,98	28,31	39,28
LA	03/11/2009	0,051	0,091	0,005	0,021	0,470	0,555	0,054	0,089	13,28	15,53	28,81
LA	10/11/2009	0,045	0,062	0,004	0,017	0,452	0,497	0,048	0,064	7,89	17,49	25,38
LA	20/11/2009	0,054	0,099	0,005	0,020	0,478	0,566	0,057	0,095	7,38	23,26	30,64
LA	24/11/2009	0,054	0,070	0,004	0,016	0,476	0,514	0,057	0,071	8,03	39,13	47,16
LA	01/12/2009	0,048	0,079	0,006	0,023	0,460	0,533	0,051	0,079	3,43	13,17	16,60
LA	10/12/2009	0,052	0,075	0,005	0,026	0,471	0,526	0,055	0,076	2,67	14,71	17,38
LA	15/12/2009	0,052	0,089	0,006	0,024	0,472	0,551	0,055	0,088	3,36	9,74	13,10
LA	21/12/2009	0,048	0,076	0,006	0,023	0,460	0,528	0,051	0,077	3,79	7,20	10,99
LA	08/01/2010	0,051	0,075	0,005	0,022	0,468	0,525	0,054	0,076	2,99	6,64	9,63
LA	12/01/2010	0,054	0,074	0,006	0,025	0,478	0,524	0,057	0,075	1,94	6,34	8,29
LA	19/01/2010	0,049	0,073	0,005	0,022	0,462	0,521	0,052	0,074	3,30	8,21	11,52
LA	26/01/2010	0,049	0,072	0,005	0,021	0,464	0,519	0,052	0,073	2,92	6,47	9,39
LA	03/02/2010	0,052	0,075	0,005	0,024	0,473	0,525	0,055	0,076	3,10	11,16	14,26
LA	10/02/2010	0,048	0,073	0,005	0,025	0,460	0,522	0,051	0,075	1,12	5,68	6,80
LA	18/02/2010	0,046	0,071	0,005	0,025	0,454	0,518	0,049	0,073	1,41	5,49	6,90
LA	23/02/2010	0,051	0,074	0,004	0,024	0,467	0,524	0,053	0,075	2,21	7,51	9,72
LA	03/03/2010	0,054	0,066	0,005	0,023	0,477	0,507	0,057	0,068	1,59	9,07	10,67
LA	10/03/2010	0,054	0,081	0,005	0,021	0,478	0,537	0,057	0,081	1,77	12,72	14,49
LA	18/03/2010	0,060	0,072	0,005	0,024	0,493	0,520	0,063	0,074	1,33	9,92	11,26
LA	25/03/2010	0,052	0,063	0,006	0,023	0,472	0,500	0,055	0,065	1,95	17,67	19,62
LA	10/04/2010	0,059	0,071	0,004	0,021	0,490	0,518	0,062	0,073	2,21	29,79	32,00
LA	13/04/2010	0,050	0,080	0,005	0,022	0,467	0,535	0,053	0,080	1,95	11,34	13,30
LA	21/04/2010	0,049	0,074	0,007	0,026	0,461	0,525	0,051	0,076	2,05	14,16	16,21
LA	27/04/2010	0,057	0,081	0,005	0,025	0,485	0,536	0,060	0,081	1,42	15,05	16,47
LA	06/05/2010	0,054	0,082	0,006	0,030	0,477	0,539	0,057	0,082	3,52	18,29	21,82
LA	14/05/2010	0,054	0,072	0,006	0,026	0,477	0,520	0,057	0,074	2,38	14,43	16,81
LA	20/05/2010	0,051	0,075	0,005	0,025	0,469	0,525	0,054	0,076	2,50	18,25	20,75
LA	25/05/2010	0,054	0,078	0,005	0,022	0,478	0,532	0,057	0,079	2,50	17,48	19,98
LA	02/06/2010	0,064	0,072	0,005	0,021	0,502	0,520	0,066	0,074	2,69	21,16	23,85
LA	18/06/2010	0,046	0,082	0,005	0,024	0,453	0,538	0,049	0,082	1,96	15,98	17,94
LA	24/06/2010	0,051	0,075	0,006	0,025	0,468	0,526	0,054	0,076	3,58	20,16	23,75
LA	30/06/2010	0,062	0,069	0,005	0,024	0,498	0,513	0,065	0,071	2,83	19,34	22,18
LA	07/07/2010	0,059	0,091	0,005	0,025	0,490	0,554	0,061	0,089	1,67	16,04	17,72
LA	15/07/2010	0,054	0,079	0,005	0,023	0,477	0,533	0,057	0,079	1,96	21,23	23,19
LA	20/07/2010	0,051	0,080	0,005	0,024	0,469	0,534	0,054	0,080	1,02	24,70	25,72
LA	27/07/2010	0,047	0,076	0,005	0,024	0,458	0,527	0,050	0,076	1,64	17,77	19,41
LA	03/08/2010	0,053	0,074	0,005	0,020	0,473	0,523	0,056	0,075	4,35	25,29	29,64
LA	13/08/2010	0,063	0,083	0,004	0,021	0,501	0,541	0,066	0,083	4,99	23,80	28,79

Table 2 (Part 1 of 3). Flow cytometric and extrapolated single-cell properties and total and relative biomass of bacterial subpopulations. St: Station; L: LNA subpopulation; H: HNA subpopulation; SSC: relative side scatter; FL1: relative green fluorescence; Diam: cell diameter (µm); Vol: cell volume (μm^3); Biom: Biomass ($\mu g\ C\ l^{-1}$).

