Bacterioplankton in the Seine River (France): impact of the Parisian urban effluent[1]

JOSETTE GARNIER[2]
Laboratoire d'écologie, U.A. 258, Centre national de la recherche scientifique, École Normale Supérieure, 46, rue D'Ulm, 75005 Paris, France

AND

PIERRE SERVAIS AND GILLES BILLEN
Groupe de microbiologie des milieux aquatiques, Université Libre de Bruxelles, Campus de la Plaine, CP 221, BD du Triomphe, 1050 Bruxelles, Belgique

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Bacterial abundance and biomass were studied in April, July, and October 1989 at 13 stations along 300 km of the course of the river Seine, including Paris and its suburbs. Monthly investigations were carried out at five stations downstream from Paris where the river receives the effluent of an important waste water treatment plant (Achères). As a result of an input of allochthonous bacteria from the effluent of the plant, an increase in bacterial abundance and biomass was observed below Achères (from about 5×10^9 to 15×10^9 cells L^{-1} and from 100 to 750 μg C L^{-1}). This was followed by a rapid decrease. The allochthonous bacteria comprised a high proportion of large bacteria, which disappeared at a much higher rate than the small bacteria (0.0366 vs. 0.0125 h^{-1}). Paradoxically, these large bacteria grew at a rate twice that of the smaller cells in culture experiments (0.129 vs. 0.065 h^{-1} in June and 0.118 vs. 0.071 h^{-1} in October). These large bacteria must therefore be subjected to intense losses (grazing, sedimentation, etc.). Higher rates of discharge of the river, i.e., a shorter residence time of the water masses, appeared to transmit the Achères signal farther, leading to faster transport of the bacterial populations.

Key words: bacterioplankton ecology, size fractions, river ecosystem.

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Les variations temporelles et spatiales des abondances et biomasses bactériennes ont été étudiées dans le fleuve français, la Seine, sur un secteur long de 300 km. La zone d'influence de la station d'épuration d'Achères, à l'aval de Paris a été plus particulièrement analysée. En raison d'un apport massif de bactéries allochtones par les effluents d'Achères, il se produit à l'aval de la station un accroissement important tant des abondances (d'environ 5×10^9 à 15×10^9 cellules L^{-1}) que des biomasses (de 100 à 750 μg C L^{-1}). Les valeurs diminuent ensuite rapidement. Les bactéries allochtones se composent d'une proportion importante de bactéries de grande taille qui disparaissent plus rapidement que les petites (0,0366 vs. 0,0125 h^{-1}). Comme le montrent les expériences en culture, ces bactéries de grande taille croissent pourtant à un taux environ deux fois plus élevé que les petites (0,129 vs. 0,065 h^{-1} en juin et 0,118 vs. 0,071 h^{-1} en octobre). Ces grosses bactéries doivent donc être soumises à des processus de pertes importantes (broutage, sédimentation ...). Il apparaît que le signal de la station d'épuration d'Achères est observé d'autant plus loin que le débit de la rivière est élevé, le transport de la population bactérienne étant plus rapide.

Mots clés : bactérioplancton, écologie de la taille, écosystem fluvial.

[Traduit par la rédaction]

Introduction

The degradation of organic matter is a major process in the oxygen balance of river systems that has been studied for a long time, particularly when there is organic enrichment from waste-water discharges. The approach, first proposed by Streeter and Phelps (1925) and which still prevails in most recent studies (Lesouëf and André 1982), does not take into account the complex dynamics of planktonic bacteria but only considers a first-order kinetics of organic matter degradation with respect to organic matter concentration.

Methods and concepts developed in the last decade for studying biomass and activity of bacterioplankton have led to a better understanding of carbon metabolism in marine systems (Williams 1981; Azam *et al.* 1983; Sherr *et al.* 1988), in lakes (Riemann and Søndergaard 1986; Simon and Tilzer 1987; Garnier 1989), and in estuaries (Admiraal *et al.* 1985; Relexans *et al.* 1988; Riemann *et al.* 1990). Only few studies of microbial ecology were devoted to rivers with an ecosystemic view (Wissmar *et al.* 1981; Healey *et al.* 1988).

A comprehensive study of major hydrodynamic, chemical, and biological processes involved in the functioning of the ecosystem of the river Seine is in progress. The goal is to establish a model of oxygen balance and organic-matter degradation, specifically taking into account the dynamics of the bacterial compartment.

As a part of this study, we investigated the temporal and spatial variations of planktonic bacteria in the river Seine. This paper analyzes these variations in response to the impact of a waste water treatment plant (Achères). Particular emphasis is given to the different behavior of small and large bacteria in the area surrounding the effluent outfall

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[2]Present address: Groupe de microbiologie des milieux aquatiques, Université Libre de Bruxelles, Campus de la plaine, CP 221, BD du Triomphe, 1050 Bruxelles, Belgique.

