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# Contribution of Heterotrophic Bacterial Production to the Carbon Budget of the River Seine (France)

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**Abstract.** Bacterial activity was measured in the river Seine by two methods, $^3$H-thymidine incorporation into DNA and $^3$H-leucine incorporation into proteins. Both incorporation rates are characterized by low values upstream of Paris, a large increase just downstream of the outfall of the Achères treatment plant effluents, and then decreasing values further downstream. The covariation of both activities is demonstrated by the constancy of the molar ratio (leucine to thymidine incorporation rate) in the range of 6 to 8 for all the samples, except in the perturbed area where it is higher (15 to 35). These high values of molar ratio are linked to the introduction into the river of large sized bacteria ($\geq 1$ μm) with higher incorporation rates per cell or biomass unit than the small autochthonous bacteria ($<1$ μm). Growth rates of large bacteria were on average 3.7 times higher than those of small bacteria. Bacterial production was calculated with experimentally determined conversion factors ($0.5 \times 10^{18}$ cells per mole of thymidine incorporated and 900 gC per mole of leucine incorporated) and by taking into account the activity of both size classes of bacteria measured through fractionation experiments (post-incubation filtration). Production estimated in the perturbed area downstream of Achères was very high, up to 60 μgC liter$^{-1}$h$^{-1}$ in the summer. Carbon consumption by bacteria in the area perturbed by the Achères effluents was calculated assuming a growth yield of 0.2 and compared to the load of biodegradable organic matter discharged by the treatment plant. In summer, an additional supply of organic matter is required to account for the intense bacterial activity, suggesting the importance of phytoplankton production in the carbon budget.

## Introduction

In aquatic environments, the utilization of organic matter by planktonic bacteria is a major process governing the functioning of these ecosystems, especially when they receive significant quantities of allochthonous organic matter. Degradation of or-

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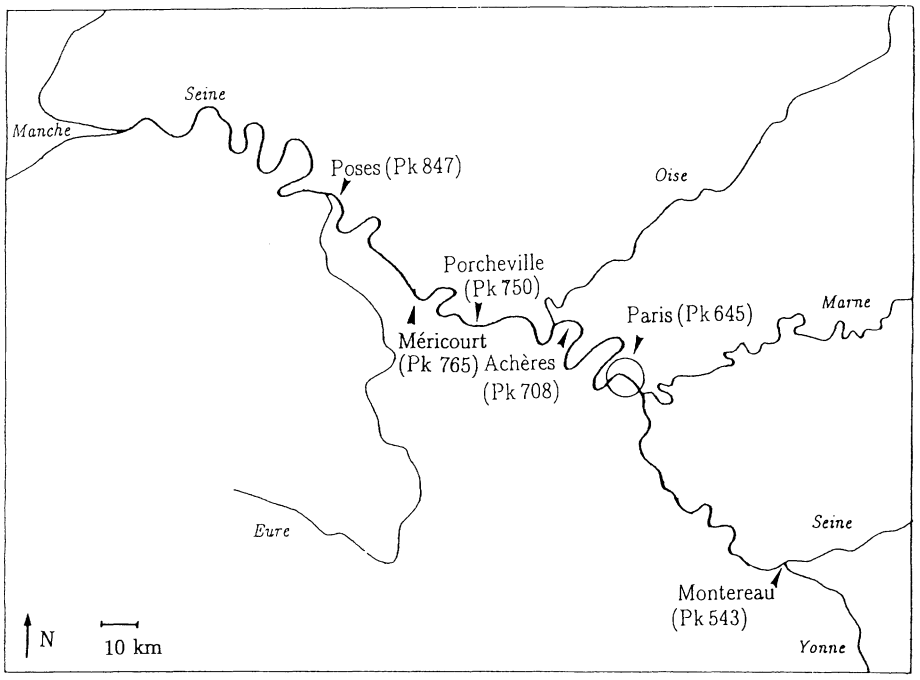


**Fig. 1.** Sampling sites in the studied zone in the river Seine.

ganic matter contributes to the purification of ecosystems and is therefore a major process controlling water quality.

The river Seine, downstream of Paris, is an example of an aquatic environment considerably altered by large inputs of organic matter. At 60 km downstream of Paris, the river is strongly perturbed by the effluent of the wastewater treatment plant at Achères. Around 80% of the wastewater produced by the ten million inhabitants of Paris and its suburbs reaches this plant; 70% is treated in the plant by physico–chemical and biological processes before being discharged into the river; the remaining 30% is discharged without any treatment [13].

