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# Growth, grazing and carbon flux of high and low nucleic acid bacteria differ in surface and deep chlorophyll maximum layers in the NW Mediterranean Sea

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ABSTRACT: Growth and grazing mortality of marine heterotrophic bacteria were measured in the summer of 2000 in coastal waters of the NW Mediterranean Sea. Serial-dilution experiments were performed with water from surface and deep chlorophyll maximum (DCM) layers. Bacterial abundances (mean ± SD) were very similar at the surface ($7.2 \pm 2.9 \times 10^5$ cells $ml^{-1}$) and DCM ($7.4 \pm 1.1 \times 10^5$ cells $ml^{-1}$). Intrinsic bacterial growth rates (mean ± SD) were 0.88 ± 0.43 $d^{-1}$ in the surface layer and 0.71 ± 0.23 $d^{-1}$ at the DCM. Grazing rates on bacteria (mean ± SD) were 0.75 ± 0.23 and 0.58 ± 0.29 $d^{-1}$, in the surface and DCM layers, respectively. Nucleic acid content analysis by flow cytometry revealed different intrinsic growth rates and grazing pressure on bacteria of high (HNA) and low (LNA) content depending on their location in the water column. Generally, growth and grazing rates were balanced in both groups in both layers. At the surface, HNA bacteria revealed significantly higher intrinsic growth rates than LNA bacteria (1.18 ± 0.60 and 0.47 ± 0.28 $d^{-1}$, respectively). Average growth rates at the DCM were higher for LNA (0.90 ± 0.46 $d^{-1}$) than for HNA (0.36 ± 0.23 $d^{-1}$), but not significantly. At the surface, grazing rates on HNA bacteria were also significantly higher than on LNA bacteria (1.02 ± 0.31 and 0.37 ± 0.19 $d^{-1}$, respectively). At the DCM, the opposing tendency, though not statistically significant, was observed (0.26 ± 0.17 and 0.77 ± 0.50 $d^{-1}$). Bacteria were responsible for a large portion of the C flux through the system. Bacterial C flux was funneled mostly by HNA bacteria at the surface (70%) and by LNA at the DCM (80%). HNA bacteria were the most active component of the bacterial community in the surface layer. However, we found that LNA bacteria were also active, particularly at the DCM, suggesting differences in structure and functioning of the corresponding microbial networks. The most significant result was the clear relation between depth and activity of each bacterial fraction.

KEY WORDS: HNA bacteria · LNA bacteria · Bacterial growth · Grazing mortality of bacteria · Carbon flux · Deep chlorophyll maximum layer



## INTRODUCTION

Heterotrophic bacteria constitute a fundamental component of marine ecosystems, with important roles in the biogeochemical cycles of key elements (Sherr & Sherr 2000). Nowadays the link of bacterial diversity or physiology and ecological functionality is a field of intense research. The diversity of bacterial communities in different pelagic habitats cannot be precisely assessed microscopically due to their minute size and the limited variety of morphological features. Molecular approaches are the most appropriate techniques for diversity and physiological studies. However, they are still too laborious for processing the large sets of samples needed in many ecological studies. As a first approach, flow cytometry measurements combined with nuclear staining provide information on the nucleic acid content of bacterial populations (Li et al.

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1995). This parameter can be a valuable tool for distinguishing the assemblages and/or physiological status of populations. The initial studies taking into consideration this parameter indicated that bacteria with high nucleic acid (HNA) content were the active fraction (e.g. Gasol & del Giorgio 2000). However, recent studies suggest that bacteria with low nucleic acid (LNA) content are not metabolically inactive and can be an integral part of the microbial food web (Zubkov et al. 2001, Jochem et al. 2004, Longnecker et al. 2005).

In the present study, we focused on the role of HNA and LNA bacteria in the carbon flux of the ecosystem. Heterotrophic bacteria play a predominant role in the dynamics of carbon. On the one hand, they are the main consumers of dissolved organic carbon (DOC). On the other hand, they are heavily preyed upon by heterotrophic nanoflagellates, contributing to a fast cycling of carbon within the microbial food web. We combined dilution experiments with flow cytometry measurements in order to measure growth and grazing rates for bacterial populations and to estimate bacterial carbon flux. We chose the serial-dilution method (Landry & Hassett 1982) as it manipulates the bacterial and bacterial grazer communities as a whole. The serial-dilution method, which originally was devised for protozoan grazing on phytoplankton, has also been applied in measuring bacterial growth and grazing (e.g. Tremaine & Mills 1987, Murrell & Hollibaugh 1998, Rivkin et al. 1999, Sakka et al. 2000, Jochem et al. 2004).

Our research was carried out within the framework of a biological-oceanographic cruise (ARO2000) to the NW Mediterranean Sea along the coast of Catalonia (Spain). Experiments were performed with samples from several stations and from 2 different layers: the surface and the deep chlorophyll maximum (DCM). Our experiments permitted us to gain new insights into the growth dynamics of bacterial populations and how they are affected by grazing in distinct habitats of the water column.