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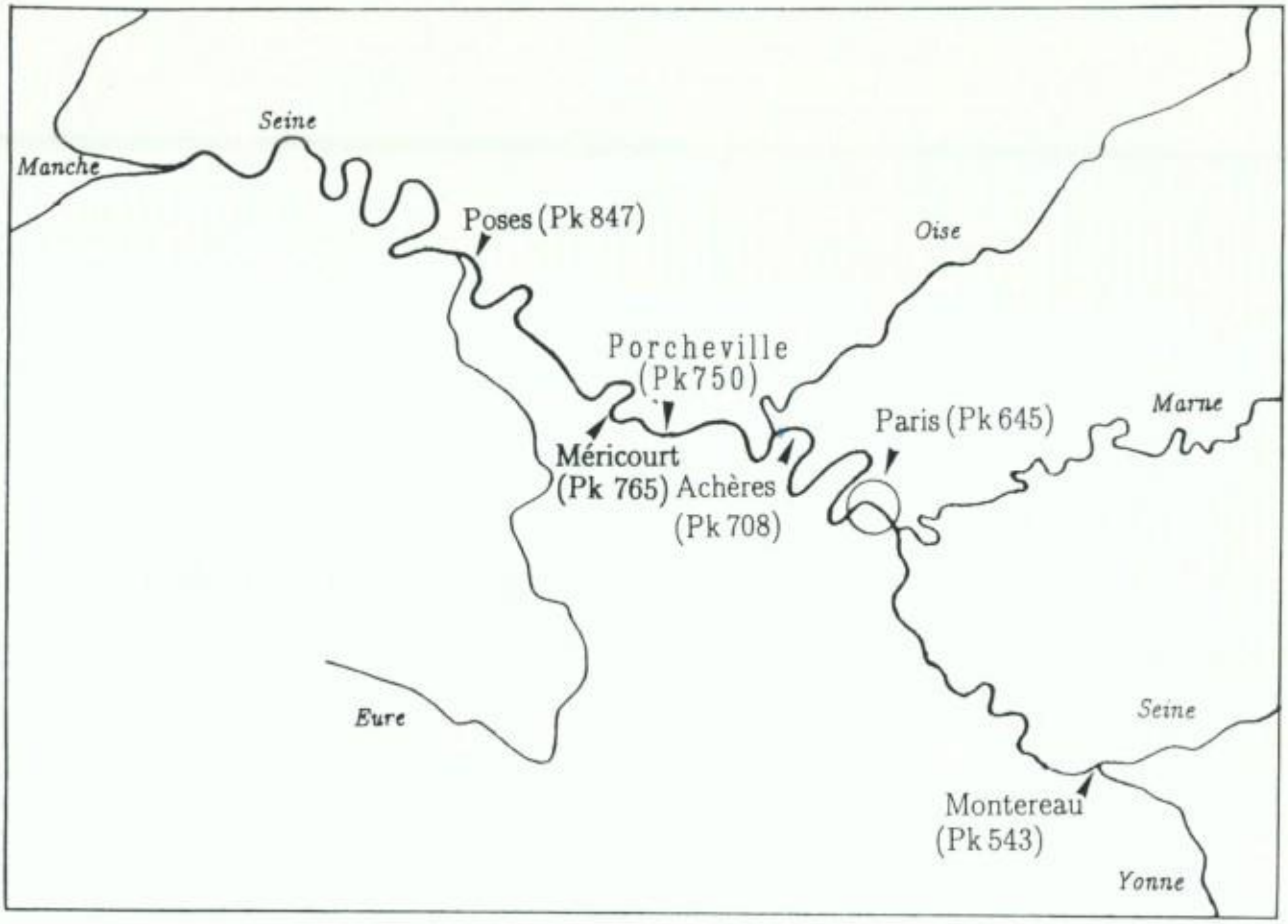

FIG. 1. Study area showing the main station locations.

of the plant. We present evidence that large allochthonous bacteria brought into the river by the effluent of the plant are an active component of the microbial community.

Study area

The watershed of the Seine River has a temperate rainfall pattern with an oceanic influence downstream. The mean tendency of the hydrographic conditions in the last 50 years is characterized by the highest rates of discharge occurring in winter (monthly mean at Paris: 550 $m^3\ s^{-1}$ in February) and the lowest in summer (50 $m^3\ s^{-1}$ in August), increasing from October onwards. However, during the period studied (April 1989 - March 1990), discharge was highest in April 1989, decreased in May, and remained low until March 1990 because of dry meteorological conditions.

The bacterioplankton of the Seine River was studied for a distance of 300 km between Montereau, located at the confluence with the river Yonne, and Poses, 150 km from the mouth of the Seine estuary (Fig. 1). About 60 km downstream from Paris, the river receives an urban effluent from the waste water treatment plant of Achères. About 80% of the waste water produced by the 10 million inhabitants of Paris and its suburb reaches this plant; 70% is treated by the conventional activated sludge process, before being discharged into the river; the remaining 30% is discharged without treatment. The study has therefore also focused on the reach downstream from Paris (Andrésy-Méricourt), at the effluent outfall of the water-treatment plant of Achères.

Material and methods

Sampling program

Three sampling programs were established. In April, July, and October 1989, the course of the river was studied at 13 stations between Montereau and Poses. In addition, five stations, one upstream from the outfall of the Achères effluent and four downstream, were investigated almost every month, and weekly investigations were carried out, from May to October, at a median station of the reach (Porcheville).

The stations are named by a kilometric unit, Pk, used by the Financial Agency of the "Seine Normandie" Bassin. Pk is set at 1000 at Honfleur, the mouth of the estuary, and then decreases upwards: Poses is located at Pk 847 and Montereau at Pk 543. The water-treatment plant of Achères is at Pk 708.

At each station, before sampling, the tubing was abundantly rinsed with the river water. To ensure that the samples were representative, at the two stations where the effluent was shown to yield to heterogeneity of the river (Pk 713 and Pk 721), 10 L of water was taken from two depths (0.5 and 5 m) at three points along a transverse section of the river and mixed (Chesterikoff *et al.* 1991). At the other stations, the 10 L was pumped in the middle of the river at two depths (0.5 and 5 m) and mixed.

