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MICROHETEROTROPHIC COMMUNITIES IN THE WATER COLUMN OF THE RHONE RIVER PLUME

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ABSTRACT: As tidal range is very small in the Mediterranean, the Rhône River plume ($1600m^3\ s^{-1}$) spreads as a thin layer of low density water over the seawater. Schematically, the water column of the plume area is constituted by three layers. The upper freshwater layer is about 1-1.5m deep and carries heavy loads of organic and mineral particles as well as dissolved components. The intermediate layer, of 0.5-1m, is a discontinuity zone, showing strong density and dissolved nutrients gradients. The third layer, seawater, receives mostly particles from the other layers. In the Rhône water layer, as well as in the seawater, bacterial production and biomass are relatively constant, whatever more important in the freshwater. The three superposed layers showed three distinct microbial communities. The river water community is composed of freshwater bacteria, with low activity. The interface layer community can be described as a juvenile community of oligotrophic marine bacteria. The marine water community was characterized by low numbers of bacteria whith growth limited by organic substrate availability.

Introduction

Contact zones between two water masses are called fronts. The physical and biological phenomena occurring at such interfaces are of great interest to oceanographers as well as ecologists. From an oceanographical point of view, biomass is higher at fronts at spatio-temporal mesoscales, and this plays a major role in the global productivity of oceans. From an ecological perspective, the properties of the contact zone are determined by the fluxes of material between water masses, and the different densities, which affects the buoyancy of the organisms.

Microorganisms can respond rapidly to external events and then, the structure of microbial communities at fronts should be different from the surrounding between water masses. So, as for macroscopic communities at the interface, that are distinct from those of the two adjacent water masses (Frontier, 1986), bacterial communities may also differ in the frontal zone (Ducklow, 1983; Egan and Floodgate, 1985).

Coastal plumes are excellent sites for studying such situations, as they are characterized by distinct salinity structures. The Rhône River plume ($1600m^3.\ s^{-1}$) is especially suitable because of the lack of tide in the Mediterranean Sea. The freshwater forms a thin lens (1-1.5m) of low salinity water with high-nutrient concentration, overlaying oligotrophic seawater. The plume-sea boundary is about 1m deep with strong physico-chemical gradients.

We have already shown that the bacterial production is greater at the plume-sea boundary (Soto and Bianchi, 1988, Kirchman *et al.*, 1989). In this report we focus on (i) the nature of the microorganisms at the interface, compared with those of the river water and the seawater; (ii) the ecology of microbial communities in the three layers.

Materials and Methods

At a station close to the mouth of the Rhône River several depth profiles were taken during a cruise in May 1988. As the results were very similar for the studied parameters we shall described one typical profile. Water samples were pumped up to the boat at intervals of 50 cm. from the surface to a depth of 2.5m, as described previously (Kirchman, *et al.*, 1989).

The temperature and salinity were determined in the three layers with electrods (Yellow Springs Instrument Co). Ammonium concentrations were measured on board (Strickland and Parsons, 1972). Concentrations of nitrite, nitrate and phosphate were measured with an automatic Technicon analyser (Strickland and Parsons, 1965). Amino acid concentrations were de-

