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BACTERIAL ABUNDANCE AND GROWTH IN OLIGOTROPHIC MEDITERRANEAN SEAWATER AND RHONE RIVER PLUME

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ABSTRACT: In order to compare microbial characteristics of the oligotrophic Mediterranean seawater and the eutrophic Rhône river freshwater, samples were taken at five depths of a marine station and of two stations in the plume area with a low salinity upper layer of 2-5 meters. No clear difference was seen either in bacterial densities (5-10 $\times$ 10^5 bacteria $\cdot$ ml^{-1}) nor in bacterial growth rates (0.05-0.08 h^{-1}) between oligotrophic and eutrophicated water. But, the bacterial production in the Rhône water was higher (5 - 8 times) than in the oligotrophic water. Counts of bacteria and heterotrophic micropredators showed oscillations between predator and prey populations along depth profiles.

RÉSUMÉ: Au niveau du débouché du fleuve Rhône (1000 à 2000 $m^3 \cdot S^{-1}$ de débit) se crée un panache d'eau dessalée et chargée en particules organiques et minérales, qui s'écoule sur l'eau Méditerranéenne réputée pour son oligotrophie. Afin de définir et de comparer les caractéristiques microbiologiques de ces 2 types d'eau, des échantillons ont été prélevés à 5 niveaux de 3 stations: une typiquement marine et deux autres situées dans le panache, montrant une couche de surface (2-3 mètres) dessalée. Les effectifs bactériens varient peu d'une station à l'autre (5 à 10 $\times$ 10^5 cellules $\cdot$ ml^{-1}). L'eau oligotrophe est caractérisée par une évolution parallèle des effectifs bactériens et de leurs prédateurs. La pauvreté nutritionnelle de l'eau ne permet pas une production bactérienne intense (0.11×10^9 bactéries $\cdot$ $l^{-1} \cdot h^{-1}$) et le taux de broutage presente de faibles valeurs (0.4 à 1.2×10^4 bactéries $\cdot$ $ml^{-1} \cdot h^{-1}$). Aux deux autres stations de prélèvement, dans la couche dessalée, les effectifs des bactéries et des prédateurs montrent des relations inverses, classiques des évolutions entre proie et prédateur. L'halocline constitue une interface en dessous de laquelle, en quelques mètres, se retrouvent les caractéristiques microbiennes relevées à la station oligotrophe. La production bactérienne varie enre 0.12 et 0.44 $\times$ 10^9 bactéries $\cdot$ $l^{-1} \cdot h^{-1}$ avec des taux de prédations plus élevés que dans l'eau dessalée ($5\text{-}8 \times 10^4$ bactéries $\cdot$ $ml^{-1} \cdot h^{-1}$). Par contre, quelle que soit la qualité de l'eau, le taux de croissance reste sensiblement le même (0.05-0.08 h^{-1}) et du même ordre de grandeur que ceux déjà relevés dans de semblables zones de front.

Introduction

Within oceanic waters, frontal regions demonstrate large variability in abundance and activity of bacterial populations (Ducklow and Hill, 1985; Egan and Floodgate, 1985), allowing studies through the "bacterial window"

(Ducklow, 1984). In such discontinuities there is a clear increase in bacterial production and the grazing of these bacteria by heterotrophic microprotozoa (Palumbo et *al*, 1984) initiates the microbial loop (Azam et *al*, 1983).

One of these frontal systems is the Rhône river plume, which constitutes an important freshwater input (1000-2000 $m^3 \cdot s^{-1}$), and is heavily loaded with organic and mineral particles (1-10 $mg \cdot l^{-1}$), (Aloïsi et *al*, 1979). As it enters the salty (38‰) oligotrophic Mediterranean seawater, the plume appears to be an ideal "model" for bacteriological studies and at the present time no bacterial study has been done in this particular ecological site. In order to approach microbial characteristics of the stratified water in the plume area versus these of the typical Mediterranean seawater, bacterial densities and production as well as heterotrophic micropredators abundance were measured along depth profiles at three sampling stations (Fig. 1).