Bacterial enumeration

Samples (20 mL) for direct epifluorescence microscopic counts were preserved in 2% formalin immediately following collection from the river or from the culture (Hobbie *et al.* 1977); they were stored at 4°C until analysis (within 1-2 months).

A subsample of 200-500 μL was brought to 2 mL with 0.22 μm filtered water (Millex, 0.22 μm), stained for 4 min with acridine orange at a final concentration of 2.5×10^{-4} g mL^{-1}, and filtered at a low vaccum (less than 100 mbar (1 bar = 100 kPa)) through a black polycarbonate membrane (Nuclepore, 0.22 μm porosity, 25 mm diameter) placed on a cellulose acetate filter (Millipore, 0.45 μm porosity). The membrane was mounted between a slide and a glass cover slip, with a nonfluorescence immersion oil (Olympus).

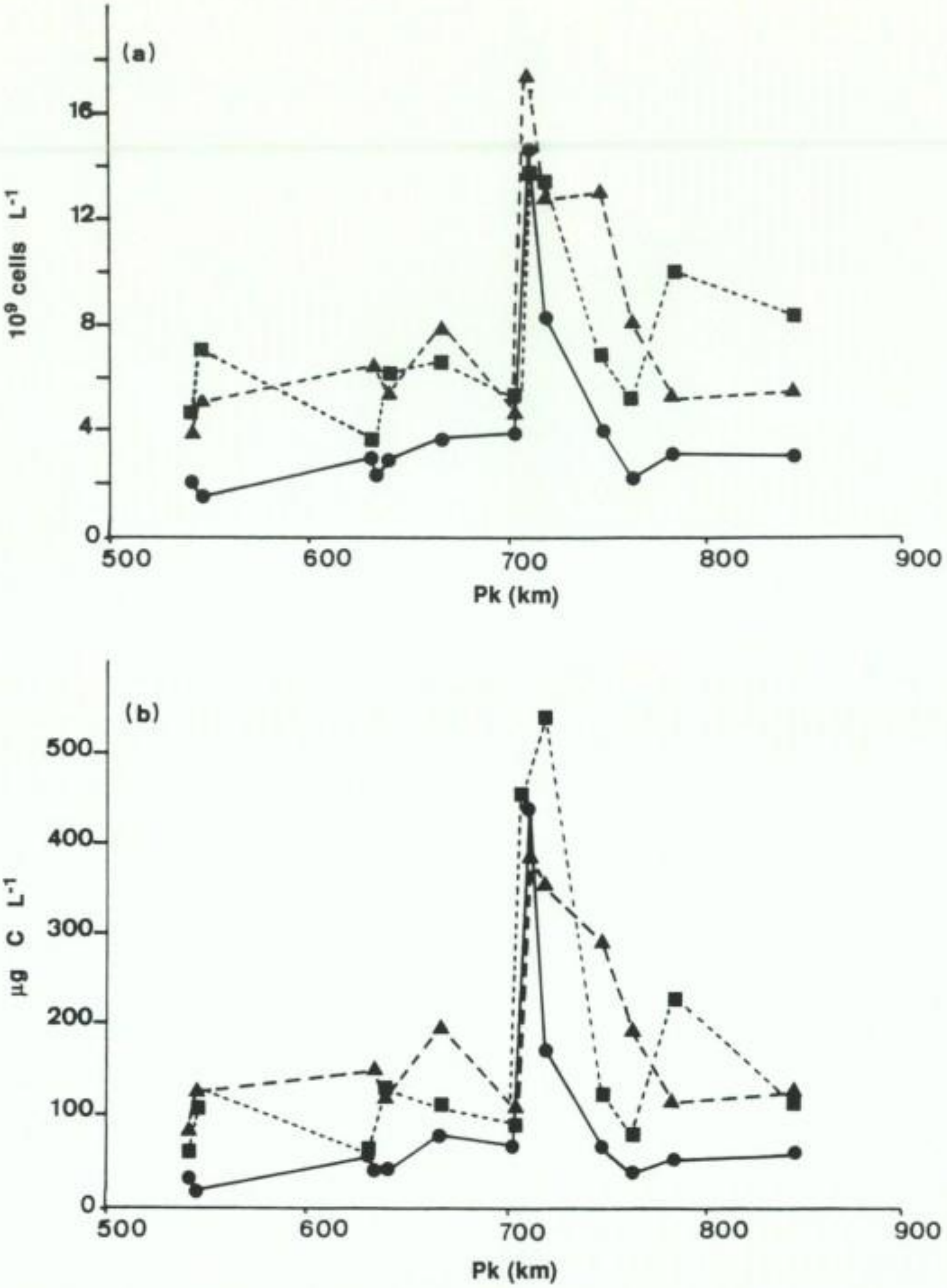

FIG. 2. Longitudinal variations of (*a*) bacterial abundance (10^9 cells L^{-1}) and (*b*) biomass (µg C L^{-1}) between Montereau (Pk 546) and Poses (Pk 846) in April (▲), July (■), and October 1989 (●). Pk, in kilometres (see text).

Counting was performed with a Leitz microscope (Laborlux) fitted with a 3-Lambda-Ploemopak illuminator for epifluorescence and a 100-W Hg lamp. A 100× Fluotar oil immersion objective was used at a total magnification of 1000×.