## MATERIALS AND METHODS

**The study area.** Experiments were carried out during the first of 2 ARO2000 cruises. This cruise lasted from 30 May to 9 June 2000 and covered the narrow shelf area off the Catalan coast (Spain). Our dilution experiments were carried out at stations following the frontal slope–edge current flowing from the Gulf of Lyon southward, from 42°29.48′N, 4°03.07′E to 41°17.08′N, 2°43.00′E (Fig. 1). At the end of spring, waters from the Rhone River slightly freshen the surface layers of this current (Fig. 2). In this area, nutrient concentrations (nitrate, phosphate, silicate) are generally around or below the detection limit in the surface layer and start to increase at a depth coinciding with the DCM (Estrada 1985). Chlorophyll *a* fluorescence profiles were used to localize the DCM at about 40 to 65 m depth (Fig. 2).

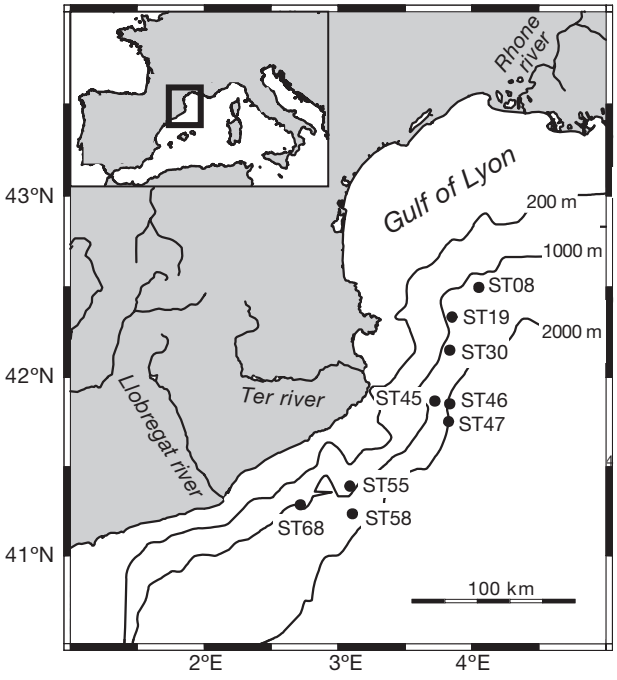


Fig. 1. Positions of stations with experiment numbers and depth contour lines

**Experimental setup.** Growth and grazing were estimated in 11 dilution experiments at 8 stations located in the slope–edge current, as described by Latasa et al. (2005). Seven experiments were performed with water from the surface (4 to 8 m), and 4 experiments were performed with water from the DCM (Fig. 1, Table 1), following the method described by Latasa et al. (2005). The initial seawater was retrieved by means of a modified 30 l Niskin bottle. The spring inside the bottle was Teflon-coated, the spigot had an inner diameter of 10 mm, which allowed a gentler exit of water than the standard 4 mm spigot, and the air vent had been moved to the opposite side of the bottle to avoid bubbling. All these modifications minimized the effects of experimental manipulation on the delicate flagellates and ciliates. The Niskin bottle was mounted on a wire and closed by a messenger. A CTD equipped with a fluorometer and mounted on a rosette sampler served for characterizing the water column and for locating the depth of the DCM.

Whole seawater was diluted to 75, 50 and 25% in 2 l polycarbonate bottles with gravity-filtered seawater (Pall-Gellman Suporcap with serial filters of 0.8 and 0.2 µm pore size and 1000 $cm^2$ effective area). This