Material and Methods

Sampling: One station was sampled per day on 21, 22 and 23 of May 1986 (n/o "Korotneff"). Sampling depths, based on salinity and turbidity values, were: 0, 5, 10, 20 and 50 meters for station 1; 0, 2, 5, 10 and 35 meters for station 2 and 0, 2, 5, 10 and 20 meters for station 3.

Bacterial densities: 0.5 to 2 ml of sample, preserved with formalin 2% (v/v), were stained with DAP1 (Porter et Faig, 1980) and filtered on 0.22 um black Nucleopore filter. Microscopical counts (using epifluorescence method) were done using an image analysis "Systeme III".

Grazers numbers: To estimate bacterial predators, counts were done by inverse microscopy after sedimentation, on aliquots of water samples, without any attempt of identification.

Bacterial growth rates and production: The increase in bacterial abundance were followed during 12 hours incubation of unfiltered and 3 um screened 100 ml subsamples from the surface level of each station and from 20 m and 5 m for station 1 and stations 2 and 3 respectively.

The counts were used to calculate bacterial growth rates and bacteria removed by predation using Gak et al (1972) method where:

$P = N_2 - N_1 + G$ (1)

P = bacterial production; N_1 = bacterial number in an unfiltered sample at TO; N_2 = bacterial number in the same sample at TX (X = hours of incubation); G = grazing. If

$u = (\ln n_2 - \ln n_1) / t$ (2)

Where, u = bacterial growth rate; n_1 = bacterial number in filtered sample at TO; n_2 = bacterial number

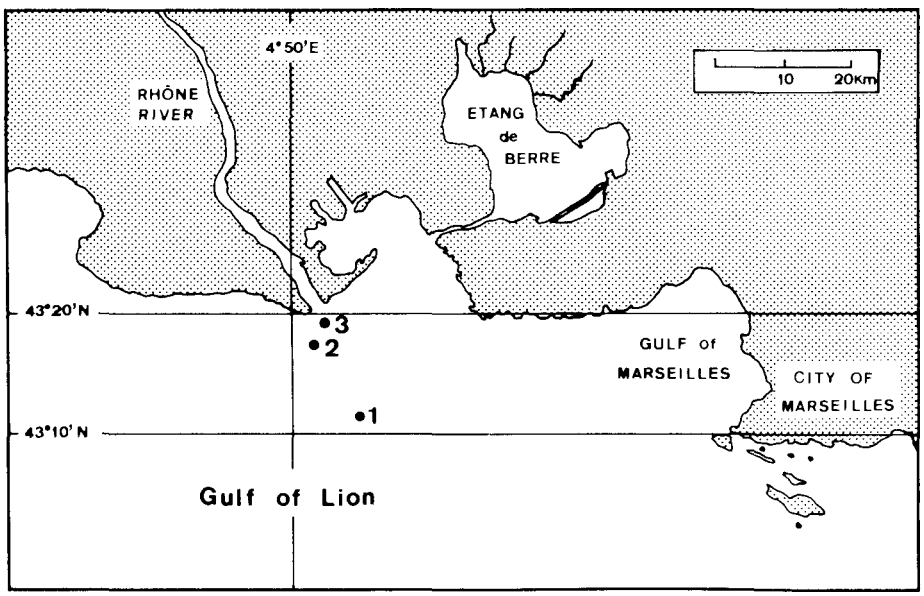

Figure 1. Map showing the mouth of Rhône river and the position of the three sampling stations.

in the same sample at TX, then $G = u\ (n_2\text{-}N_2/(n_2/n_1) - 1)$ (3) and $G = ((\ln n_2 - \ln n_1)/t)\ ((n_2\text{-}N_2)/(n_2/n_1)\text{-}1)$ (4)

Incorporation of ^{14}C-leucine into bacterial proteins : 100 ml duplicates were sampled as for increase in cell number measurements, and incubated 4 hours with ^{14}C leucine (3.3 nmol.ml^{-1}, specific activity : 300 mCi.mmol^{-1}). Abiotic adsorption was determined in a formalin – killed sample (2% final concentration) before adding the labelled compound. Amount of ^{14}C leucine incorporated into proteins was determine using the hot TCA extraction method of Kirchman et *al* (1985). Filters were counted in a liquid scintillator counter.