As a part of a multidisciplinary study of the river Seine, the dynamics of the heterotrophic bacterial community was studied on a 300 km stretch between Montereau, located at the confluence of the river Yonne, and Poses, 150 km from the mouth of the Seine estuary (Fig. 1). A spectacular increase of bacterial abundance and biomass has been shown in the area downstream of Paris and especially at the effluent outfall from the Achères treatment plant [13]. This increase is due mainly to the introduction into the river of a significant proportion of large sized bacteria ($\geq 1$ μm in the largest dimension) which differs in size from the small autochthonous bacteria ($<1$ μm in the largest dimension) present upstream. These large bacteria have higher incorporation rates per cell or biomass unit but rapidly disappear [14].

In order to quantify bacterial carbon flux in the river Seine, bacterial production was measured along longitudinal profiles. During the 15 last years, various methods have been proposed for measuring bacterial heterotrophic production in aquatic

ecosystems; these are based principally on the use of radioactive tracers [1]. The tritiated thymidine incorporation method [10, 11] is now widely used to estimate bacterial production in water [9]. More recently, Kirchman et al. [16] suggested the use of tritiated leucine incorporation to evaluate protein synthesis rate as an estimate of bacterial production. Theoretically, the two methods differ by the fact that thymidine incorporation into DNA is related to an increase in cell numbers while leucine incorporation into proteins measures an increase of bacterial biomass. Several authors have applied the two methods in parallel; they usually found good concordance between the methods in marine ecosystems [7, 8, 15, 33] and in lakes [24]. Up to now, few studies have been done in rivers; therefore, thymidine and leucine incorporation have never been applied simultaneously to estimate bacterial production in rivers. Fractionation experiments were used to estimate separately the activity of large and small sized bacteria. On the basis of these experiments, bacterial production was calculated using experimentally determined conversion factors.

This paper analyzes the contribution of heterotrophic bacterial activity to the carbon budget of the river Seine by comparing bacterial production in the river to the input of biodegradable organic carbon from the Achères treatment plant.

## Methods

### *Sampling Program*

At five different periods of the year (October 1989, April, June and September 1990, March 1991), the river was studied at 5 to 16 stations between Montereau and Poses. The stations are named by a kilometric unit, Pk, used by the Water Agency of the "Seine Normandie" basin. Pk is equal to 1000 at Honfleur, the mouth of the estuary, and then decreases upwards. Poses is located at Pk 846, the Achères treatment plant at Pk 708, and Montereau at Pk 546.

In order to collect samples in the same water mass during its transfer down the river, the sampling times were planned according to the residence time of the water mass between the different stations. At each station, when the river was shown to be transversely homogeneous [6], 10 liters of river water were pumped, through PVC-tubing, from the middle of the river at two depths (0.5 m and 5 m) and mixed in a bucket. The tubing was previously rinsed at each depth for 5 min with circulating river water. To ensure the representativeness of the samples at the two stations below the outfall of the Achères treatment plant, where the river was shown to be heterogenous [6], the samples were pumped from two depths (0.5 m and 5 m) at 3 points of the transverse section (10 m from each shore and in the middle) and mixed.

### *Bacterial Numbers and Biomass*

Bacterial numbers were determined by epifluorescence microscopy at 1000× magnification after DAPI staining, following the procedure of Porter and Feig [23]. The bacteria were counted and classified into 24 size classes (four shapes and six lengths) by comparison to an eye piece graticule; biovolume in each class was calculated.

Biomass was estimated from abundance and biovolume distribution using the biovolume dependent conversion factor proposed by Simon and Azam [33], which ranges from $4 \times 10^{-13}$ gC $\mu m^{-3}$ for smaller bacteria (0.026 $\mu m^3$) to $1.3 \times 10^{-13}$ gC $\mu m^{-3}$ for larger ones (>0.4 $\mu m^3$).

As a bimodal size distribution of bacteria has been observed in the river Seine [13], abundance and biomass have been calculated for two size classes, namely small bacteria (greatest dimension less than 1 $\mu m$) and large bacteria (1 $\mu m$ and more).

## *Incorporation of $^3$H-Leucine*

Incorporation of $^3$H-leucine (Amersham, 120–140 Ci mmole$^{-1}$) was measured in each river sample at four concentrations of leucine which ranged from 2 to 77 nM (2 nM $^3$H-leucine in each case with 0–75 nM nonradioactive leucine) [28]. Four 5-ml subsamples were incubated 20 ± 3 min in the dark at *in situ* temperature. Incubation was stopped by addition of cold (0°C) trichloroacetic acid (TCA) (5% final concentration). The samples were heated at 85°C for 30 min, then cooled and filtered through a 0.2 μm pore size cellulose acetate membrane (Sartorius) [16]. Radioactivity associated with the filters was measured with a Packard Tri-Carb scintillation counter. The reciprocal of the fraction of $^3$H-leucine incorporated per hour at each leucine concentration was plotted against leucine concentration (radioactive and not radioactive) and the linear regression line calculated ($P$ was always $<0.05$). The incorporation rate was estimated as the reciprocal of the slope of this regression line.