Bacteria were counted on 12 fields on one preparation or on 8 fields when preparations were duplicated. Bacteria (250–500) were counted for each preparation. The bacteria were classified by four shape criteria (coccoids, rods, ellipsoids, and vibrio like), and within each, six or seven size classes were distinguished. Measurements were made during the counting with an eyepiece graticule and calculations of biovolumes were based on the formula for geometric shapes. Biovolumes were converted into a carbon unit, using a conversion factor that varies in relation to the cell biovolume, from 4×10^{-13} g C μm^{-3} for smaller bacteria (0.026 μm^3) to 1.3×10^{-13} g C μm^{-3} for larger ones (>0.4 μm^3) (Simon and Azam 1989).

Culture experiments

In June and October 1989, at the Porcheville station, river-water culture experiments were performed according to Ammerman *et al.* (1984). However, the inoculum was filtered through a 2-µm Nuclepore membrane filter to eliminate grazers and diluted 10-fold into 0.22 µm filtered river water. Considering the size of the bacteria, the filtration of the inoculum through 2 µm was assumed to be the best compromise to separate the bacterial populations from the grazers community (see Fig. 5). The culture was incubated in the dark at *in situ* temperature (20 and 15°C in June and October, respectively). About 10 samples were taken during 20 h

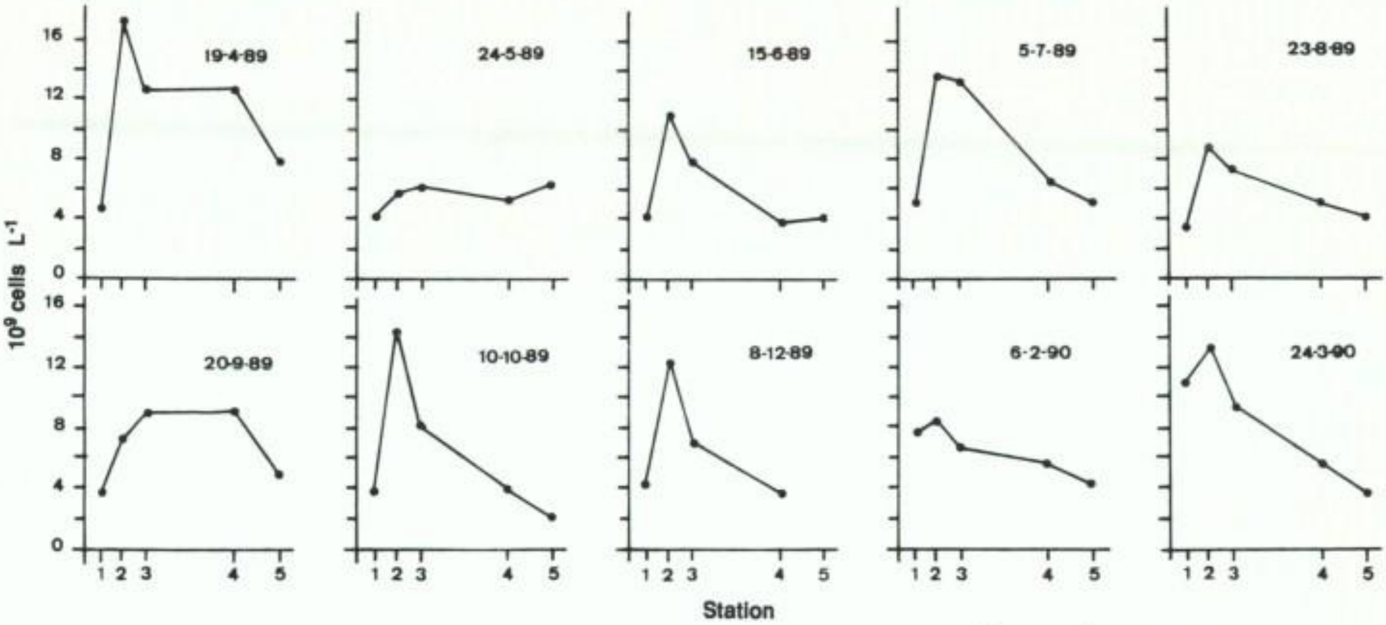

FIG. 3. Longitudinal and seasonal (day · month · year) variations of bacterial abundance (10^9 cells L^{-1}) around the water-treatment plant of Achères (Pk 708). Station 1, upstream from Achères, la Frette (Pk 706); station 2, downstream from Achères (Pk 713); station 3, Poissy (Pk 721); station 4, Porcheville (Pk 750); station 5, Méricourt (Pk 765). Pk, in kilometres.

TABLE 1. Average profiles of total bacterial abundance and biomass around the perturbed zone of the water-treatment plant of Achères (Pk 708). Distribution of the relative abundance and biomass of small (< 1 μm) and large (≥ 1 m) bacteria

Station (Pk)	Total bacterial abundance			Biomass		
	$\times 10^9$ cells L^{-1}	% < 1 μm	% ≥ 1 μm	μg C L^{-1}	% < 1 μm	% ≥ 1 μm
706	5.4	82.5	17.5	107.9	61.8	38.2
713	11.2	69.7	30.3	489.5	32.9	67.1
721	9.7	73.3	26.7	322.3	44.7	55.3
750	6.0	85.9	14.1	106.1	68.6	31.4
765	4.9	88.4	11.6	83.3	72.2	27.8

for determination of bacterial abundance and biomass as described above.