St	DATE	L SSC	H SSC	L FL1	H FL1	L Diam	H Diam	L Vol	H Vol	L Biom	H Biom	T Biom
LA	18/08/2010	0,053	0,090	0,004	0,023	0,474	0,553	0,056	0,088	5,67	16,38	22,05
LA	27/08/2010	0,059	0,084	0,005	0,022	0,490	0,542	0,062	0,083	10,62	20,20	30,82
LA	10/09/2010	0,053	0,071	0,004	0,018	0,476	0,517	0,056	0,073	10,56	14,05	24,61
LA	17/09/2010	0,063	0,103	0,004	0,024	0,499	0,572	0,065	0,098	1,46	10,44	11,90
LA	24/09/2010	0,053	0,085	0,005	0,023	0,473	0,544	0,055	0,084	1,98	6,57	8,56
LA	30/09/2010	0,062	0,114	0,004	0,026	0,497	0,587	0,064	0,106	2,45	13,56	16,01
LA	08/10/2010	0,051	0,077	0,004	0,019	0,469	0,530	0,054	0,078	3,28	7,17	10,45
LA	14/10/2010	0,052	0,079	0,004	0,024	0,472	0,532	0,055	0,079	2,25	10,73	12,99
LA	18/10/2010	0,054	0,081	0,004	0,023	0,477	0,537	0,057	0,081	1,69	10,43	12,12
LA	28/10/2010	0,057	0,071	0,004	0,022	0,486	0,518	0,060	0,073	3,10	11,89	14,98
LA	03/11/2010	0,052	0,080	0,004	0,023	0,473	0,535	0,055	0,080	3,28	10,53	13,81
LA	11/11/2010	0,051	0,073	0,004	0,021	0,469	0,521	0,054	0,074	4,07	18,02	22,08
LA	18/11/2010	0,049	0,079	0,004	0,021	0,464	0,533	0,052	0,079	2,10	9,75	11,85
LA	25/11/2010	0,049	0,064	0,004	0,018	0,463	0,503	0,052	0,067	2,46	9,54	12,00
LA	30/11/2010	0,051	0,076	0,004	0,021	0,468	0,528	0,054	0,077	2,51	10,10	12,61
LA	09/12/2010	0,059	0,074	0,004	0,019	0,490	0,523	0,062	0,075	1,77	5,59	7,36
LA	14/12/2010	0,052	0,071	0,005	0,021	0,470	0,517	0,054	0,073	3,00	9,49	12,49
LA	28/12/2010	0,052	0,074	0,005	0,023	0,471	0,524	0,055	0,075	2,98	8,57	11,55
LA	07/01/2011	0,049	0,085	0,005	0,025	0,464	0,544	0,052	0,085	1,22	2,86	4,08
LA	12/01/2011	0,048	0,077	0,005	0,027	0,459	0,530	0,051	0,078	1,59	4,04	5,63
LA	17/01/2011	0,057	0,082	0,006	0,028	0,486	0,538	0,060	0,081	2,40	7,98	10,38
LA	25/01/2011	0,055	0,101	0,005	0,027	0,480	0,569	0,058	0,097	1,75	8,31	10,06
LA	04/02/2011	0,056	0,073	0,007	0,029	0,481	0,521	0,058	0,074	2,15	6,29	8,44
LA	08/02/2011	0,053	0,073	0,006	0,028	0,474	0,522	0,056	0,075	1,54	8,20	9,74
LA	16/02/2011	0,050	0,072	0,006	0,029	0,466	0,520	0,053	0,074	1,33	16,07	17,41
LA	23/02/2011	0,050	0,067	0,006	0,027	0,467	0,508	0,053	0,069	2,34	6,63	8,97
LA	01/03/2011	0,053	0,082	0,005	0,026	0,475	0,539	0,056	0,082	1,41	7,90	9,31
LA	10/03/2011	0,052	0,076	0,005	0,024	0,470	0,528	0,054	0,077	1,29	8,75	10,04
LA	17/03/2011	0,053	0,086	0,006	0,028	0,475	0,546	0,056	0,085	1,13	7,78	8,91
LA	24/03/2011	0,052	0,081	0,005	0,028	0,471	0,537	0,055	0,081	0,82	9,41	10,23
