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Summer relationships between bacterioplankton abundance and chlorophyll-*a* concentration in a eutrophic coastal system of Alboran Sea (SE of Spain)

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Abstract : The relationship between chlorophyll-*a* concentration and bacterial abundance was analysed in a permanent station in coastal waters of Alboran Sea (SE of Spain), during a summer period. Bacterial abundance and chlorophyll-*a* concentration dynamics showed a positive empirical relationship in sub-surface waters, but no relationships were found in deep waters. The estimation of bacterial size determined as biovolume, showed bigger cells in deep samples as a feature independent of phytoplankton production. This suggested that bacterial production was mainly linked to the growth of phytoplankton healthy cells in surface layers and with other processes as physical dynamics, nutrient supply from sediments and other allochtonous carbon sources in bottom samples.

Résumé : *Relations entre l'abondance du bactérioplancton et la concentration en chlorophylle a, pendant la période estivale, dans un système côtier eutrophique de la mer d'Alboran (SE de l'Espagne).*
La relation entre la concentration en chlorophylle-*a* et l'abondance bactérienne a été analysée en un point permanent dans les eaux côtières de la Mer d'Alboran (SE de l'Espagne) pendant une période estivale. La dynamique de l'abondance bactérienne et des concentrations en chlorophylle-*a* a montré une relation positive dans les eaux superficielles, mais aucune relation dans les eaux profondes. Les plus grandes cellules bactériennes se trouvent en profondeur, ce fait démontre l'indépendance de la production phytoplanctonique et du volume bactérien. Ceci suggère que la production bactérienne serait principalement liée à la croissance du phytoplancton dans les eaux de surface, tandis que la production bactérienne dans les eaux profondes serait liée à d'autres variables, parmi lesquelles la dynamique physique, l'arrivée d'éléments nutritifs du sédiment et d'autres sources de carbone benthique sont considérées comme importantes.

Keywords : Bacterioplankton, Chlorophyll, Bacterial size, Mediterranean.

Introduction

Bacterioplankton are recognized as a large and active component of marine ecosystems (Pomeroy, 1974). However, the study of heterotrophic pelagic bacteria is relatively recent and it is unclear which factors control the dynamics of the bacterioplankton community (Wikner & Hagström, 1991).

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An important tool in the understanding of pelagic ecosystem dynamics is to examine the relationship between physico-chemical parameters and plankton communities. Many authors related bacterial abundance with some environmental variables in freshwater and marine systems, such as temperature (Shiah & Ducklow, 1994), total nitrogen and total phosphorus (Spencer, 1978; Currie, 1990), degree of eutrophication and organic matter pollution or trophic state estimated as chlorophyll *"a"* concentration (Rao *et al.*, 1979; Aizaki *et al.*, 1981; Bird & Kalff, 1984;

Cole *et al.*, 1988). A general dependence of bacteria on phytoplankton production (Bird & Kalff, 1984; Sell & Overbeck, 1992) is now recognized by many bacteriologists and oceanographers, because phytoplankton production provides substrates and nutrients to support bacterial growth. A large percentage of phytoplankton production flows to bacterioplankton, that consume up to half of this production (Cole *et al.*, 1988). However recently, Pace & Cole (1994) found, during a summer stratification period in three oligo-mesotrophic lakes, poor relationships between vertical pattern of bacterial productivity and primary production, indicating that other factors regulating carbon sources (as sediment influences, nutrient recycling or allochtonous material loading) may determinate the patterns of distribution of bacterial productivity. We were interested in determining if the pattern of heterotrophic bacterial abundance corresponds to the pattern of phytoplankton biomass (as chlorophyll-*a* concentration), at two distinct depths in a coastal eutrophic ecosystem of Alboran Sea (SE of Spain).

Material and Methods

Study site

Samples were obtained from a shallow (10 m depth) station in Málaga harbour (South Spain). The main characteristics are the restricted circulation and the slightly eutrophic environment, which cause strong fluctuations in plankton community (Ruiz *et al.*, 1992).

Field sampling

Water samples were collected from two depths (1 m and 7 m) with a submersible pump, weekly for four months during the summer period (June to September, 1988). One-liter of water was placed in an acid-washed bottle and kept at cold and dark conditions for quantitative analysis of total chlorophyll-*a* and 50 ml were preserved with formaldehyde (2%) for bacterial epifluorescence analysis.

Laboratory methods

Chlorophyll-*a* measurements were carried out spectrophotometrically on 100% acetone extracts. Chlorophyll-*a* concentrations were calculated using the equations of Talling & Driver (1963).