Results

Spatial variations of bacterial abundance and biomass

The three longitudinal profiles studied in April, July, and October along the 300 km separating Montereau from Poses followed the same general pattern (Fig. 2); they all showed a sharp increase both in abundance and biomass in the area where the river receives effluent from the waste water treatment plant of Achères. This was followed by a marked decrease, so that values close to those found upstream from Paris were observed from Méricourt (Pk 765) to Poses (Pk 847).

Similar observations were made at shorter sampling intervals of time, for the zone perturbed by the effluent of the waste water treatment plant, although the impact of Achères appears to vary in intensity (Fig. 3).

The abundance of attached bacteria was estimated only in April 1989, at the highest rate of discharge. The attached bacteria represented 23% of the total abundance, as an average (SD = 10), over the longitudinal profile. The abundance of attached bacteria is higher when there is elevated suspended matter (Healey *et al.* 1988), which is the case at high discharge rates; therefore, the proportion of attached bacteria observed here at the highest discharge rate can be considered as maximum.

An expected linear relationship existed between abundance and biomass for most of the data (Fig. 4). However, biomass values were higher for the stations located just downstream from Achères (Pk 713 and 721), where larger bacteria were observed.

The shift in size, as affected by the plant effluent, is clearly shown by the appearance of a second peak in size distribution towards higher values (≥ 1 μm in greatest dimension), whereas upstream from the plant and farther downstream, the size distribution was unimodal (at a size < 1 μm) (Fig. 5). These observations allowed two size classes of bacteria to be distinguished, i.e., small bacteria (greatest dimension < 1 μm) and large bacteria (≥ 1 μm).

An increase or a decrease in total bacterial abundance and biomass was related to an increase or a decrease in abundance and biomass of the two size classes (Table 1). Small cells dominated the bacterial populations at all the stations and represented on average at least 69.7% of the total abundance. However, the proportion of large bacteria increased at the station downstream from the waste water treatment plant of Achères (Table 1). Regarding the biomass, the pro-

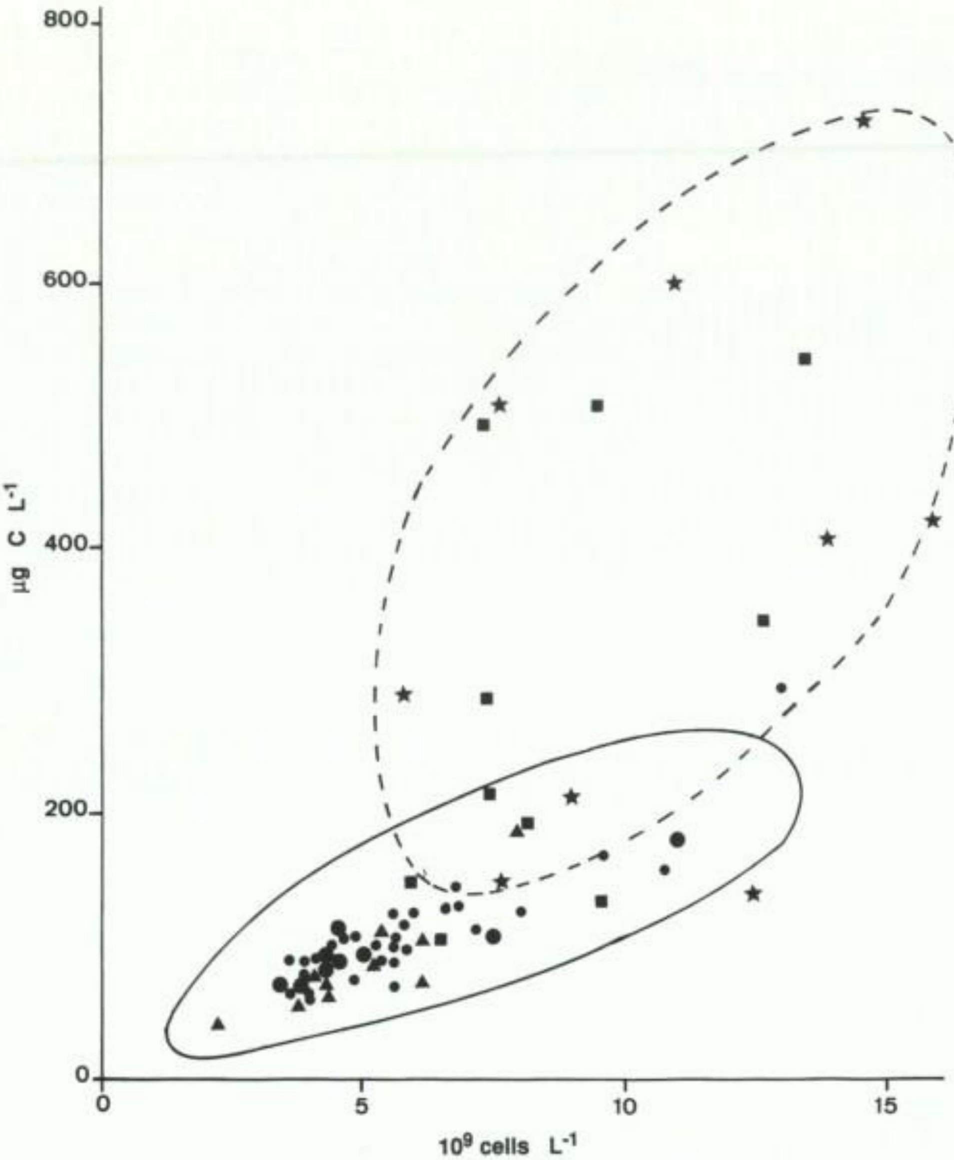

FIG. 4. Relationship between bacterial biomass (μg C L^{-1}) and abundance (10^9 cells L^{-1}). Pk 706 (●), pK 713 (★), Pk 721 (■), Pk 750 (●), Pk 765 (▲).

portion of small bacteria represented at least 60% above the water treatment plant of Achères but decreased notably down to 32.9% just below. As a whole, for both abundance and biomass, proportions identical with those found above Achères were again observed at the median station of the reach, Porcheville, Pk 750 (Table 1).