LA	29/03/2011	0,051	0,081	0,006	0,028	0,469	0,537	0,054	0,081	1,52	11,33	12,85
LA	08/04/2011	0,047	0,077	0,005	0,026	0,456	0,530	0,050	0,078	1,09	12,81	13,90
LA	13/04/2011	0,053	0,074	0,005	0,025	0,474	0,523	0,056	0,075	1,54	17,39	18,93
LA	20/04/2011	0,054	0,077	0,005	0,025	0,478	0,529	0,057	0,077	2,38	9,49	11,87
LA	27/04/2011	0,052	0,077	0,006	0,029	0,471	0,530	0,055	0,078	0,73	8,07	8,80
LA	05/05/2011	0,046	0,070	0,005	0,025	0,453	0,515	0,049	0,072	1,27	13,93	15,20
LA	11/05/2011	0,056	0,070	0,017	0,066	0,482	0,515	0,059	0,072	0,92	13,96	14,88
LA	19/05/2011	0,049	0,069	0,014	0,059	0,464	0,514	0,052	0,071	0,84	15,82	16,66
SS1	14/05/2009	0,051	0,064	0,008	0,033	0,470	0,502	0,054	0,066	2,17	5,77	7,94
SS1	08/06/2009	0,053	0,079	0,006	0,032	0,474	0,533	0,056	0,079	1,25	10,93	12,18
SS1	14/07/2009	0,048	0,072	0,004	0,020	0,461	0,518	0,051	0,073	4,30	6,18	10,48
SS1	06/08/2009	0,045	0,061	0,003	0,014	0,448	0,496	0,047	0,064	6,99	6,67	13,66
SS1	11/09/2009	0,046	0,053	0,005	0,017	0,454	0,473	0,049	0,056	13,54	13,40	26,94
SS1	19/10/2009	0,049	0,052	0,004	0,018	0,463	0,471	0,052	0,055	6,04	5,96	12,01
SS1	17/11/2009	0,046	0,046	0,004	0,018	0,452	0,455	0,048	0,049	9,49	13,14	22,62
SS1	10/12/2009	0,050	0,058	0,005	0,021	0,467	0,488	0,053	0,061	6,32	8,90	15,22
SS1	19/01/2010	0,053	0,058	0,006	0,023	0,475	0,488	0,056	0,061	5,73	9,90	15,63
SS1	17/02/2010	0,047	0,059	0,004	0,019	0,457	0,491	0,050	0,062	2,50	3,13	5,63
SS1	16/03/2010	0,047	0,057	0,005	0,021	0,456	0,484	0,050	0,060	2,63	3,21	5,84
SS1	20/04/2010	0,053	0,063	0,006	0,022	0,473	0,500	0,055	0,066	5,76	8,83	14,58
SS1	13/05/2010	0,052	0,054	0,005	0,020	0,472	0,476	0,055	0,056	18,00	19,83	37,83
SS1	08/06/2010	0,056	0,066	0,006	0,025	0,483	0,506	0,059	0,068	2,02	6,21	8,24
SS1	15/07/2010	0,053	0,053	0,005	0,016	0,475	0,473	0,056	0,055	17,99	19,94	37,93
SS1	09/08/2010	0,050	0,062	0,005	0,018	0,466	0,497	0,053	0,064	15,94	19,61	35,55
SS1	15/09/2010	0,047	0,056	0,005	0,019	0,456	0,482	0,050	0,059	12,31	12,13	24,44
SS1	19/10/2010	0,048	0,069	0,005	0,023	0,458	0,514	0,050	0,071	6,21	9,35	15,56
SS1	30/11/2010	0,053	0,053	0,004	0,019	0,474	0,475	0,056	0,056	4,04	8,16	12,21
SS1	12/12/2010	0,050	0,053	0,005	0,022	0,466	0,473	0,053	0,055	5,07	6,14	11,21
SS1	18/01/2011	0,051	0,074	0,005	0,025	0,469	0,524	0,054	0,075	4,22	8,75	12,97
SS1	24/02/2011	0,052	0,063	0,005	0,024	0,471	0,501	0,055	0,066	3,52	7,57	11,09
SS1	15/03/2011	0,053	0,058	0,004	0,021	0,473	0,488	0,055	0,061	1,66	4,02	5,68