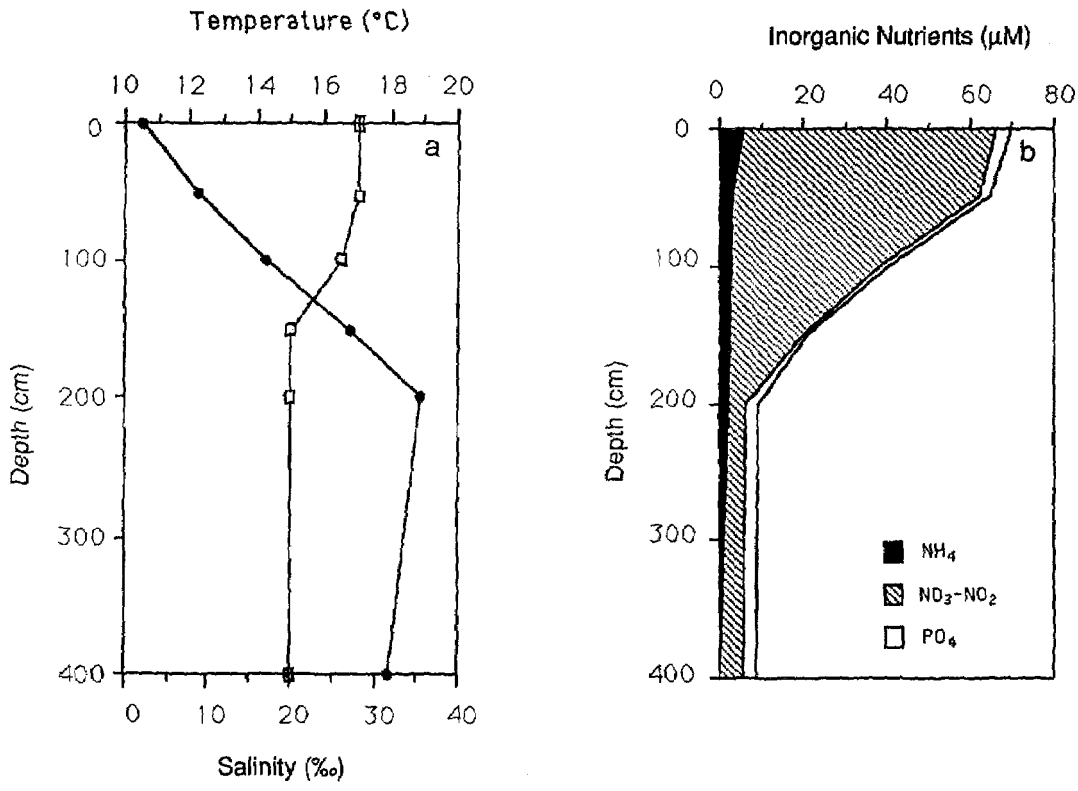

Figure 1. Vertical distribution of (a) Temperature, salinity, and (6) nutrient salts in the three layers.

termined using high pressure liquid chromatography (HPLC and the method described by Lindroth and Mopper (1979). We used a Kontron Compact System LCS 620 equipped with a Beckman column (Ultrasphere ODS 5µ 4.6×250mm).

Bacteria were counted by epifluorescence microscopy using DAPI (Porter and Feig, 1980) and image analysis (Van Wambeke, 1988). Microflagellates were counted using a double staining method (Sherr and Sherr, 1983); autotrophic microflagellates and other phototrophic organisms were distinguished by their autofluorescing chlorophyll.

In order to differentiate the nutritional nature of viable bacteria in each layer, counts were done using two nutrient rich media: marine agar (Difco) and freshwater nutrient agar (Difco), and two oligotrophic media: Rhône River water and Mediterranean seawater enriched with 10 mgl^{-1} of yeast extract, without distort the oligotrophy (Olsen et Bakken, 1987), and 15g of agar. Serial dilutions of water samples were prepared to 10^{-5} in 9ml amounts of sterile seawater and were inoculated (0.2ml) in duplicate on each medium plate. Incubation time was 15 days.

Incorporation rates of (methy $l^{3}H$) Thymidine (Fuh-

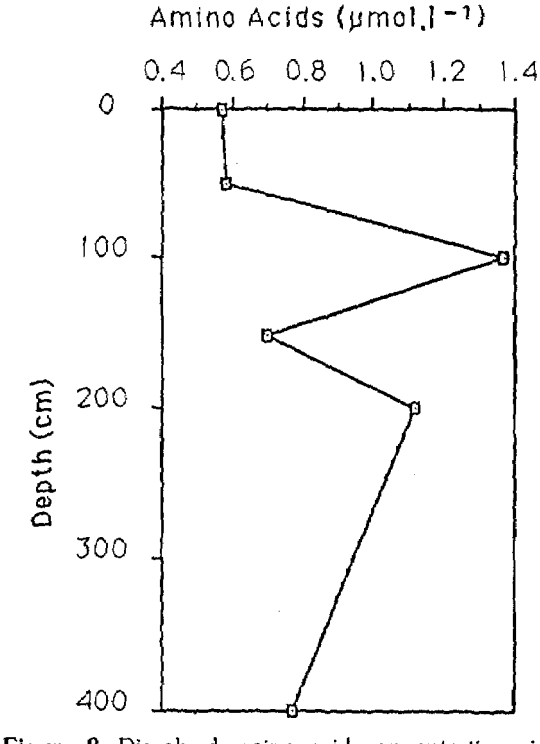

Figure 2. Dissolved amino acid concentrations in the three layers.

rman and Azam, 1982) and (^{14}C) Leucine (Kirchman *et al.*, 1985) were performed as described by Kirchman *et al.* (1989), except that the final concentration of labelled compounds which was 20 nM.