The discharge of the waste water treatment plant presented little variation during the course of the year, from 25 to 32 m^3 s^{-1}, and represented from 4 to 60% of the discharge of the river. The abundance of large bacteria, just below the water treatment plant, increased rapidly with increasing percentage of discharge, showing that these larger bacteria were concentrated under low discharge of the river (Fig. 6). This concentration effect was not observed for small bacteria.

Temporal variations of abundance and biomass

At Porcheville, abundance and biomass, respectively, varied from 3.6×10^9 to 13×10^9 cells L^{-1} and from 69 to 294 μg C L^{-1} from April 1989 to March 1990 (Fig. 7*a*). The decrease in both abundance and biomass, from 13×10^9 to 5×10^9 cell L^{-1} and from 294 to 100 μg C L^{-1} respectively, was associated with a considerable reduction in discharge rate, from 936 to 287 m^3 s^{-1} (Fig. 7*a*). Although both the rates of discharge and their amplitude of variations were relatively low from May 1989 to March 1990, the increases in bacterial populations coincided most of time with augmentations in discharge rates. Similar coincidences were also observed between bacteria and discharges upstream from the water treatment plant of Achères (Fig. 7*b*).

Growth and disappearance rates of small and large bacteria

Water culture experiments conducted with Porcheville water show a distinct exponential growth for small- and large-sized bacteria (Fig. 8). Although these experimental conditions do not lead to the determination of an *in situ* growth rate of the populations, calculations show that the large bacteria were able to growth at rates about twice (0.129 and 0.118 h^{-1} in June and October, respectively) that of small ones (0.065 and 0.071 h^{-1} in June and October, respectively).

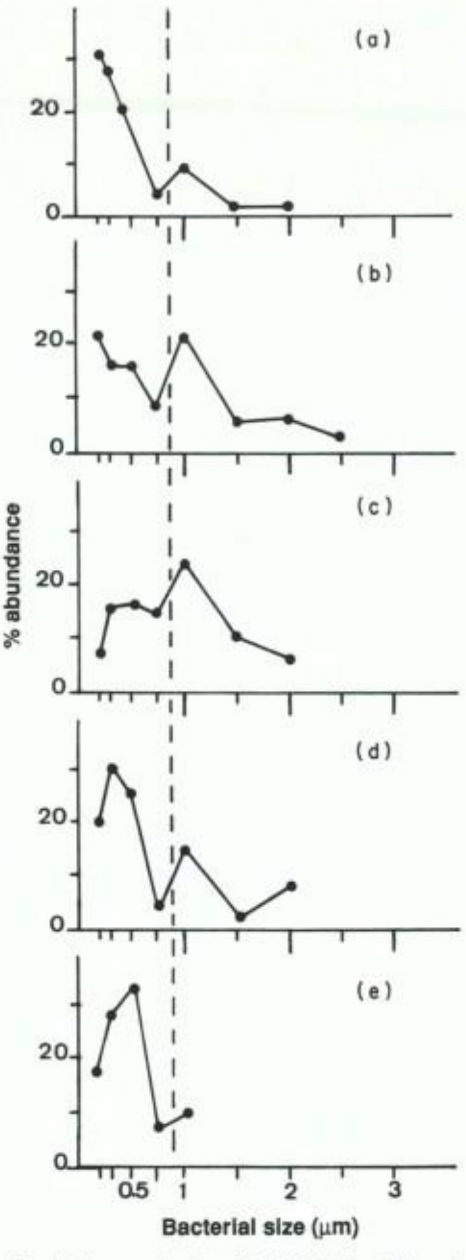

FIG. 5. Typical example (on 5 July 1989) of size distribution of bacteria at different stations, represented as percent of total abundance as a function of maximal cell length. The broken line separates the <1-μm fraction from the ≥ 1-μm fraction. (*a*) Upstream from Achères, la Frette (Pk 706); (*b*) downstream from Achères (Pk 713); (*c*) Poissy (Pk 721); (*d*) Porcheville (Pk 750); and (*e*) Méricourt (Pk 765). Pk, in kilometres.

On the other hand, from the observed decreases of bacterial abundance downstream from Achères, the rate of observed change in bacterial abundance (apparent disappearance rate) can be calculated for small and large bacteria according to the relationship, which assumes an exponential decrease,

$$k\ (\mathrm{h}^{-1}) = -(\ln N_2 - \ln N_1/T_{2-1})$$

where N_1 and N_2 represent the abundance at two consecutive stations and T_{2-1} is the residence time (hours) between the two stations. The residence time is determined as the distance between two stations divided by the mean current velocity.