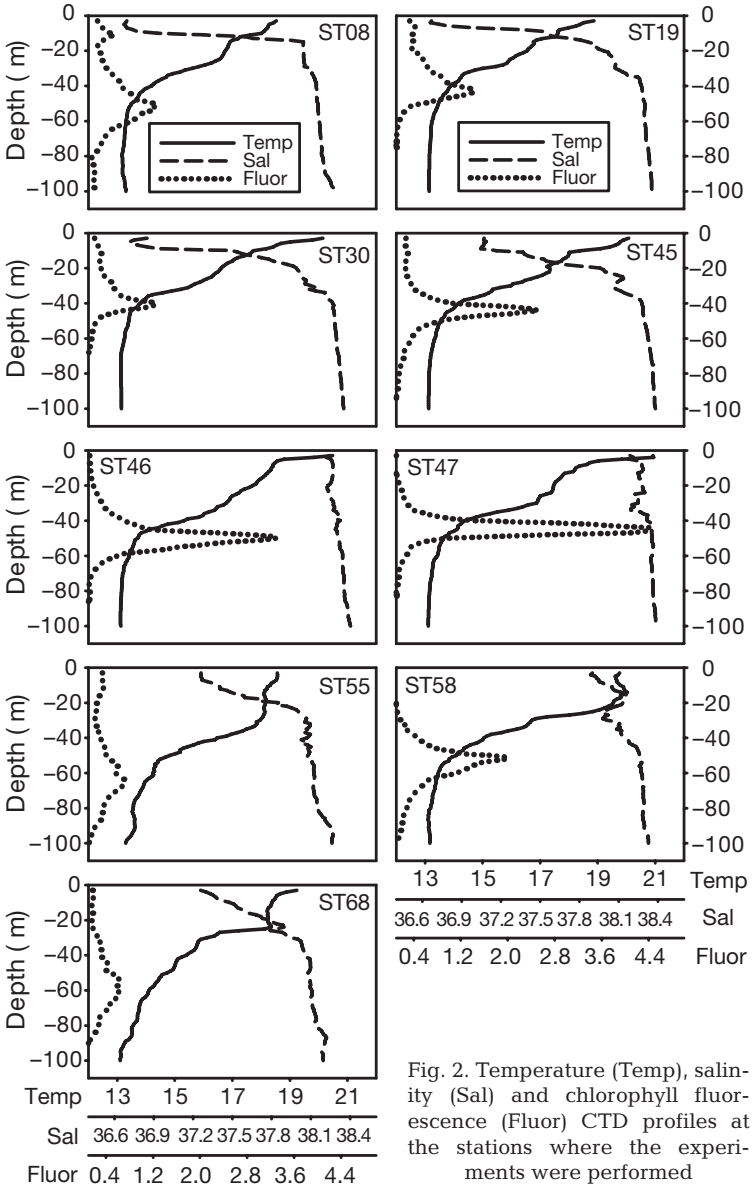


Fig. 2. Temperature (Temp), salinity (Sal) and chlorophyll fluorescence (Fluor) CTD profiles at the stations where the experiments were performed

filtration procedure did not increase the amount of DOC in a previous test conducted during a decaying diatom bloom. The dilution series plus 2 undiluted bottles were spiked with *f*/2 nutrient medium (Guillard 1975) to a final added concentration of 3 µM $NO_3$, 0.12 µM $PO_4$ and 0.36 µM $SiO_4$. $NH_4$ was also added to a final concentration of 3 µM. An extra bottle with undiluted water without amended nutrients was included in each experiment. Bacterial demand for DOC was assumed to be met by background concentrations and DOC released from actively growing phytoplankton and feeding grazers. Samples were incubated on-deck in a methacrylate incubator protected with blue screens to mimic the intensity and quality of either sub-surface or DCM irradiance. The incubator was temperature controlled with on-line surface seawater and a recirculation cooling system. All materials, including measurement cylinders, funnels, silicone tubing and incubation bottles, were thoroughly rinsed with 5% HCl and Milli-Q water. After 24 h, samples were taken for analysis by flow cytometry. Initial samples were taken in triplicate.

**Flow cytometry.** For flow cytometry, 2 ml samples were immediately fixed with paraformaldehyde plus glutaraldehyde (1 and 0.05% final concentrations), incubated for 10 min at room temperature and then stored frozen in liquid nitrogen until analysis. For the measurements, the samples were thawed, stained with a dilution of the nucleic acid fluorochrome Syto 13 (dimethyl sulfoxide, 2.5 µmol $l^{-1}$, Ref. S-7575, Molecular Probes), left for 10 min in

Table 1. Station, position, depth (m), chlorophyll *a* concentration (chl *a*, µg $l^{-1}$), bacterial concentration (×$10^5$ cells $ml^{-1}$) and growth and grazing rates ($d^{-1}$) for high nucleic acid (HNA), low nucleic acid (LNA) and total bacteria corresponding to the experiments

| Stn | Position | Depth | Chl *a* | Bacteria | Growth | | | Grazing | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | HNA | LNA | Total | HNA | LNA | Total |
| ST08 | 42°29′N, 4°03′E | Surface | 0.174 | 4.54 | 1.470 | 0.862 | 1.231 | 0.729 | 0.350 | 0.596 |
| ST19 | 42°20′N, 3°51′E | Surface | 0.165 | 6.10 | 2.066 | 0.878 | 1.453 | 1.358 | 0.615 | 1.017 |
| ST45 | 41°52′N, 3°43′E | Surface | 0.152 | 8.56 | 0.819 | 0.320 | 0.639 | 0.946 | 0.489 | 0.785 |
| ST47 | 41°45′N, 3°49′E | Surface | 0.045 | 6.08 | 0.673 | 0.470 | 0.599 | 0.816 | 0.484 | 0.675 |
| ST55 | 41°23′N, 3°05′E | Surface | 0.123 | 6.44 | 0.408 | 0.020 | 0.233 | 0.827 | 0.177 | 0.524 |
| ST58 | 41°14′N, 3°06′E | Surface | 0.085 | 5.65 | 1.767 | 0.509 | 1.265 | 1.547 | 0.402 | 1.097 |
| ST68 | 41°17′N, 2°43′E | Surface | 0.114 | 13.30 | 1.132 | 0.363 | 0.800 | 0.929 | 0.084 | 0.556 |
| ST30 | 42°09′N, 3°50′E | 40 m | 0.509 | 6.75 | 0.285 | 0.973 | 0.699 | 0.424 | 1.106 | 0.834 |
| ST46 | 41°51′N, 3°50′E | 40 m | 0.970 | 8.96 | 0.082 | 1.495 | 0.993 | 0.065 | 1.295 | 0.816 |
| ST55 | 41°23′N, 3°05′E | 60 m | 0.440 | 7.36 | 0.610 | 0.741 | 0.702 | 0.361 | 0.391 | 0.393 |
| ST58 | 41°14′N, 3°06′E | 50 m | 1.024 | 6.42 | 0.453 | 0.405 | 0.433 | 0.174 | 0.306 | 0.260 |