Results

The three layers (freshwater, interface and seawater) were clearly distinguished by their temperature, salinity and nutrient concentrations (Figs 1a and 1b). The river water layer was about 50cm deep, whereas the interface was 1.5m. The seawater conditions of high salinity and low nutrient content were established below 2m depth.

In the low salinity water and seawater, the amino acid concentration was less than 0.8 µM, while it showed a clear increase. (1.4 µM) at 1m depth, which corresponded to the interface water (Fig. 2).

The bacterial densities decreased from freshwater (5.5×10^6 cells ml^{-1}) to seawater (2×10^6 cells ml^{-1}). The drop of bacterial counts was clear in the interface zone (Fig. 3a). The population densities of microbial predators (350 cells ml^{-1}) were similar in freshwater and in seawater (Fig. 3a). The population densities of heterotrophic flagellates were a little greater at 50 and 150 cm (Fig. 3a). The heterotrophic bacterial activity, as measured by thymidine or leucine incorporation rates, were greatest in the interface water (Fig. 3b). In the overlaying river water and in the seawater, the bacterial activities showed similar values, which were about half of the rates measured in the interface water.

Viable counts of heterotrophic bacteria, obtained with the oligotrophic river water medium, were higher than numbers of colony forming units (CFU) from the other culture media in the samples from the surface, 50cm and 100cm depths (Fig. 4). At the contact