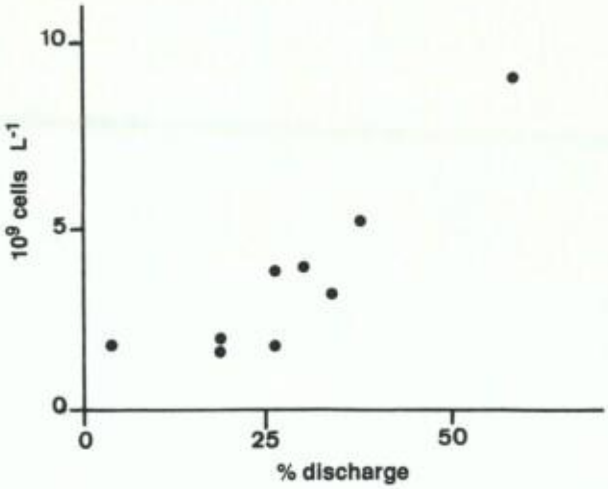

FIG. 6. Relationship between abundance (10^9 cells L^{-1}) of large bacteria (≥ 1 μm) and the percentage of the effluent discharge relative to the river discharge.

The disappearance rates of large bacteria (k_l) were from 3 to 6 times higher than for the small ones (k_s), along the three zones considered from Achères to Méricourt (k_s equalling 0.0125, 0.0112, and 0.0038 h^{-1} vs. 0.0366, 0.0335, and 0.0243 h^{-1} for k_l, Table 2). A test of signs on paired samples shows that the values of k_l are indeed significantly higher than those of k_s ($\alpha = 0.05$; among the 30 paired samples, 23 values of k_l were higher than those of k_s).

Discussion

Spatial and temporal variations of planktonic bacteria

Downstream from the water-treatment plant of Achères, a large increase in abundance of both small- and large-sized bacteria was observed.

To check whether this increase could be explained only by the direct input from the plant, we calculated the concentration of small and large bacteria within the effluent pipes, considering the bacterial abundances and the associated discharges above and below Achères, and also the discharges from the pipes. In October 1989, when cell counts were performed on the effluent waters, the calculated abundances (42.8×10^9 cells L^{-1} with 63% small bacteria) agreed well with the observations (48.6×10^9 cells L^{-1} and 59% small bacteria). This shows that the station choosen below Achères can be considered as representative of the mixing of the river water upstream from the station with the effluent and that no growth or losses have occurred between the effluent outfall and that station. An important population of allochthonous bacteria, characterized by large bacteria, but also comprising small ones ressembling those found upstream in the river, was brought into the river by the treatment plant.

The paradoxical effect of bacterial increase with increasing river discharges observed farther below Achères (see Fig. 7*a*) can be explained by a shorter residence time of the water masses, leading to a faster transport of the bacteria added by the effluent waters: the higher the discharge, the farther is the transmission of the signal. The similar pattern observed upstream from the Achères waste water treatment plant (see Fig. 7*b*) can be explained by other wastewater inputs occurring within Paris.