the dark and run through a Becton Dickinson FACScalibur bench cytometer with a laser emitting at 488 nm. Samples were run at low speed (ca. 18 µl $min^{-1}$), and data were acquired in a log mode for 10000 events. A latex-bead solution (10 µl) was added ($10^6$ beads $ml^{-1}$, yellow-green 1 µm Polysciences latex beads) to every sample as an internal standard. The bead solution was sonicated before use to destroy any aggregates, and its concentration was calibrated with a TrueCount bead solution (TrueCount, Ref. 340335, BD Biosciences). Both internal-standard-bead and TrueCount-bead solutions were counted by microscopy to confirm their concentrations. Bacteria were detected by their signatures in a plot of right-angle light scatter (RALS) versus green fluorescence emitted by Syto-13-stained nucleic acid. In this plot we could separate HNA bacteria from LNA bacteria (Gasol & del Giorgio 2000).

**Data analyses.** Net growth rates of bacteria were estimated in each bottle as $k_D = 1/t \times (dC/dt)$, where $C$ is the bacterial concentration. Net growth in the dilution treatments with added nutrients was used to estimate the intrinsic growth ($\mu_n$ = bacterial growth with added nutrients) and grazing ($g$) from the regression of $k_D$ against dilution ($D$), $k_D = \mu_n - g \times D$ (Fig. 3). The rate of bacterial intrinsic growth without added nutrients ($\mu_0$) was calculated as $\mu_0 = g + k_{D_0}$, where $k_{D_0}$ is the net growth rate in the undiluted seawater samples incubated without nutrient additions. Unless specifically identified as being with added nutrients, the term bacterial growth rate refers throughout the text to the intrinsic rate estimated without added nutrients ($\mu_0$).

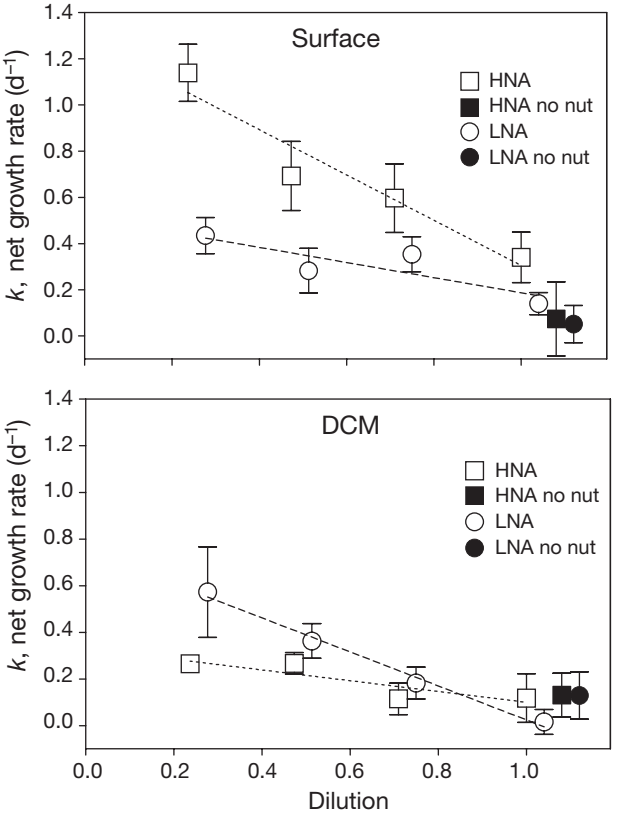


Fig. 3. Pooled results of the dilution experiments in surface and deep chlorophyll maximum (DCM) layers (error bars: ±SE). Different data series are slightly displaced along the *x*-axis to prevent overlap. HNA: high nucleic acid; LNA: low nucleic acid; nut: nutrients

Bacterial C flux was estimated based on Frost's (1972) equations. Bacterial C incorporation was calculated as $C_{incorporated} = \mu \times B_0(e^{(\mu - g)t} - 1) \times (\mu - g)^{-1}$, and bacterial C grazed was calculated as $C_{grazed} = g \times B_0(e^{(\mu - g)t} - 1) \times (\mu - g)^{-1}$. $\mu$ and $g$ were the intrinsic growth and grazing rates obtained from the dilution experiments. $B_0$ was the initial bacterial C concentration derived from bacterial concentration. $B_0$, $\mu$ and $g$ were employed for total bacteria and also separately for HNA and LNA bacteria. We chose a cellular carbon content of 15 fg C $cell^{-1}$ as an appropriate value for bacterial cells in marine oligotrophic environments (Ducklow 2000). These calculations should be considered with some caution because the RALS we measured indicate dissimilar bacterial sizes and, probably, carbon content.

Chlorophyll *a* concentration was measured by HPLC in each experiment, as described by Latasa et al. (2005).