## *Incorporation of $^3$H-Thymidine*

Incorporation of $^3$H-thymidine (Amersham 40–50 Ci mmol$^{-1}$) was measured at 20 nM. A 10-ml sample was incubated in the presence of thymidine for 30 ± 3 min. After incubation, cold TCA was added (5% final concentration), and samples were filtered through a 0.2 μm pore size cellulose acetate membrane.

Six experiments were performed at thymidine concentrations which ranged from 20 to 100 nM. In these experiments, the maximum incorporation rates were calculated as explained for leucine in the previous section. The saturating concentration was found to be greater than 20 nM in the river Seine samples; on average, incorporation rates at 20 nM represented 0.48 ± 0.05 ($P < 0.05$) of the incorporation rates at the saturating concentration. Hence, incorporation rates at the saturating concentration were calculated from our measurements at 20 nM on the basis of this average ratio. All the data on thymidine incorporation mentioned in this paper are therefore incorporation rates at the saturating concentration.

## *Bacterial Production Estimations*

Converting leucine and thymidine incorporation into bacterial production requires conversion factors. These have been experimentally determined according to the procedure of Riemann et al. [25]. In a culture of bacteria from the river Seine, the increase in cell numbers and biomass was followed over 15 to 20 hours in parallel with thymidine and leucine incorporation. Fig. 2 presents an example of calibration for both types of incorporation. The conversion factor for thymidine incorporation, equal to the reciprocal of the slope of the regression line, is expressed in terms of cells produced and measures an increase in bacterial abundance. The factor for leucine incorporation is expressed in terms of biomass produced.

Several determinations have been performed, and the average conversion factors were $0.5 \pm 0.1 \times 10^{18}$ ($P < 0.05$, $n = 12$) cells per mole of thymidine incorporated and 900 ± 200 ($P < 0.05$, $n = 4$) gC per mole of leucine incorporated. This thymidine conversion factor is in the lower range of the experimental values found in the literature [2, 21] but is consistent with theoretical considerations [2, 24]. For leucine incorporation, information on empirical conversion factors into bacterial production is very limited because, in most studies, incorporation rates are converted into cell production rather than into biomass production. The few published experimental conversion factors in biomass production [5, 17] are higher than our value; this may be due to a significant isotopic dilution, as those authors worked with a low leucine concentration (≤10nM), whereas, in this study, leucine incorporation was measured at saturating concentration.

Bacterial production (BPL) expressed in gC liter$^{-1}$h$^{-1}$ was directly calculated from leucine incorporation by multiplying the incorporation rate by 900. Bacterial production (BPT) estimated from thymidine incorporation is usually calculated with the following relationship:

$$\text{BPT (gC liter}^{-1}\text{h}^{-1}) = \text{Thy inc rate (mole liter}^{-1}\text{h}^{-1}) \times 0.5 \times 10^{18} \times \text{C (gC cell}^{-1})$$

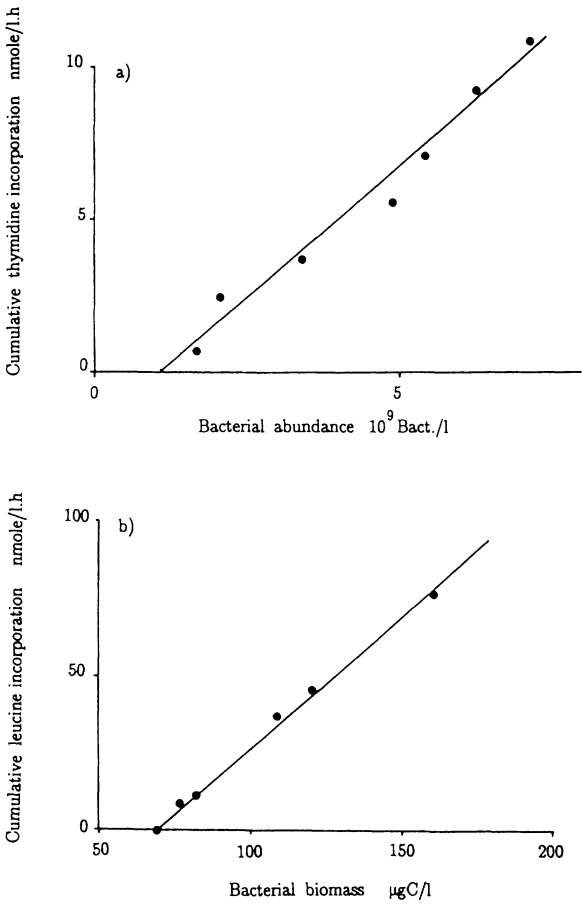


**Fig. 2.** Example of experimental determination of the conversion factor for thymidine incorporation (a) and for leucine incorporation (b) in a batch culture of bacteria from the river Seine.