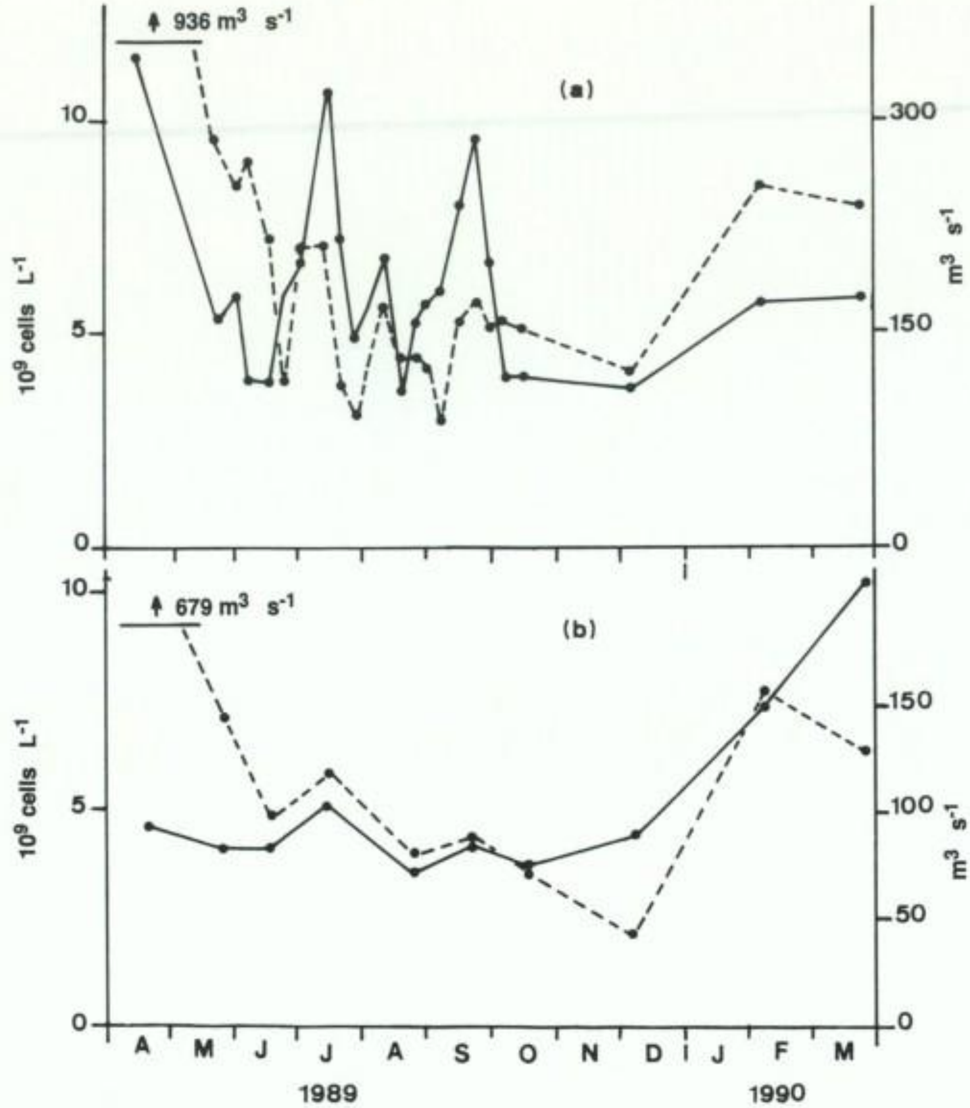

FIG. 7. Temporal variations, from April 1989 to March 1990, of bacterial abundance (10^9 cells L^{-1}, ——) and discharge ($m^3\ s^{-1}$, ----) (*a*) at Porcheville (Pk 750), (*b*) upstream from Achères, la Frette (Pk 706).

Behavior of small and large bacteria

The data presented show that large bacteria below the Achères plant were characterized by both higher growth rates and higher disappearance rates than the smaller bacteria.

The calculations of growth and disappearance rates on the two size classes assume that there was no recruitment from one class to another, i.e., that the two size classes can be considered as independent populations. This is supported by the bimodal size distribution of the bacteria shown in Fig. 5.

The higher disappearance rates of larger bacteria in the river downstream from Achères are unlikely to be biased by their decrease in size. Changes in bacterial shape and size have, however, been shown in extreme nutritional conditions, bacteria becoming larger when cultured on rich medium or smaller when starved (Novitsky and Morita 1976, 1977; Torella and Morita 1981). Considering the minimum values here of the concentrations of biodegradable dissolved organic carbon (1.5 mg C L^{-1}, P. Servais, unpublished data), bacterial populations were far from being in extreme conditions of starvation. The higher disappearance rates for large bacteria would be better explained by grazing and sedimentation. Large cells can be grazed on more efficiently (McManus and Fuhrman 1986; Pace 1988; Gonzales *et al.* 1990). In addition, larger bacteria downstream from Achères often tended to form loose aggregates, which might have accentuated these two loss processes.

In addition to their high disappearance rates, large bacteria have been shown to grow at a higher rate than smaller ones in culture experiments. This difference could be attributed to an increase in size of small bacteria exposed to favorable nutritional conditions in the experimental approach, i.e., a reduction of competition for resources after dilution (Kirchman *et al.* 1982; Ammerman *et al.* 1984; Ducklow and Hill 1985). Nevertheless, preliminary *in situ* experiments of specific bacterial activity on the fractions <1 and ≥ 1 μm have confirmed that large bacteria had a higher growth rate (Servais and Garnier 1990). The water culture could in fact favor the development of populations of larger bacteria, with higher growth rates (Fig. 8), which might have been maintained at a low level by limiting factors (grazing, competition, etc.) in the natural environment.

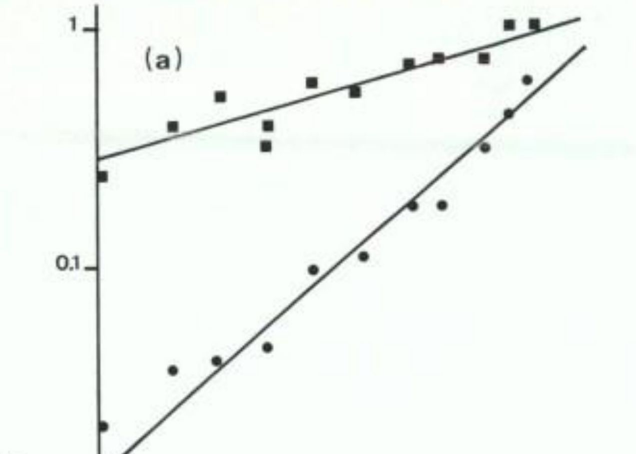

FIG. 8. Evolution of bacterial abundance (ln scale) in river water cultures as a function of time (h): (*a*) June 1989 (20°C); (*b*) October 1989 (15°C). Small bacteria (■), large bacteria (●).

The large allochthonous bacteria, originating from the effluents of the waste water treatment plant, obviously have the ability to grow in the environmental conditions of the river. Considering their higher growth rates, these large bacteria should constitute a very active component of the bacterial community, significantly contributing to organic-matter degradation. However, as a result of their higher disappearance rate, they only can act in a limited area of the river below the effluent outfall.

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TABLE 2. Apparent disappearance rates per hour of small (k_s) and large bacteria (k_l), between two consecutive stations downstream from the water-treatment plant of Achères

Stations (Pk)*	k_s	k_l
713 and 721	0.0125 (−0.0281 – 0.0315)	0.0366 (−0.0363 – 0.0841)
721 and 750	0.0112 (−0.0078 – 0.0368)	0.0335 (0.0060 – 0.0629)
750 and 765	0.0038 (−0.0375 – 0.0330)	0.0243 (0.0060 – 0.0536)

NOTE: Values in body of table are the average. The range is given in parentheses.

*Pk 713, downstream from Achères; Pk 721, Poissy; Pk 750, Porcheville; Pk 765, Méricourt.

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