## RESULTS

### The water column

The water column presented clear thermal stratification (Fig. 2). There was a temperature decrease of 4 to 6°C between surface and DCM depths. At the stations above the slope, surface layers showed weak influence of the Rhone River plume in the upper 10 to 30 m (0.2 to 1.5 lower salinity). This influence was clearer in the north, decreasing towards the southern stations (Figs. 1 & 2). A general surface temperature increase from 18.5 to 19.8°C was measured from north to south. The DCM was located between 40 and 65 m and coincided with the lowest part of the thermocline (Fig. 2). Average chlorophyll *a* concentrations (mean ± SD) in the water used for the serial-dilution experiments were 0.12 ± 0.05 µg $l^{-1}$ at the surface and 0.74 ± 0.30 µg $l^{-1}$ at the DCM (Table 1). Growth responses of phytoplankton were 70% higher in the nutrient-enriched bottles than in the non-enriched bottles, indicating that nutrient concentrations were limiting for phytoplankton (Latasa et al. 2005).

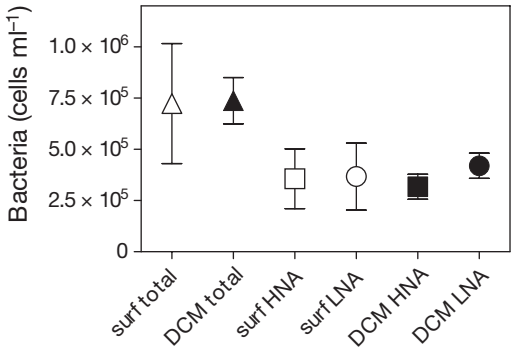


Fig. 4. Concentrations of total bacteria and high nucleic acid (HNA) and low nucleic acid (LNA) bacteria at the beginning of the serial-dilution experiments (error bars: ±SD). DCM: deep chlorophyll maximum; surf: surface

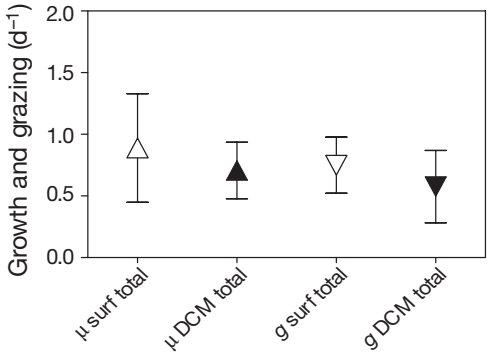


Fig. 5. Growth (μ) and grazing (g) rates of total bacteria (error bars: ±SD). DCM: deep chlorophyll maximum; surf: surface

### Bacterial concentration

Concentrations of total bacteria (mean ± SD) at the beginning of the experiments ranged between 4.5 × $10^5$ and 13.3 × $10^5$ cells ml$^{-1}$ (7.2 ± 2.9 × $10^5$) at the surface and between 6.4 × $10^5$ and 9.0 × $10^5$ ml$^{-1}$ (7.4 ± 1.1 × $10^5$) at the DCM (Fig. 4). These values match the concentrations measured by Pedrós-Alió et al. (1999) and Vaqué et al. (2001) in the same area. Proportions of HNA bacteria (mean ± SD) varied between 34 and 55% (49.4 ± 5.3%) of total bacterial concentrations in the surface layer and from 39 to 47% at the DCM (43.0 ± 2.9%; Fig. 4). At the end of the experiments, the proportion of HNA bacterial cells in the undiluted non-enriched incubations were on average 51.1 ± 6.0% in the surface layer and 43.2 ± 2.2% at the DCM.

### Bacterial size

RALS can be used as a proxy of cell size (Lebaron et al. 1999, Jaquét et al. 2001, Servais et al. 2003). According to this parameter (data not shown), HNA bacteria were significantly larger than LNA bacteria only in the surface layer (*t*-test, $p < 0.01$). RALS of both HNA and LNA bacteria in the surface were higher than at the DCM (*t*-test, $p < 0.001$), which suggests that bacteria of both groups were somewhat smaller at the DCM.

### Growth and grazing

All experiments presented an increase of net growth rate with dilution, indicating that grazing was reduced with dilution. Moreover, net growth was proportional to dilution without a clear departure from linearity, thus permitting the use of a linear regression approach (Fig. 3). We found a close coupling between growth and grazing ($r^2 = 0.74$). Growth and grazing rates on the overall bacterial population were quite similar in surface and DCM layers (Fig. 5, Table 1). Average intrinsic growth rates (mean ± SD) were 0.88 ± 0.43 d$^{-1}$ in the surface layer and 0.71 ± 0.23 d$^{-1}$ at the DCM. Average grazing rates on total bacteria were 0.75 ± 0.23 d$^{-1}$ in the surface layer and 0.58 ± 0.29 d$^{-1}$ at the DCM.