where C is the average carbon content per cell for the whole bacterial population of the environment. In the river Seine, the situation is more complex because of the presence of two bacterial populations of different size and specific activity [13, 14]. Bacterial production was thus calculated as the sum of the production calculated for each population:

$$\text{BPT (gC liter}^{-1}\text{h}^{-1}) = \text{Thy inc rate } (<1\ \mu\text{m}) \times 0.5 \times 10^{18} \times \text{C } (<1\ \mu\text{m}) + \text{Thy inc rate } (\geq 1\ \mu\text{m}) \times 0.5 \times 10^{18} \times \text{C } (\geq 1\ \mu\text{m})$$

## *Post-filtration vs. Pre-filtration*

In order to estimate incorporation rates in two size classes, post-filtration was deliberately used instead of pre-filtration procedure, as experiments have shown that pre-filtration considerably reduce the activity with respect to the data obtained with post-filtration; the reduction averaged 70% (Table 1). Another experiment showed that the reduction was not due to a modification of the biological environment (e.g., elimination of particulate organic matter or reduction of indirect grazing effects) but to a mechanical effect of filtration. Indeed, leucine incorporation of a prefiltered (1 µm) and enriched (yeast extract) sample measured over time was reduced when, in the course of the experiment, the

**Table 1.** Percentage of incorporation (%) in the prefiltered fraction of <1 μm bacteria (Pre) with respect to the postfiltered fraction of <1 μm bacteria (Post) for uptake of amino acid uptake and leucine incorporation in different river waters

| | Pre/Post (%) | |
|---|---|---|
| Rivers | Amino acid uptake | Leucine incorporation |
| Seine | 22 | 12 |
| | 33 | ND |
| | 32 | ND |
| | 65 | ND |
| Eau d'Heure | ND[a] | 8 |
| Sambre | ND | 38 |

[a]Not determined

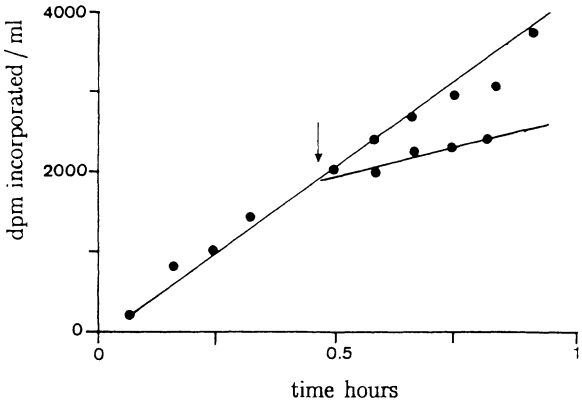


**Fig. 3.** Incorporation of leucine as a function of time in a <1 μm filtered and enriched river water sample. After 30 min, half of the sample was filtered again through a 2 μm pore size membrane, and the incorporation of leucine was then measured in parallel in both subsamples. Lower plot represents filtered sample after 0.5 h.

sample was filtered again through a membrane of higher porosity (2 μm), which, however, did not cause any reduction of biomass (Fig. 3).

## *Size Fractionation Experiments*

In September 1990 and March 1991, bacterial production was measured in both cell size classes (<1 μm and ≥1 μm). The volume of river water incubated in the presence of radioactive tracers was doubled. After incubation (post-filtration procedure), the sample was fractionated into two size classes by filtration of half the sample through a 1 μm pore size membrane (Nucleopore), both subsamples were then filtered through a 0.2 μm pore size membrane (cellulose acetate) after TCA addition (5% final concentration). Incorporation rates were thus determined in the total and less than 1 μm fractions, the activity in the fraction ≥1 μm being obtained by difference.

## *Determination of Biodegradable Dissolved Organic Carbon (BDOC)*

The biodegradable fraction of dissolved organic carbon (DOC) was determined using a bioassay procedure [32]. It consists of following the DOC decrease in the water sample, sterilized and reinocu-

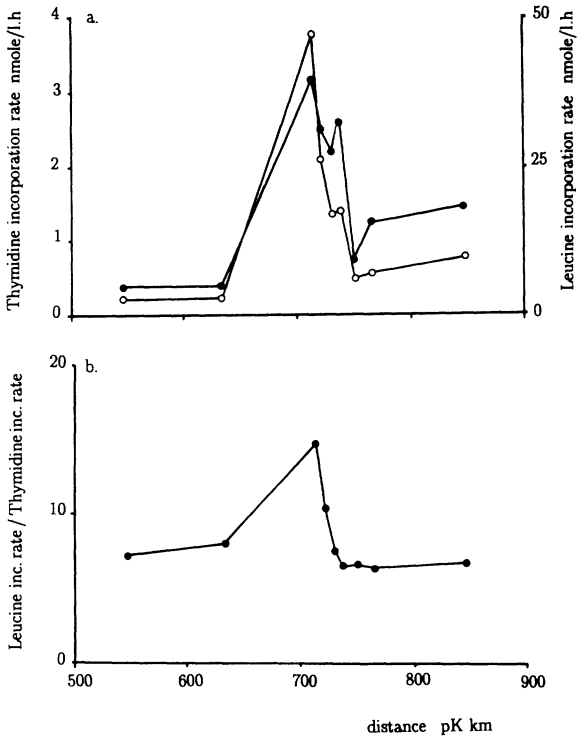


**Fig. 4.** Longitudinal fluctuations along the river Seine in September 1990 of (a) $^3$H-thymidine incorporation rate, ●, and $^3$H-leucine incorporation rate, ○; (b) the molar ratio (leucine incorporation rate/thymidine incorporation rate).