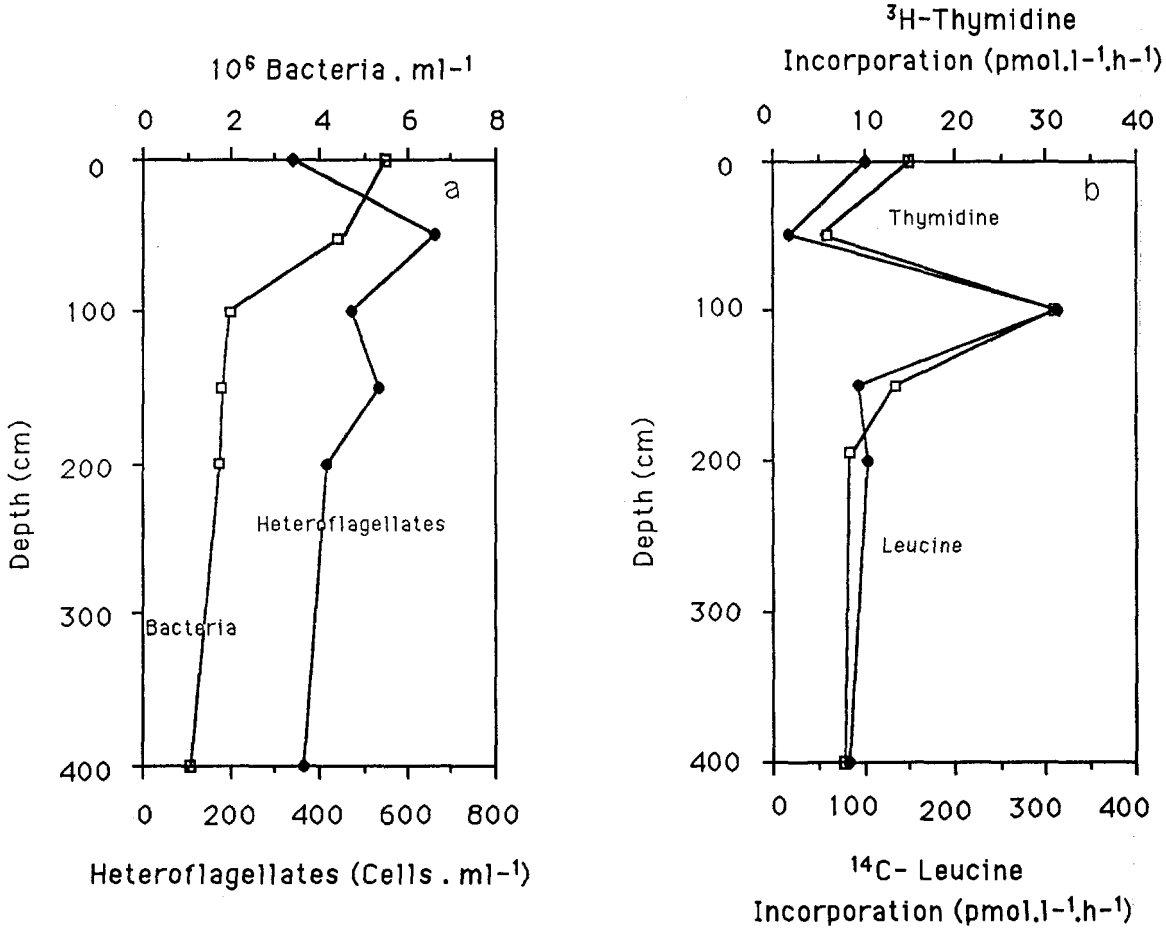

Figure 3. (a) Densities of bacteria and heteroflagellates, (b) ^{3}H-Thymidine and ^{14}C-Leucine incorporation rates into heterotrophic bacteria.

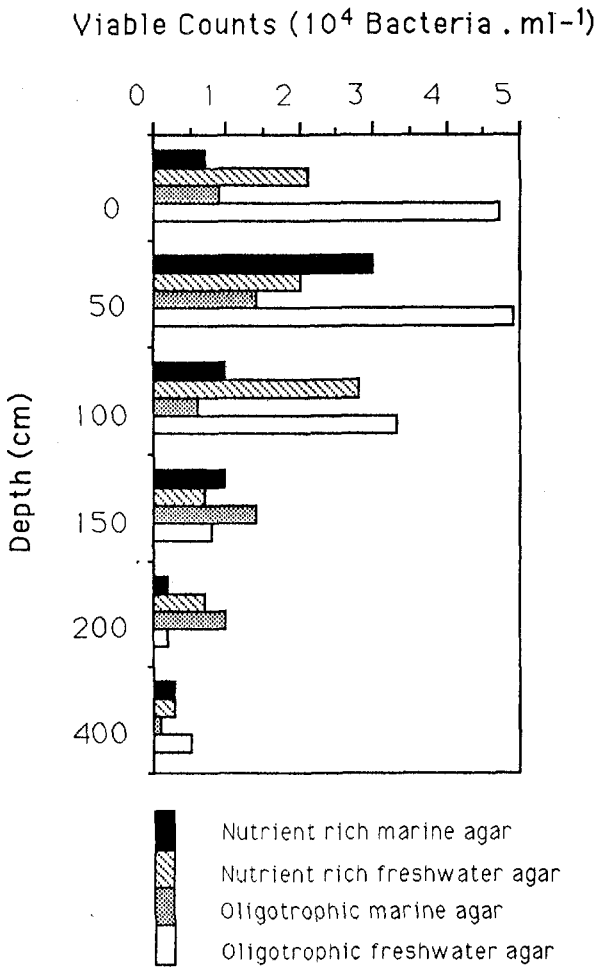

Figure 4. Viable counts performed on two oligotrophic and two nutrient rich freshwater and seawater culture media.

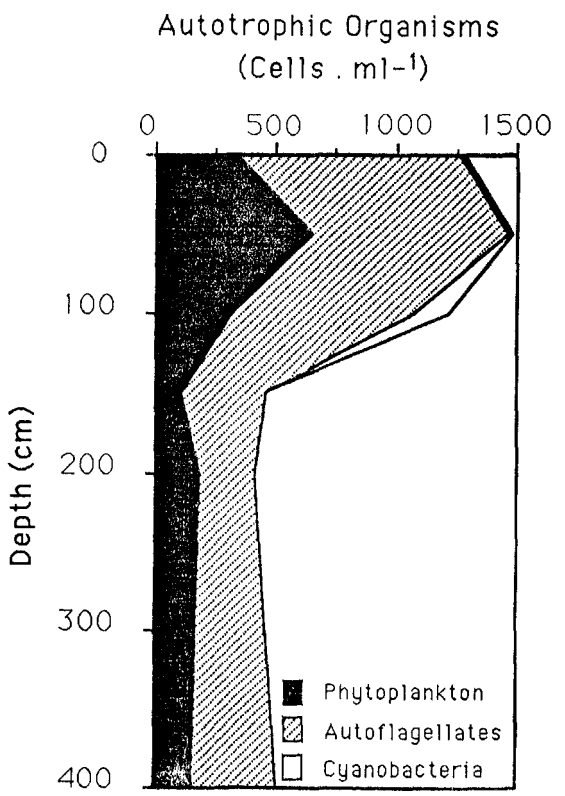

Figure 5. Densities of autotrophic organisms in the three layers.

zone between interface water and seawater (150 and 200cm) viable counts on all media decreased and the greatest numbers of CFU were found on seawater oligotrophic medium (Fig. 4).