The populations of HNA and LNA bacteria presented different growth rates between them and between surface and DCM layers (Fig. 6A, Table 2). At the surface, the average growth rate of HNA bacteria was 1.18 ± 0.60 d$^{-1}$, significantly higher than the average 0.47 ± 0.28 d$^{-1}$ for LNA bacteria. In contrast, at the DCM, the average growth rate of HNA bacteria was only 0.36 ± 0.23 d$^{-1}$, lower, although not statistically significant, than the 0.90 ± 0.46 d$^{-1}$ estimated for LNA bacteria at this layer.

Grazing rates on HNA and LNA populations were also different between populations and in both layers (Fig. 6B, Table 2). At the surface, the average grazing rate on HNA bacteria was 1.02 ± 0.31 d$^{-1}$, significantly higher than at the DCM (0.26 ± 0.17 d$^{-1}$). The average grazing rate on LNA bacteria was 0.37 ± 0.19 d$^{-1}$ at the surface and 0.77 ± 0.50 d$^{-1}$ at the DCM.

In summary, we measured larger differences between HNA and LNA bacteria at the surface than at the DCM. Comparing between both layers, we observed larger differences between HNA at the surface and HNA at the DCM than between LNA at the surface and LNA at the DCM. Thus, surface HNA bacteria showed the highest turnover, followed by the DCM LNA bacteria. LNA at the surface and HNA at the DCM, although active, appeared to be less active than HNA at the surface and LNA at the DCM.

### Bacterial C flux

C flux through bacteria can be estimated if values for growth and grazing rates and bacterial biomass are known. The average bacterial biomass (mean ± SD)

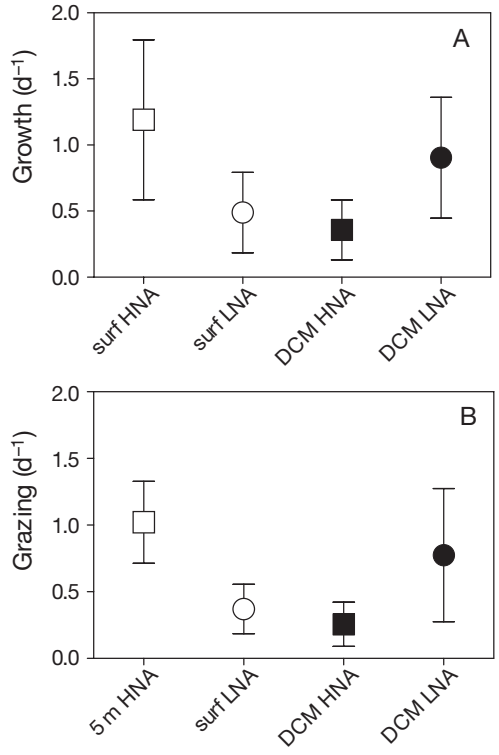


Fig. 6. (A) Growth and (B) grazing rates of high nucleic acid (HNA) and low nucleic acid (LNA) bacteria at the surface (surf) and deep chlorophyll maximum (DCM) (error bars: ± SD)

was 10.9 ± 4.40 and 11.1 ± 1.69 µg C l$^{-1}$ for surface and DCM populations, respectively. Using the equations of Frost (1972), surface bacteria synthesized on average 10.2 ± 6.4 µg C l$^{-1}$ d$^{-1}$. The bacterial C consumed by grazers was 9.0 ± 3.2 µg C l$^{-1}$ d$^{-1}$. At the surface, about 70% of this C was funneled through HNA bacteria. At the DCM, 8.7 ± 4.3 µg bacterial C l$^{-1}$ d$^{-1}$ were synthesized and 6.9 ± 4.0 µg C l$^{-1}$ d$^{-1}$ consumed. Here the situation was the opposite, 80% of the C was funneled through LNA bacteria.

Table 2. Probability values of for differences in growth and grazing rates between low (LNA) and high (HNA) nucleic acid bacteria at the surface and deep chlorophyll maximum (DCM; paired Student's *t*-test), and of LNA and HNA between the surface and DCM (Student's *t*-test)

| | Growth | Grazing |
|---|---|---|
| **Between LNA and HNA** | | |
| Surface | 0.003 | 0.001 |
| DCM | 0.195 | 0.158 |
| **Between surface and DCM** | | |
| HNA | 0.029 | 0.001 |
| LNA | 0.102 | 0.080 |

## DISCUSSION

### Active LNA bacteria

We conclude from our experiments that LNA bacteria were actively growing (Table 1, Fig 6A). This appears to be contrary to several observations in the literature where LNA have been described as bacterial resting stages or as dead, dying, or barely active bacteria (Gasol et al. 1995, Zweifel & Hagström 1995, Lebaron et al. 2001, 2002). Nevertheless, there have also been reports of active LNA bacteria (Zubkov et al. 2001, Jochem et al. 2004, Wetz & Wheeler 2004, Longnecker et al. 2005).