Results and Discussion

The salinity of the staion 1, outside the plume, was 37.2 ‰ at the surface level, with only a slight increase to 38‰ (50 m). While there was a strong halocline between two and five meters, drawing a freshwater layer of 23.2 and 19.6 ‰ respectively, at stations 2 and 3 (fig. 2 A, B, C).

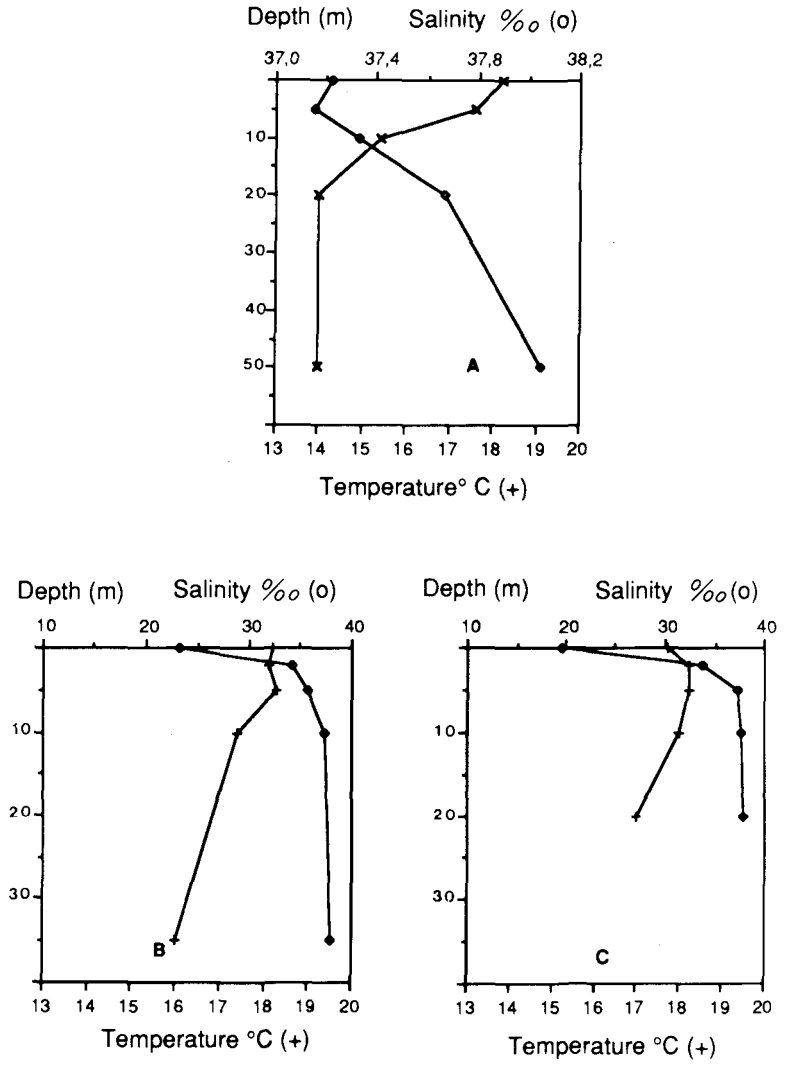

Fig. 2. Temperature and salinity values. A : station 1 ; B : station 2 ; C : station 3.

Bacterial densities were of the same order at the marine and plume station (5 to 8 × 10^6 bacteria·ml^{-1}) being similar values published in estuaries areas (Malone et *al*, 1986) (fig. 3 A, B, C). The bacterial abundance did not show any correlation with temperature and/or salinity. But, for the marine station (figure 3 A) bacterial and grazer numbers fluctuations are positively correlated (r = 0.96) demonstrating that, in case of oligotrophic situation the number of bacterial predators depend on their prey density (Sieburth and Davis, 1982). For the station 2 and station 3, the bacterial abundance was depending on many physical, chemical and biological parameters. The physical barrier constituted by the halocline, probably induced an area of passive accumulation of the particules and organisms (Egan and Floodgate, 1984 ; Ducklow and Hill, 1985), which are able to develop complex trophic relationships. So, on figure 3 B, at the interface layer between fresh and sea waters, bacterial and grazer populations showed the classical negative correlation of prey-predator relationships (Andersen and Fenchel, 1985; Sieracki and Sieburth, 1985). Beneath oligotrophic Mediterranean seawater (35 and 20 m for stations 2 and 3 respectively) both popula-