For epifluorescence analysis, 2 ml of the preserved sample were stained with Acridine Orange (0.01 % final concentration) and filtered through 0.2 µm Nuclepore filters (Hobbie *et al.*, 1977). Filters were examined with a Leitz Dialux microscope equipped with a 2∂-Ploemopack epifluorescence system (100w HBO lamp and a filter set type I2). The criteria for bacterioplankton enumeration was to count at least 300 cells per sample.

Occasionally we obtained measurements of bacterial size. Then several pictures (tri-X pan, 400 ASA) of random selected fields were taken. The slides were projected and the image outlined and processed with a video-interactive image analyser (VIDS IV-AMS). Biovolume for each cell was calculated using the formula of Krambeck *et al.* (1981).

Data treatment

Bacterial abundance data and chlorophyll-*a* concentrations were examined with a simple linear regression analysis. The data were transformed to logarithms to satisfy the assumptions of ordinary least squares (Draper & Smith, 1981; Bird & Kalff, 1984). Regression coefficients were tested for significance using a variance analysis. Previously, the requierement of normality were stated by a Kolmogorov-Smirnov test (KS-test).

Results and discussion

Figure 1 shows the temporal variation in chlorophyll-*a* ($\mu g\ l^{-1}$) and bacterial cell abundance (cell ml^{-1}) in the water column at surface (A) and bottom (B) depths, during the sampling period. The seasonal chlorophyll-*a* concentration and bacterial abundance data follow a multi-peak pattern. The results show that bacterial blooms, up to $1x10^6$ cells ml^{-1}, were usually associated with chlorophyll peaks. Bacterial populations decrease at the same time that phytoplankton blooms fall. A crossed correlogram analysis of all data (1 and 7 m) indicates a significative correlation with $\Delta t = 0$ and $p < 0.01$, suggesting that these variables are in phase.

In figure 2 we show the regression line between both variables using all data (A) and two sampling depths separately (B and C). The requierement of normality was checked using a Kolmogorov-Smirnov test and we could not reject the normality hypothesis. We found a significant positive relationship between heterotrophic bacterial abundance and chlorophyll-*a* concentrations at subsurface waters (F-test: $p < 0.001$). However we do not observe the same positive relationship in samples collected at 7 meters depth (F-test: $p > 0.1$).

Many authors found a strong empirical relationship between chlorophyll-*a* concentration and heterotrophic bacterial abundance in subsurface waters of marine and freshwater ecosystems (Aizaki *et al.*,1981; Linley *et al.*, 1983; Bird & Kalff, 1984 among others). These results support the concept of a tight coupling between bacterial and algal production. In our case, we observe the same strong relationship at 1 meter depth. The slope value obtained in the regression equation (0.746) is similar to that obtained by other authors i.e. in the range of 0.6 to 0.8 (Bird & Kalff, 1984; Cole *et al.*, 1988). From this close relation between bacterial abundance and chlorophyll-*a*, we suggest that bacterial productivity at 1 meter depth is supported by the release of organic carbon derived from the activity of phytoplankton community although some authors reveal that this source of carbon could be insufficient for bacterial requierements (Baines & Pace, 1991).