SS1	18/04/2011	0,055	0,074	0,005	0,024	0,479	0,524	0,057	0,075	4,19	10,18	14,37

Table 2 (Part 2 of 3). Flow cytometric and extrapolated single-cell properties and total and relative biomass of bacterial subpopulations. St: Station; L: LNA subpopulation; H: HNA subpopulation; SSC: relative side scatter; FL1: relative green fluorescence; Diam: cell diameter (µm); Vol: cell volume (μm^3); Biom: Biomass ($\mu g\ C\ l^{-1}$).

St	DATE	L SSC	H SSC	L FL1	H FL1	L Diam	H Diam	L Vol	H Vol	L Biom	H Biom	T Biom
SS1	09/05/2011	0,054	0,056	0,005	0,022	0,476	0,483	0,056	0,059	3,45	10,20	13,65
SS2	14/05/2009	0,052	0,074	0,006	0,031	0,471	0,524	0,055	0,075	2,93	11,01	13,94
SS2	08/06/2009	0,050	0,061	0,005	0,020	0,465	0,495	0,053	0,063	2,84	5,74	8,58
SS2	14/07/2009	0,045	0,065	0,004	0,017	0,451	0,505	0,048	0,067	4,12	4,50	8,61
SS2	06/08/2009	0,044	0,048	0,004	0,016	0,448	0,461	0,047	0,051	5,84	4,08	9,92
SS2	11/09/2009	0,045	0,064	0,005	0,018	0,449	0,503	0,047	0,067	9,21	10,90	20,11
SS2	19/10/2009	0,049	0,049	0,004	0,016	0,462	0,464	0,052	0,052	24,03	17,54	41,57
SS2	17/11/2009	0,047	0,042	0,004	0,018	0,456	0,441	0,050	0,045	9,68	10,98	20,66
SS2	10/12/2009	0,051	0,053	0,006	0,022	0,469	0,474	0,054	0,056	6,04	8,37	14,41
SS2	19/01/2010	0,046	0,055	0,006	0,025	0,453	0,479	0,049	0,057	5,63	9,22	14,85
SS2	17/02/2010	0,046	0,047	0,005	0,020	0,454	0,458	0,049	0,050	2,89	2,53	5,42
SS2	16/03/2010	0,046	0,046	0,005	0,022	0,454	0,454	0,049	0,049	2,00	1,99	3,99
SS2	20/04/2010	0,052	0,060	0,005	0,023	0,470	0,493	0,055	0,063	6,48	4,78	11,26
SS2	13/05/2010	0,048	0,056	0,005	0,022	0,459	0,482	0,051	0,059	12,87	20,48	33,36
SS2	08/06/2010	0,053	0,072	0,007	0,028	0,475	0,520	0,056	0,073	4,22	3,68	7,90
SS2	15/07/2010	0,051	0,057	0,006	0,019	0,470	0,484	0,054	0,059	8,73	9,45	18,18
SS2	09/08/2010	0,047	0,060	0,005	0,019	0,455	0,493	0,049	0,063	16,07	10,90	26,97
SS2	15/09/2010	0,043	0,054	0,005	0,019	0,443	0,477	0,045	0,057	10,01	7,68	17,68
SS2	19/09/2010	0,045	0,056	0,005	0,024	0,452	0,482	0,048	0,059	4,46	5,69	10,14
SS2	30/11/2010	0,048	0,049	0,004	0,019	0,460	0,463	0,051	0,052	3,61	5,47	9,07
SS2	12/12/2010	0,047	0,048	0,004	0,019	0,457	0,459	0,050	0,051	4,51	6,26	10,77
SS2	18/01/2011	0,053	0,053	0,006	0,024	0,473	0,475	0,056	0,056	6,29	10,01	16,30
SS2	24/02/2011	0,052	0,055	0,005	0,021	0,471	0,478	0,055	0,057	4,83	7,22	12,05
SS2	15/03/2011	0,053	0,057	0,006	0,028	0,475	0,485	0,056	0,060	1,88	4,36	6,23