lated with bacteria, during a 30 day incubation. BDOC is calculated as the difference between DOC at the beginning and at the end of the incubation.

## Results

### *Leucine vs. Thymidine Incorporations Rates*

As already shown by a preliminary study [31], thymidine incorporation rates along the course of the river are low upstream of Paris; there is a large increase in the area surrounding Paris, with maximal values in the range of 1 to 3 nmol liter$^{-1}$ h$^{-1}$ just downstream of the Achères sewage treatment plant; a decrease towards values found upstream is then observed. An example of such a profile is presented in Fig. 4 for September 1990. Leucine incorporation rates present the same general pattern (Fig. 4a). The covariation of both activities is illustrated by the constancy of the molar ratio (leucine incorporation rate/thymidine incorporation rate); it is in the range of 6 to 8 for all the samples except in the perturbed area, where it is higher (Fig. 4b).

In the perturbed area downstream of Achères, the increase of leucine incorporation is larger than that of thymidine incorporation. The molar ratio is higher in this area; the maximum values (15 in September 1990 (Fig. 4b) and 35 in March 1991 were recorded just downstream of the effluent outfall.

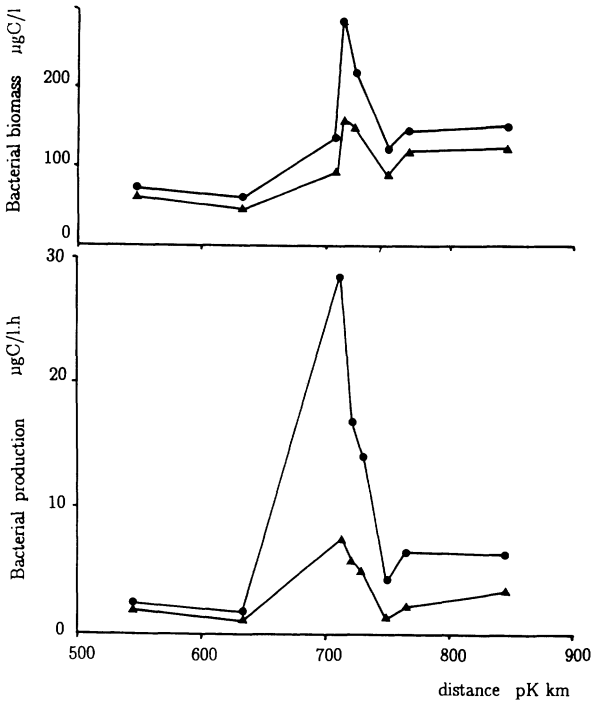


**Fig. 5.** Longitudinal fluctuations of bacterial biomass and production (calculated from thymidine incorporation rates) of the whole bacterial population, ●; and of the <1 µm bacteria, ▲.

*Bacterial Production*

The fractionation experiments carried out in September 1990 and March 1991 show the distribution of bacterial activity in both cell size classes. Fig. 5 presents typical profiles of bacterial biomass and activity for the whole bacterial population and for the <1 µm bacteria. Upstream of Paris, small bacteria are the most important componant in biomass and activity. Just downstream of Achères the increase in biomass is relatively smaller than that for production; the biomass of small bacteria represents roughly half the total, while their activity represents about a quarter of total activity.

For two longitudinal profiles (September 1990 and March 1991), when thymidine incorporation was measured in the two size classes, bacterial production calculated from the thymidine incorporation rate agreed well ($r = 0.87$, $n = 20$) with bacterial production calculated from leucine incorporation (Fig. 6).

The measurements of biomass and production provide the opportunity to compare the growth rates of small and large bacteria. In Fig. 7, growth rates calculated from thymidine incorporation (cellular production/cell number) of large bacteria have been plotted against those of small bacteria for all stations sampled in September 1990 and March 1991. A significant correlation is observed ($r = 0.85$, $n = 18$). The regression line indicates an average ratio of growth rates (large/small) of 3.7, in good agreement with the results on incorporation rates per cell or biomass unit presented by Garnier et al. [14].