In the area of our study (the NW Mediterranean Sea) only reports of inactive or less active LNA bacteria have been produced until now (Gasol et al. 1995, Lebaron et al. 2001, 2002, Vaqué et al. 2001). All these studies, however, were carried out in coastal surface layers. We found similar results in our surface samples with river influence (see also below). It seems that in these environments HNA cells are recurrently the most active bacterial component. A strong difference arose with our DCM samples in which LNA bacteria showed very active growth. Vaqué et al. (2001), in the only study in our area distinguishing HNA and LNA bacteria in which subsurface water samples were collected, encountered a well-mixed layer with almost homogeneous chlorophyll *a* concentrations from the surface to deeper samples in 5 of the 6 stations studied (Table 1 in Vaqué et al. 2001). Thus, our data from the DCM cannot be directly compared with the data reported by Vaqué et al. (2001). We conclude that our findings do not contradict previous results in our area, and LNA could be an active component in specific areas or habitats.

The most likely explanation for the different roles assigned to 'LNA bacteria' is that this group includes a wide variety of bacterial forms. Dead and less active bacteria contain low amounts of nucleic acid, but small bacteria (e.g. SAR 11) also might be characterized as LNA in the flow cytometer. The nucleic acid content of a bacterial cell as differentiated in the flow cytometer appears to reflect very different causes, which can be phylogenetic affiliation or physiological condition.

### Contrast between the surface and DCM layers

The DCM is a typical feature of this oligotrophic Mediterranean area and is situated just above the nutricline. It is a result of increased cellular pigment content due to photoadaptation as well as increased cell numbers (Estrada 1985, Latasa et al. 1992). Therefore, it would be a Type 1 DCM, as defined by Cullen (1982).

It was clear that there was strong interaction between depth and growth and grazing rates of HNA and LNA bacteria (interaction $p < 0.005$, 2-way ANOVA). There are clear indications that our dilution experiments from both layers were performed with different HNA and LNA populations. Most of our surface samples were sampled in an area with weak influence of the Rhone River plume. One could infer a more coastal character of the surface samples due to the admixture of fluvial water. The larger size of surface bacteria might indicate a more coastal origin of this population (Fukuda et al. 1998, Morán et al. 2007, this issue). RALS at Time 0 indicated that bacteria were significantly larger at the surface than at the DCM, and V. Balagué et al. (unpubl.), using molecular techniques, found different bacterial assemblages at the surface and DCM during our cruise. The difference in bacterial populations between both layers has previously been reported (Acinas et al. 1997, 1999, Zubkov et al. 2001, Van Mooy et al. 2004), and it is also reflected in other plankton components without vertical migration, such as phototrophic bacterioplankton (Partensky et al. 1999), phytoplankton (Venrick 1988, Scharek et al. 1999), or microzooplankton (Dolan & Marrasé 1995).

The distinct bacterial growth and grazing rates imply differences between both habitats, not only in composition, but also in structure and functioning of the microbial communities. Because of the marked change in irradiance regime, phytoplankton physiology is logically very different in both layers. However, reports on distinct activity of heterotrophs are less frequent. Vázquez-Domínguez et al. (2005) observed similar bacterial production in surface and DCM layers during a transect in the Atlantic Ocean, and Pedrós-Alió et al. (1999) estimated very similar growth rates at the surface and DCM in our study area in June 1993, 1995 and 1996. Taking all bacteria into account, growth and grazing rates were basically the same at the surface and DCM in our case. However, the differentiation of HNA and LNA revealed a distinction in the composition and behavior of the 2 types of bacterioplankton in both layers. The reasons for the distinct ecological role of both kinds of bacteria at the surface and DCM can be multiple. Our results could fit into the hypothesis formulated by Zubkov et al. (2004), by which LNA cells consume only the labile fraction of organic nutrients, while HNA also feed on more refractory sources of nutrients, which could be more abundant in coastal waters. An alternative hypothesis could be that HNA production is more associated to phytoplankton production (DOC), while LNA bacteria have higher needs of the inorganic nutrients present at the DCM.

### Bacterial C flux

Bacterial fluxes (growth and grazing) were on the order of 10 µg C $l^{-1}$ $d^{-1}$ in surface waters. Comparing this number with phytoplankton fluxes of ca. 4.6 µg C $l^{-1}$ $d^{-1}$ reported in Latasa et al. (2005) for phytoplankton, bacterial production would represent >200% of primary production, a much higher value than the often cited 20% (Ducklow 1999). The value of primary production was based on photosynthesis versus irradiance (*P-E*) experiments that rendered an average (±SD) of 3.48 ± 2.02 mg of C $mg^{-1}$ chlorophyll *a* $h^{-1}$, well within the range of the values obtained in the NW Mediterranean for 6 yr in the spring–summer period (3.37 ± 1.25 mg of C $mg^{-1}$ chlorophyll *a* $h^{-1}$; Estrada et al. 1993). To examine this discrepancy we investigated the 3 parameters involved in the estimation of C fluxes: bacterial C biomass and growth and grazing rates. Average bacterial biomass was around 11 µg C $l^{-1}$. Our average bacterial concentrations of $7.3 \times 10^5$ bacteria $ml^{-1}$ are at the higher end of the $2.0 \times 10^5$ to $7.1 \times 10^5$ bacteria $ml^{-1}$ range reported for the same area by Pedrós-Alió et al. (1999), Vaqué et al. (2001) and Sala et al. (2002). Therefore, although high, bacterial abundances should not be considered an artifact in our estimations of bacterial C flux.