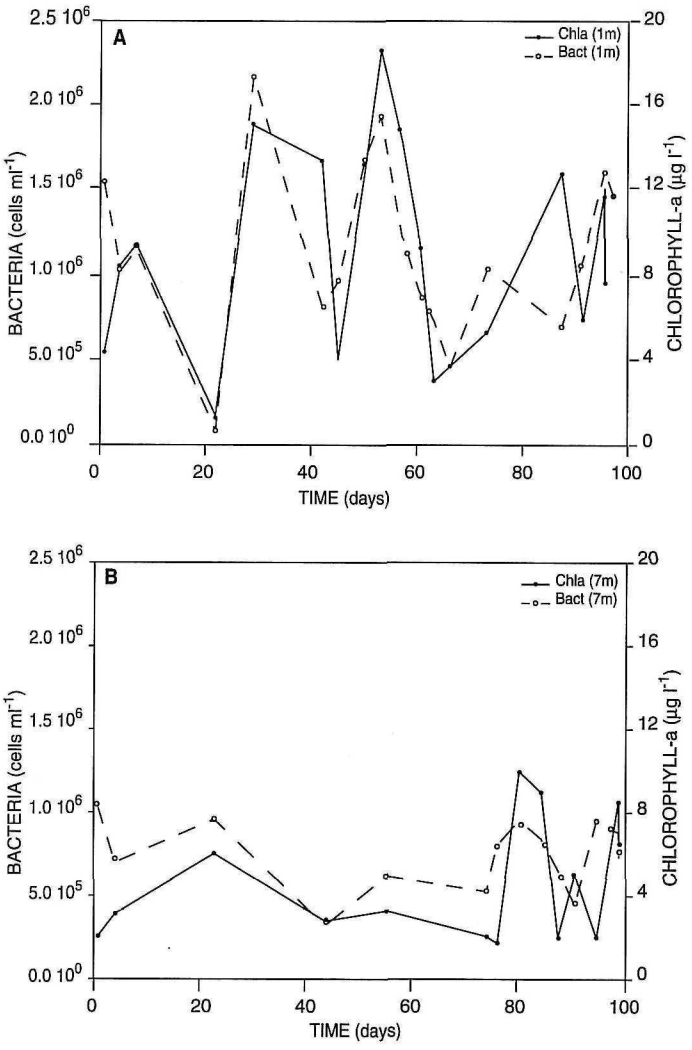

Figure 1. Time evolution of chlorophyll-*a* concentration (µg l^{-1}) and bacterial abundance (cell ml^{-1}) at one meter (A) and seven meter depth (B).

Figure 1. Evolution temporelle de la concentration en chlorophylle-*a* (µg l^{-1}) et de l'abondance bactérienne (cell ml^{-1}) à 1 mètre (A) et 7 mètres (B) de profondeur.

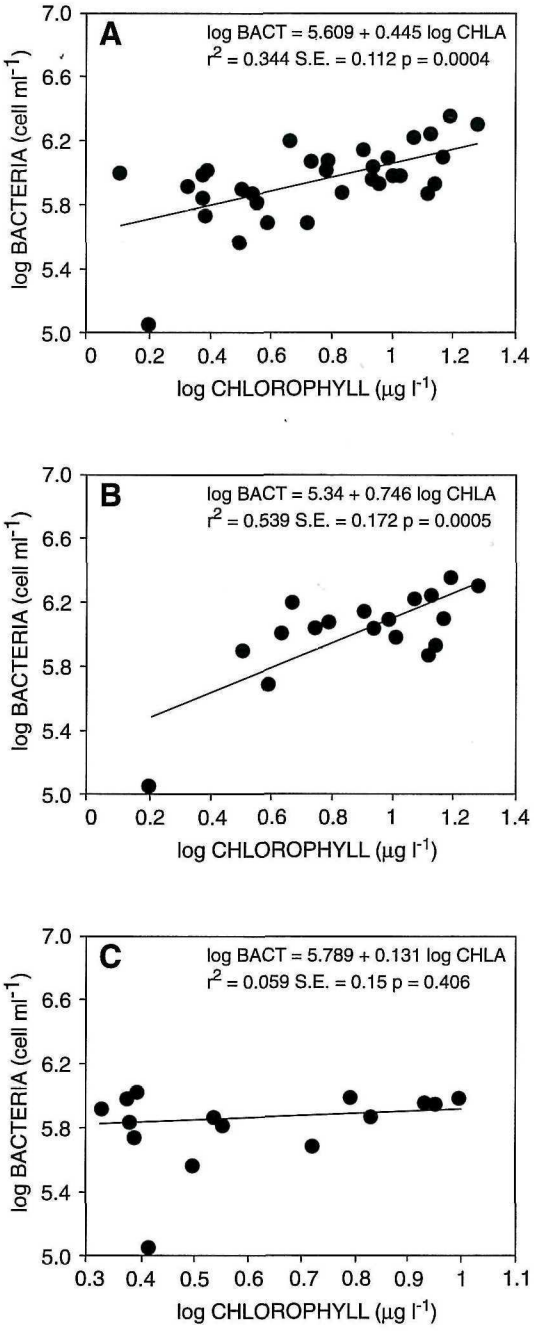

Figure 2. Relationship between chlorophyll-*a* concentration and bacterial abundance for all data points (A), one meter (B) and seven meter depth (C).

Figure 2. Relation entre la concentration en chlorophylle-*a* et l'abondance bactérienne pour tous les points (A) et aux profondeurs de 1 mètre (B) et 7 mètres (C).

On the other hand, we detected that in bottom samples (7 m) bacterial abundance was not correlated with phytoplankton biomass, implying that, in deep waters, factors other than primary productivity, such as sediment influence, were associated with bacterial abundance.

Some authors indicate that in nearshore regions, bacterial and primary production are often unrelated on vertical scales. Rao *et al.* (1979) explain a lack of relationship in a nearshore station in Lake Ontario, as an effect of diatom blooms that produce antibacterial substances. We must reject this hypothesis for our data because the main fraction of phytoplankton community in our ecosystem is non-diatoms nanophytoplankton, mainly phytoflagellates (Ruiz *et al.*, 1992). On the other hand, Furhmann *et al.* (1980)

indicate that bacterioplankton growth rate is higher when phaeopigments are high, connecting bacterial growth with dissolved organic matter from senescent phytoplankton cells. In our case, the vigorous phytoplankton blooms sink rapidly with a very short residence time for senescent cells. This is supported by the low quantity of phaeopigments found in the water column (Guerrero, 1996), so the link between bacterioplankton and degradation products of phytoplankton is not clear in this case.