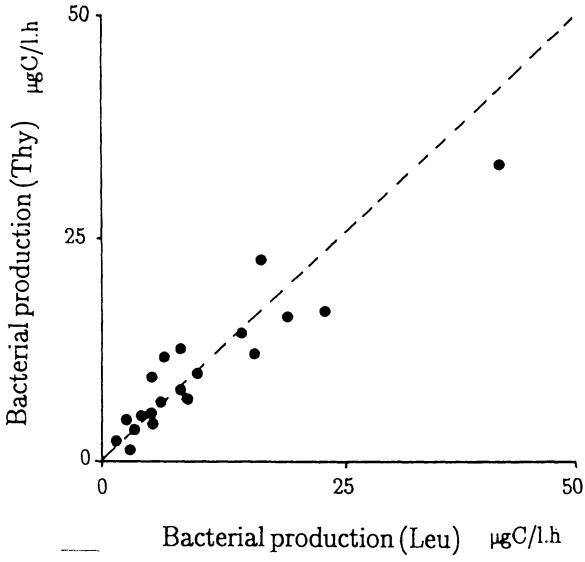


**Fig. 6.** Relationship between the values of bacterial production estimated from thymidine incorporation rate and from leucine incorporation rate.

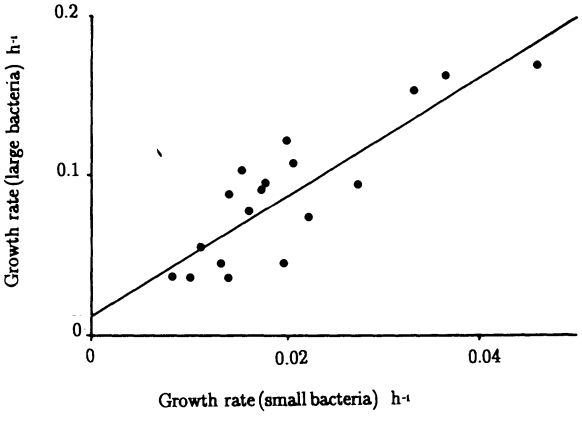


**Fig. 7.** Growth rates (calculated from thymidine incorporation rates and bacterial abundance) of large bacteria plotted against growth rates of small bacteria.

Five transects of the river were carried out between October 1989 and March 1991 in various conditions of discharge and temperature. The biomass and production results are presented in Fig. 8; production was calculated from leucine incorporation for each profile, and from thymidine incorporation when fractionation experiments were performed. Comparison of the different situations shows that the increase in production downstream of Achères is higher at low discharges and high temperatures. Consequently, at intermediate discharge (in March 1991), the increase is smaller but the production remains high over a longer distance, as the large bacteria are transported further by the rapid flow.

In all other situations, the impact of the effluents from the Achères treatment plant was already reduced at Porcheville (Pk 750) and was not detected downstream of Méricourt (Pk 765). The rapid disappearance of the large bacteria observed downstream of Achères has been interpreted as a consequence of more intense loss