Autotrophic cells (diatoms, autotrophic flagellates and cyanobacteria) showed a vertical distribution similar to that of nutrients (Fig. 5). Similarly to viable counts of heterotrophic bacteria numbers dropped abruptly at the 150 cm depth. Then, autotrophic organisms densities were always low in the water column (Fig. 5).

Discussion

The three water layers (river water, interface water and seawater), were defined by their physical and chemical parameters and constituted three different ecosystems.

All enumerated organisms, including bacteria, were more numerous in Rhône River water than in seawater. This was related to the eutrophication degree of each water mass (Figs 1 and 2). However, bacterial activities, expressed as thymidine and leucine incorporation rates, were similar in both ecosystems, demonstrating a higher activity of bacteria in marine water than in freshwater. The low number of bacteria in seawater probably resulted from the oligotrophic nature of the Mediterranean Sea. In the more eutrophicated Rhône River water, bacterial predators regulated bacterial numbers, as in many aquatic environments (Palumbo *et al.*, 1984; Azam *et al.*, 1983).

In the interface layer, site of strong physical and chemical gradients, freshwater planktonic organisms (phytoplankton as well as zooplankton) suffer from the density increase and release metabolites and cell content. This could be reflected by the peak of dissolved amino acid concentration (Fig. 2) in the interface water, So, the increase of heterotrophic bacterial activity in the interface layer (Fig. 2b), already drescribed in winter conditions by Kirchman *et al.* (1989), proba-

bly originated from the organic matter retained by the physical barrier between the two water masses (Zutic and Legovic, 1987). The increase of dissolved organic matter, we measured as dissolved amino acids, was able to support this increased bacterial growth.

Thymidine and Leucine methods may give the best estimates of rate of bacterial biomass production and reveal other aspect of the metabolism of bacterial assemblage (Kirchman *et al.*, 1986). The rates of tracer incorporation in interface layer were twice as large as those measured in the river layer and in the seawater layer. Linley *et al.* (1983) measured a bacterial production six times higher in a frontal sampling station, off the English Channel (Ushant Front), than in the mixed shelf water. On the other hand, in such ecosystems (discontinuities), microbial communities, based on experimental time scale, are in a state of unbalanced growth (Hanson *et al.*, 1986).

The decrease of viable bacterial counts from the river water to the seawater (Fig. 4) reflected the decrease of total number of bacteria (Fig. 3a). The use of oligotrophic medium, made of river water, demonstrated the presence of bacteria adapted to the river conditions (Fig. 4). Their disappearance in the bottom seawater, starting already in the interface water, is in agreement with the massive death of freshwater bacteria entrained in brakish water, hypothesized by Painchaud *et al.* (1987).

In the interface layer the more numerous bacteria were enumerated on oligotrophic marine agar. This result also agrees with the model of Painchaud *et al.* (1987) concerning bacterial fluxes in an estuary, in which the estuarine bacteria were growing rapidly because of the large amounts of undegraded organic material from freshwater organisms already evoked.

In this layer of high bacterial production, the number of bacteria, as well as the number of heterotrophic protozoa, did not increase (Fig. 3). Comparing the heterotrophic flagellates/bacteria ratio, Linley *et al.* (1983) found values similar or lower in frontal waters than in mixed and stratified waters. It is possible that, in such ecosystems demonstrating a high bacterial production, the biomass gets rapidly exported by grazing from flagellates to copepods (Painchaud *et al.*, 1987) combined to physical phenomena like currents or sinking (Le Fèvre and Frontier, 1988).

In conclusion, the three superposed ecosystems showed three specific bacterial communities. The river water community was composed of freshwater bacteria growing at the expense of autotrophic planktonic organisms. The interface layer community demonstrated a high production activity combined with low bacterial densities. This community, controlled by biomass exportation, could be described as a juvenile community composed of oligotrophic bacteria. The marine water community, characterized by lower bacterial numbers and higher bacterial activity than in the river water, was regulated by the availability of organic substrates.

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