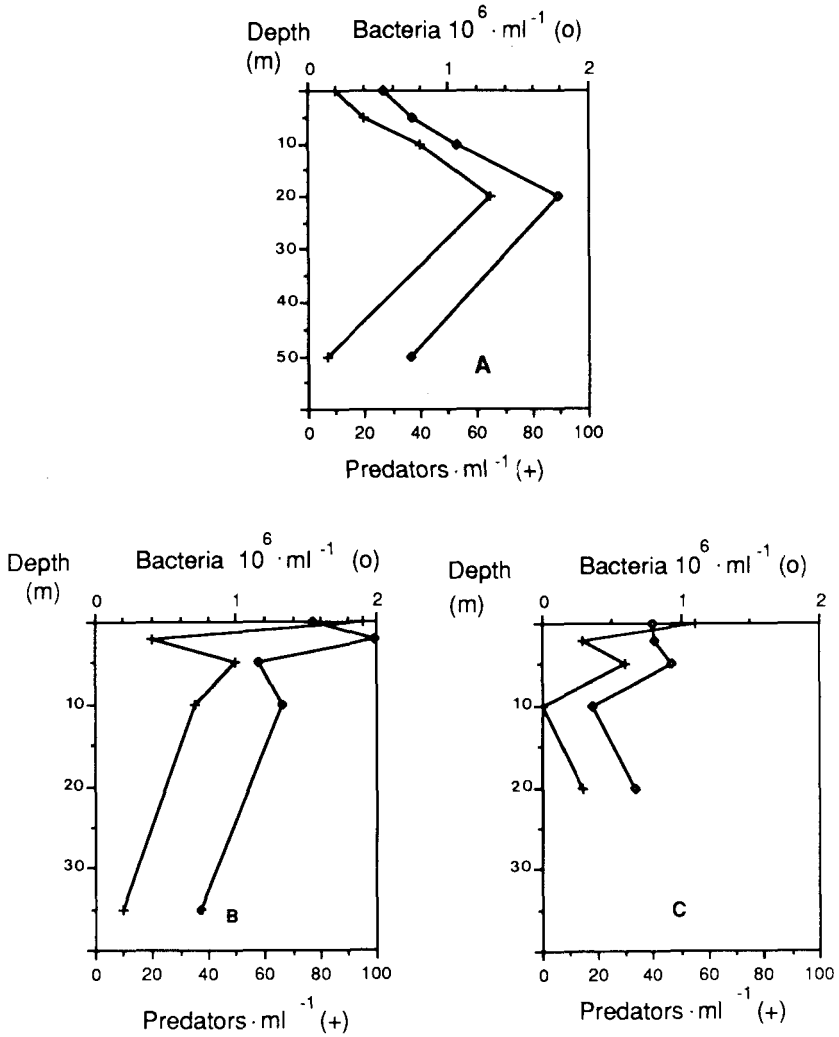

Fig. 3. Bacteria and micropredator numbers. A : station 1 ; B : station 2 ; C : station 3.

tions showed again parallel changes and densities of the same order than in oligotrophic seawater of station 1 (50m).

Growth experiments in unfiltered and screened (3 um) water allowed us to calculate the growth rate u, the grazing and the bacterial production (table 1). From the rate of incorporation of leucine in protein, the bacterial production was also calculated (table 1), using the average value of 5.4×10^{17} cells produced by mole of leucine (Kirchman et *al*, 1986).