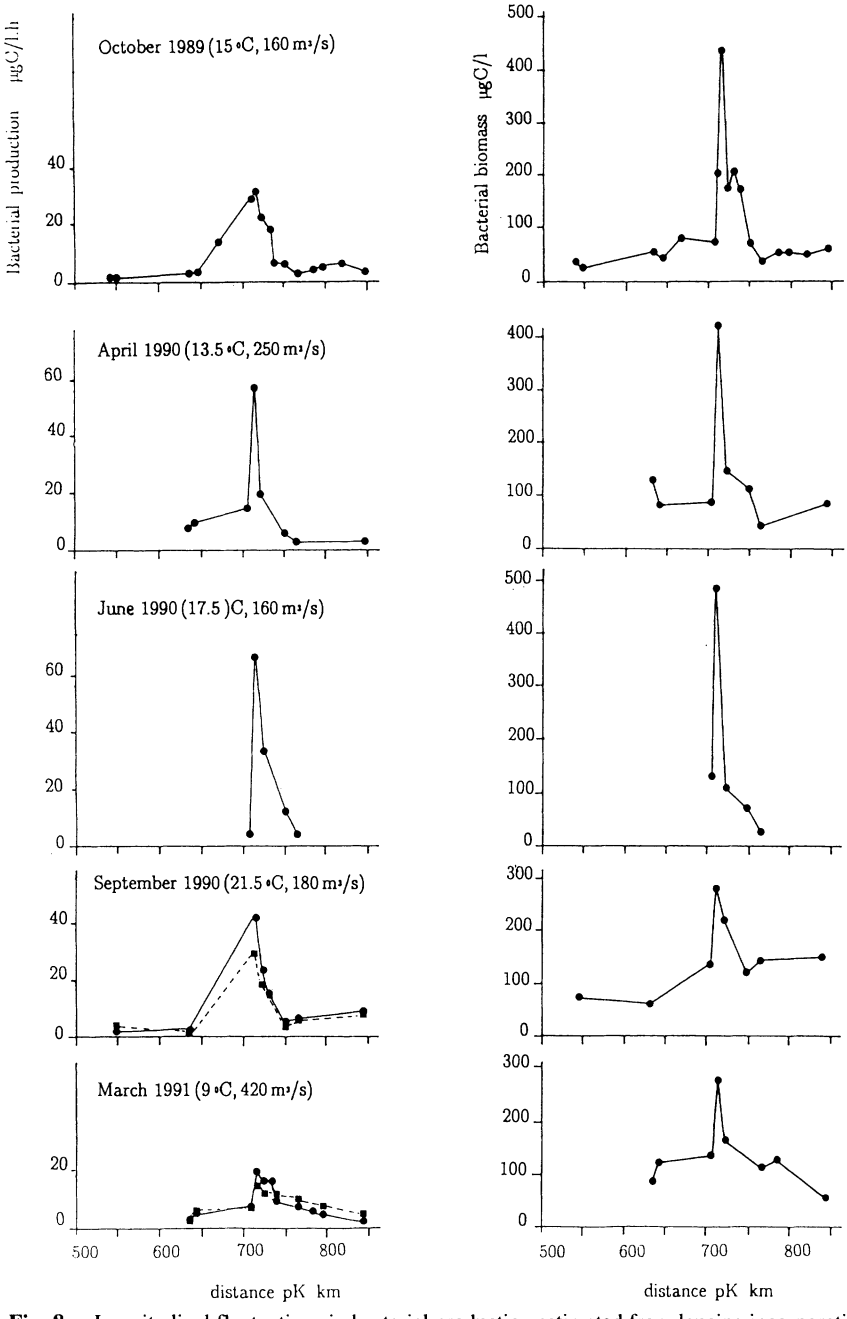


**Fig. 8.** Longitudinal fluctuations in bacterial production estimated from leucine incorporation rates, ●, and thymidine incorporation, ■, (left); and fluctuations in bacterial biomass (right) along the river Seine. Temperature and discharge of the river downstream of the confluence with the river Oise are specified in parentheses.

**Table 2.** Integrated bacterial production and total carbon uptake by bacteria in the area from Achères to Méricourt in the river Seine

| | | Bacterial production ($10^3$ kgC $h^{-1}$) | Total carbon uptake by bacteria[a] ($10^3$ kgC $h^{-1}$) |
|---|---|---|---|
| October | 1989 | 0.48 | 2.40 |
| April | 1990 | 0.61 | 3.05 |
| June | 1990 | 0.93 | 4.65 |
| September | 1990 | 0.61 | 3.05 |
| March | 1991 | 0.50 | 2.50 |

[a]Calculated considering a growth yield of 0.2

processes (e.g., sedimentation, grazing by protozoa and zooplankton) [13, 14], as compared with small bacteria.

### *Carbon Budget*

The profiles of bacterial production presented in Fig. 8 were integrated in order to estimate bacterial production in the area of the river Seine perturbed by the effluents of the Achères treatment plant (Achères-Méricourt). The integrated values were expressed in $10^3$ kg of carbon produced as bacterial biomass per hour and in the water volume contained in the considered stretch (Table 2). Values ranged between $0.5 \times 10^3$ and $1 \times 10^3$ kgC $h^{-1}$.

In order to calculate total bacterial carbon uptake from production estimates, it is necessary to know the growth yield, i.e., the fraction of bacterial carbon uptake that is used to produce bacterial biomass. Preliminary experiments performed with bacteria from the river Seine show a growth yield around 0.2 (Barillier and Garnier, unpub. data). This value has been used to calculate the total carbon which flows through bacteria in the study area (Table 2).

The organic carbon loaded into the river by the effluent of the Achères treatment plant was estimated for three sampling situations. These estimates are based on the increase of dissolved (DOC) and particulate organic carbon (POC) observed in the river just downstream of Achères, taking into account the respective discharges of the river upstream and downstream of Achères and the discharge of the effluents (Table 3). Only the biodegradable part of this organic carbon is available for bacteria. The biodegradable fraction of DOC was determined four times; it represents 50–61% of the DOC, with an average of 55%.

In the absence of experimental determination, the biodegradable fraction of POC was considered to be similar to that of DOC (55%). We can thus calculate the load of biodegradable organic carbon (dissolved + particulate) produced by the Achères effluents (Table 3). The comparison of the total carbon uptake by bacteria with the load of biodegradable organic matter from the Achères treatment plant shows that for March 1991 the total carbon uptake by bacteria matches very well with the organic load from the Achères treatment plant, but for the two other dates, the input of organic matter by Achères effluents alone cannot explain the total bacterial carbon uptake.