The lack of relationship between bacterial abundance and chlorophyll-*a* concentration in deep waters could have several explanations. In eutrophic systems, predatory control of bacterial abundance is very important (Bird & Kalff, 1984; Cole *et al.*, 1988), and the changes in bacterial number occur at hour-scales and are determined by both nutrient supply and complex trophic interactions (Wright, 1988; Gasol & Vaqué, 1993). The existence of a gradient of heterotrophic nanoflagellates abundance from surface (average value of 5.5 x 10^3 cells ml^{-1}) to bottom waters (average value of 4.2 x 10^3 cells ml^{-1}) in this system (Hippolito, pers. com.) supports the predatory control hypothesis in near sediment waters. In the same system, Bautista *et al.* (1993) showed that nanoflagellates are responsible for most of the grazing on bacteria, suggesting the importance of the microbial heterotrophic pathway in the microplankton community of coastal waters.

Moreover, we have to bear in mind the role of physical variables. We observed a strong gradient of dissolved oxygen (maximum difference from 8.6 mg l^{-1} in the upper layer to 4.0 mg l^{-1} near the bottom) that could limitate the microbial production. On the other hand, several studies have shown that temperature is an important physical parameter, positively correlated with bacterial abundance (White *et al.*, 1991; Shiah & Ducklow, 1994). A test of comparison of means (t-Student) shows a variation in bacterial abundance ($p < 0.1$) at these two sampling depths. The temperature gradient (thermocline was of 0.5 °C m^{-1}) could be a possible explanation for this difference. Then, physical dynamics in summer and interaction with sediments might act to uncouple phytoplankton and bacterial processes (Joint & Pomeroy, 1987).

Our results of figure 3 show that bacteria in deep waters are bigger (0.225 μm^3 mean size) than cells in surface waters (0.175 μm^3 mean size). In this sense, Cole *et al.* (1993) suggests that bacterial abundance and size may be regulated by different processes. If cell size instead of number is positively correlated with system eutrophy (Pedrós-Alió & Brock, 1982) then it is possible to explain this distribution according to the idea that nutrient supply from sediments and other carbon sources could be more important than phytoplankton biomass in deeper waters.

The heterogeneity in vertical distribution at centimeter scale is another important factor. Mitchell and Fuhrman

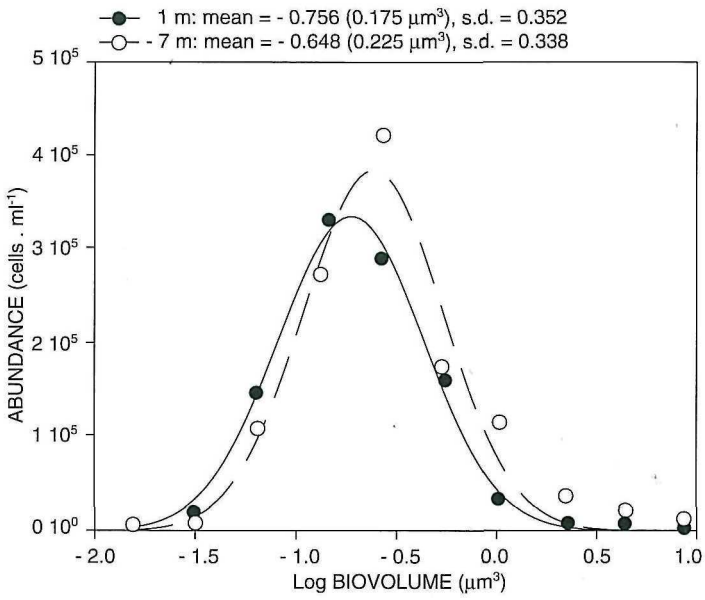

Figure 3. Bacterial size distribution at 1 m and 7 m depth. Data fit log-normal equations which parameters (mean and s.d.) are shown in each case.

Figure 3. Distribution des tailles bactériennes des eaux de surface (1 m) et profondes (7 m). Les valeurs d'abondance bactérienne dont les paramètres (moyenne et écart type) sont indiqués, sont ajustées selon une distribution normale.

(1989) found that chlorophyll-*a* changed up to 45 times m^{-1}, while bacterial abundance changed up to 35 times m^{-1}. If we consider also the encounter probability between bacterial cell and phytoplankton substrate (Vaqué *et al.*, 1989), the problem gets more complex.

In conclusion, bacteria and phytoplankton are apparently linked in sub-surface waters in our coastal zones, but not in deeper waters. However, this preliminary study requires more detailed observations over temporal and spatial scale of microbial growth, and the analysis of the fate of phytoplankton production at the microbial level is necessary to understand the ecological significance of energy flux within the food web in these eutrophic coastal ecosystems.

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