Growth rates, another parameter in the equation of C flux, were around 0.88 $d^{-1}$, higher than the values around 0.25 $d^{-1}$ reported by Pedrós-Alió et al. (1999). We used the serial-dilution method, which appears to render higher growth and grazing rates than estimated from the more widespread isotope (Gasol et al. 2002) and trace particle uptake techniques (Vaqué et al. 1994). However, growth rates of around 1.0 $d^{-1}$ and higher can be estimated from the data presented by Vaqué et al. (2001) and Sala et al. (2002). Specifically, Fig. 5 in Vaqué et al. (2001) shows that the bacterial population basically tripled (equivalent to $\mu = 1.1$ $d^{-1}$) during the days of low grazing. In their Fig. 4, Sala et al. (2002) reported specific growth rates of 0.7 to 1.0 $d^{-1}$ in control incubations where predators had been excluded by size. Pedrós-Alió et al. (2000) empirically adjusted a multiple linear regression to estimate bacterial production (BP) from temperature and chlorophyll *a* concentration in marine environments. Applying a $T = 19.6$°C (mean of our stations) and a mean chlorophyll *a* of 0.12 µg $l^{-1}$ from our study site to $\log_{10}$ BP $= 0.07 \times T + 0.40 \log_{10}$ chlorophyll *a*, the resulting bacterial production is 10.1 µg C $l^{-1}$ $d^{-1}$, a value very similar to our 10.2 µg C $l^{-1}$ $d^{-1}$ for surface waters. We do not think the same regression should be applied to high chlorophyll *a* cells of the DCM, because the specific pigment content of phytoplankton cells is higher than at the surface due to light adaptation. Finally, Gasol et al. (2002) showed growth rates well above 1.0 $d^{-1}$ in 24 h incubations of oligotrophic Atlantic samples where predators had been size excluded. Interestingly, this last

study finds high intrinsic growth rates when bacterial abundances are limited by predation and low growth rates when limited by resources. We did not see higher net growth in bottles with or without nutrients added ($p = 0.41$ and 0.79 for HNA and LNA, paired *t*-test), concluding that bacteria in our study area and season were limited by predation and not by resources. Therefore, there is compelling evidence that our intrinsic growth rates of 0.88 $d^{-1}$ are very reasonable in bacteria with no nutrient limitation in our study area during the spring–summer period.

The last parameter involved in our C estimations is grazing. Our estimates of grazing are high compared with the data reported by Vaqué et al. (2001) for the area, but are consistent with or even lower than rates reported from elsewhere (Vaqué et al. 1994, Strom 2000). An estimate of bacteria grazed per hour renders values of $2.4 \times 10^4$ and $1.9 \times 10^4$ bacteria $ml^{-1}$ $h^{-1}$ at the surface and DCM, which are in the low range of bacterivory measured with trace-particle uptake methods (Vaqué et al. 1994). In that review, the average grazing rates for open seawater were $2.9 \times 10^4$ and $4.7 \times 10^4$ bacteria $ml^{-1}$ $h^{-1}$, depending on whether trace ingestion or whole seawater methods were applied. These averages by Vaqué et al. (1994) translate into bacterial C grazed $d^{-1}$ of 10.4 and 16.9 µg C $l^{-1}$ $d^{-1}$ using a factor of 15 fg C $cell^{-1}$. Our values of 9.0 and 6.9 µg C $l^{-1}$ $d^{-1}$ fit into the lower end of this range.

Another source of variation in C estimates is the assigned C per bacteria. We have used a value of 15 fg $cell^{-1}$ (for both HNA and LNA bacteria and both layers, see also above), which could be considered conservative. Using the volumetric formula and the volume values by Pedrós-Alió et al. (1999), bacteria in our area might contain around 20 fg C $cell^{-1}$. In any case, it can be safely concluded that bacteria indeed channeled a large proportion of C in our system, doubling that funneled by surface phytoplankton during our study period. Interestingly, our estimated bacterial C biomass in the surface layer was also around twice that of phytoplankton (Latasa et al. 2005).

## CONCLUSIONS

Flow cytometry measurements of serial-dilution experiments allowed a direct comparison of growth and grazing rates of HNA and LNA bacterial populations in NW Mediterranean waters. Bacteria were responsible for a large portion of the C flux through the system, with a close coupling between growth and grazing. We found a strong dependency between activity of LNA and HNA bacteria and their location in surface or DCM layers. Our observations provide additional evidence that HNA are the most active component of the bacterial community, at least in the surface layer. Contrary to some previously published results, LNA were an active component of the bacterial plankton in both layers, particularly at the DCM. The nucleic acid content of a bacterial cell as differentiated in the flow cytometer appears to reflect different causes, which could include phylogenetic affiliation or physiological condition. Reported differences in phylogenetic composition of bacterial assemblages, as also occurs for microplankton assemblages, could be the reason for the observed ecological differences of HNA and LNA bacteria between the surface and DCM. Therefore, further characterization of HNA and LNA bacteria in different surface and DCM habitats is warranted.

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