**Table 3.** Organic carbon loaded by the effluents of the Achères treatment plant

| | Total organic carbon $10^3$ kgC $h^{-1}$ | | | Biodegradable organic carbon $10^3$ kgC $h^{-1}$ | | |
|---|---|---|---|---|---|---|
| | DOC | POC | DOC + POC | DOC | POC | DOC + POC |
| Apirl 1990 | 1.16 | 1.36 | 2.52 | 0.64 | 0.75 | 1.39 |
| Sept. 1990 | 1.42 | 0.94 | 2.36 | 0.78 | 0.52 | 1.30 |
| March 1991 | 2.36 | 2.67 | 5.03 | 1.30 | 1.47 | 2.77 |

## Discussion

The average molar ratio (leucine to thymidine incorporation rates) of 6.6 for the sampling stations outside the perturbed area (stations between Pk 708 and Pk 750 have been excluded from this calculation) is quite close to average values mentioned by some authors working in various aquatic ecosystems: 8.8 in an oligotrophic Swedish lake, 9.1 in a Danish lake [24], from 6 to 7.1 in three French lakes [18] and from 7 to 9.5 in a wide variety of aquatic ecosystems [29]. The molar ratio showed an increase with increasing cell volume because protein is constant per unit of bacterial biomass whereas DNA is usually constant per cell [33]. The introduction of a large proportion of large sized bacteria ($\geq$ 1 μm) into the river by the Achères treatment plant effluents leading to an increase in average cell size of the bacterial population [13], could be responsible for the high ratios in the perturbed zone. Moreover, the high ratios are due, in part, to the high incorporation rate per cell or biomass unit of the large bacteria, which was, on average, three times higher than that of the small ones [14].

When bacterial assemblages are composed of large and small bacteria with different incorporation rates per cell, calculation of bacterial production values from thymidine incorporation requires that we take into account the carbon content and the incorporation rates per cell of both size classes. Production calculated from thymidine incorporation is then in good agreement with that calculated from leucine incorporation; thymidine incorporation would otherwise lead to underestimates of production. From a methodological point of view, this study demonstrates that, in some cases, using $^3$H-thymidine and $^3$H-leucine incorporation in parallel and measuring bacterial activity in various size classes can help obtain a correct estimate of bacterial production.

Only a few sets of data on bacterial production in river ecosystems are available in the literature for comparison with our results from the river Seine. In the river Meuse, over an annual cycle, Servais [27] and Servais and Billen [30] found average bacterial production values of 2.3 and 3.9 μgC $liter^{-1}h^{-1}$, respectively, at two stations. These values are in the same range as those measured in the river Seine upstream of Paris and downstream of Méricourt.

Bacterial production estimates in the perturbed area downstream of Achères (up to 60 μgC $liter^{-1}h^{-1}$ in the summer period) are among the highest values quoted in the literature. In natural aquatic ecosystems studied to date, the maximum value has been 91 μgC $liter^{-1}$ $h^{-1}$ measured by Torreton et al. [34] in a eutrophic bay of a

tropical lagoon (Bietry Bay, Ivory Coast). Recently we estimated, by the $^3$H-leucine incorporation method, bacterial production as high as 300 μgC liter$^{-1}$ h$^{-1}$ in the river Senne (Belgium) downstream of Brussels, where this small river (average annual discharge of about 10 m$^3$ s$^{-1}$) receives the untreated effluent of the one million inhabitants of Brussels and its suburbs.

The growth rates for small (0.01 to 0.05 h$^{-1}$) and large (0.04 to 0.18 h$^{-1}$) bacteria in the river Seine, are in the range mentioned by Billen et al. [3, 4] for a wide variety of aquatic environments. For both populations, growth rates were high just downstream of Achères and must have been close to a maximum at *in situ* temperature, as the amino acid uptake rates were at their maximum for the two size classes [14].

There are several explanations for the discrepancy found in the bacterial carbon budget. First, the value of 0.2 considered in the calculations for the growth yield, may be a minimal value when considering the range of growth yield data (0.2 to 0.4) usually mentioned in the literature for natural aquatic bacteria [19, 20, 22, 26, 27]. However, estimates of growth yields are now under debate, and several recent studies have provided low values and evidence of seasonal fluctuations (M. Sondergaard, pers. com.). Secondly, more information about the carbon input into the river from Achères effluents, and, especially, estimates of the biodegradable fraction of particulate matter, is required. Finally, autochthonous carbon production has not been taken into account in the above calculation of the bacterial carbon budget. In spring and summer, phytoplankton growing in the upstream zones of the drainage network contributes to an important accumulation of biomass (up to 4 mgC liter$^{-1}$) downstream of the confluence of the river Seine with its two major tributaries, rivers Marne and Oise [12]. Phytoplankton decay, excretion, cell lysis, and sloppy feeding could therefore represent a significant additional source of carbon and sustain a large fraction of total carbon uptake by bacteria. Despite a large input of allochthonous organic matter, the impact of autochthonous production in spring and summer must not be neglected.

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