A little difference of counts was noted in growth rate values between seawater and water of the plume samples. Such growth rates were already described for oligotrophic and eutrophic seawaters of warm core ring of the Gulf Stream (Ducklow and Hill, 1985). They are higher values than the average value of $0.02\ ^{h-1}$ measured by Sieracki and Sieburth (1985) in oligotrophic waters, but smaller than values for coastal waters (see review of Ducklow and Hill, 1985). The grazing rates were similar in oligotrophic waters (station 1: 0 and 20 m, and 5 m at stations 2 and 3), around 0.015×10^9 bacteria $l^{-1} \cdot h^{-1}$. As expected, the higher values were measured in the eutrophic layer of the plume.

The bacterial production, estimated from the "culture" method was ten fold higher than bacterial production calculated from leucine incorporation in protein (table 1), in oligotrophic samples. But, for eutrophic estuarine water (stations 2 and 3 surface level), both methods gave the same results. This could be due to the high concentration of leucine (3 nmole/100 ml) used for measuring the heterotrophic activity, such concentration being too high for bacterial assemblages of the oligotrophic water. In the Saint Lawrence estuary, Painchaud et *al* (1987) measured the bacterial production of the same order than these obtained by leucine incorporation method, i.e. $27\text{-}71 \times 10^6$ bacteria $l^{-1} \cdot h^{-1}$. The last authors described a rapid growth of estuarine bacteria between 1 and 5 ‰ of salinity. For the surface layer of station 2 and station 3, because of the relatively low salinity (respectively 23.2 and 19.6‰) such estuarine bacteria were probably responsible of the important measured growth, and on the other hand, the halocline did not allow physical dispersion or mixing with underlayer water (Aloisi et *al*, 1979). It is interesting to note that the highest numbers of bacterial cells were produced between 15 and 25 ‰ of salinity in the Delaware estuary (Coffin and Sharp, 1987). As our results, Coffin and Sharp results afford Painchaud et *al* (1987) hypothesis.

The bacterial production, as $\mu g\ C\ l^{-1} \cdot d^{-1}$, was calculated from bacterial numbers increase in the seawater culture and from leucine incorporation, using the coefficient 10^9 cells = 12 ug C (Larsson and Hagstrom, 1979 ; Wright, 1984). When referring to the review of Coffin and Sharp (1987), the production values (table 2), determined with leucine incorporation method, give results similar than already published, both of oligotrophic and eutrophic situations. Considering values obtained from growth expermiments, values for oligotrophic samples are higher than those usually found in litterature.

In conclusion, this area of Rhône plume is the site of an important heterotrophic activity, concerning bacteria as well as their predators. The microbial loop is a probable privilegiate link for the transfert of allochtonous matter and energy to the higher trophic levels of the oligotrophic networks.

Table 1. Bacterial growth and production and grazing by micropredators.

		Growth h^{-1}	Grazing 10^9 bacteria $\cdot l^{-1} \cdot h^{-1}$	Production 10^9 bacteria $\cdot l^{-1} \cdot h^{-1}$ a	pmol leucine $l^{-1} \cdot h^{-1}$	Production 10^9 bacteria $\cdot l^{-1} \cdot h^{-1}$ b
Station 1						
	0m	0.06	0.004	0.174	45	0.024
	20m	0.05	0.012	0.112	21	0.011
Station 2						
	0m	0.08	0.051	0.409	754	0.407
	5m	0.08	0.014	0.181	45	0.024
Station 3						
	0m	0.09	0.083	0.275	1 468	0.153
	5m	0.07	0.017	0.061	452	0.046

a : form Gak et *al.*(1972) calculation b: form leucine incorporation into protein (5.4×10^7 cells/mole leucine ; Kirchman et *al.* 1986)

Table 2. Estimations of bacterial production ($\mu gC \cdot l^{-1} \cdot d^{-1}$).

		Production a	Production b
Station 1			
	0m	50.11	6.90
	20	32.25	3.15
Station 2			
	0m	117.88	117.2
	5m	52.03	6.90
Station 3			
	0m	79.20	44.04
	5m	17.52	13.24

a : calculated from increase in cell numbers b : calculated from leucine incorporation into protein For both calculations : 10^9 cells=12μg C (Larsson and Hagström, 1979).

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