SS2	18/04/2011	0,053	0,074	0,005	0,023	0,473	0,524	0,055	0,075	8,21	13,23	21,44
SS2	09/05/2011	0,049	0,054	0,005	0,021	0,463	0,478	0,052	0,057	10,76	16,92	27,68
SS3	14/05/2009	0,048	0,063	0,006	0,028	0,458	0,499	0,050	0,065	1,14	7,43	8,57
SS3	08/06/2009	0,044	0,053	0,004	0,020	0,447	0,474	0,047	0,056	3,60	6,01	9,61
SS3	14/07/2009	0,044	0,052	0,004	0,014	0,445	0,471	0,046	0,055	5,45	4,58	10,03
SS3	06/08/2009	0,041	0,069	0,004	0,017	0,437	0,513	0,044	0,071	9,40	7,84	17,24
SS3	11/09/2009	0,047	0,064	0,005	0,019	0,457	0,502	0,050	0,066	9,61	9,54	19,15
SS3	19/10/2009	0,048	0,047	0,004	0,016	0,459	0,457	0,051	0,050	15,74	10,90	26,64
SS3	17/11/2009	0,043	0,048	0,004	0,020	0,445	0,460	0,046	0,051	4,63	6,12	10,75
SS3	10/12/2009	0,045	0,052	0,005	0,022	0,450	0,470	0,048	0,054	5,59	5,73	11,32
SS3	19/01/2010	0,045	0,054	0,006	0,023	0,451	0,476	0,048	0,056	5,75	7,37	13,12
SS3	17/02/2010	0,044	0,052	0,005	0,022	0,446	0,472	0,047	0,055	3,84	3,27	7,11
SS3	16/03/2010	0,045	0,043	0,006	0,023	0,449	0,445	0,048	0,046	2,62	2,29	4,91
SS3	20/04/2010	0,048	0,049	0,006	0,022	0,460	0,463	0,051	0,052	7,84	14,75	22,59
SS3	13/05/2010	0,044	0,050	0,005	0,020	0,447	0,466	0,047	0,053	15,38	13,55	28,93
SS3	08/06/2010	0,056	0,071	0,007	0,030	0,482	0,516	0,059	0,072	2,18	6,18	8,36
SS3	15/07/2010	-	-	-	-	-	-	-	-	-	-	-
SS3	09/08/2010	0,045	0,060	0,004	0,016	0,451	0,492	0,048	0,062	11,38	8,24	19,61
SS3	15/09/2010	0,044	0,061	0,005	0,020	0,448	0,495	0,047	0,063	8,60	8,17	16,77
SS3	19/10/2010	0,046	0,074	0,006	0,025	0,453	0,524	0,049	0,075	4,21	7,52	11,73
SS3	30/11/2010	-	-	-	-	-	-	-	-	-	-	-
SS3	12/12/2010	0,047	0,053	0,005	0,020	0,458	0,475	0,050	0,056	5,03	5,91	10,94
SS3	18/01/2011	0,045	0,082	0,006	0,028	0,450	0,539	0,048	0,082	3,49	6,00	9,49
SS3	24/02/2011	0,047	0,047	0,005	0,019	0,457	0,456	0,050	0,050	4,48	4,32	8,80
SS3	15/03/2011	0,055	0,056	0,006	0,030	0,480	0,483	0,058	0,059	1,60	6,11	7,72
SS3	18/04/2011	0,054	0,085	0,005	0,024	0,477	0,544	0,057	0,084	11,38	15,59	26,97
SS3	09/05/2011	0,049	0,052	0,005	0,022	0,463	0,473	0,052	0,055	11,40	11,99	23,39

Table 2 (Part 3 of 3). Flow cytometric and extrapolated single-cell properties and total and relative biomass of bacterial subpopulations. St: Station; L: LNA subpopulation; H: HNA subpopulation; SSC: relative side scatter; FL1: relative green fluorescence; Diam: cell diameter (µm); Vol: cell volume (μm^3); Biom: Biomass ($\mu g\ C\ l^{-1}$).