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# Bacterial Production in a Mesohumic Lake Estimated from [$^{14}$C]Leucine Incorporation Rate

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**Abstract.** Incorporation of [$^{14}$C]leucine into proteins of bacteria was studied in a temperate mesohumic lake. The maximum incorporation of [$^{14}$C]leucine was reached at a concentration of 30 nM determined in dilution cultures. Growth experiments were used to estimate factors for converting leucine incorporation to bacterial cell numbers or biomass. The initially high conversion factors calculated by the derivative method decreased to lower values after the bacteria started to grow. Average conversion factors were $7.09 \times 10^{16}$ cells mol$^{-1}$ and $7.71 \times 10^{15}$ μm$^3$ mol$^{-1}$, if the high initial values were excluded. Using the cumulative method, the average conversion factor was $5.38 \times 10^{15}$ μm$^{-3}$ mol$^{-1}$. The empirically measured factor converting bacterial biomass to carbon was 0.36 pg C μm$^{-3}$ or 33.1 fg C cell$^{-1}$. Bacterial production was highest during the growing season, ranging between 1.8 and 13.2 μg C liter$^{-1}$ day$^{-1}$, and lowest in winter, at 0.2–2.9 μg C liter$^{-1}$ day$^{-1}$. Bacterial production showed clear response to changes in the phytoplankton production, which indicates that photosynthetically produced dissolved compounds were used by bacteria. In the epilimnion bacterial production was, on average, 19–33% of primary production. Assuming <50% growth efficiency for bacteria, the allochthonous organic carbon could have also been an additional energy and carbon source for bacteria, especially in autumn and winter. In winter, a strong relationship was found between temperature and bacterial production. The measuring of [$^{14}$C]leucine incorporation proved to be a simple and useful method for estimating bacterial production in humic water. However, an appropriate amount of [$^{14}$C]leucine has to be used to ensure the maximum uptake of label and to minimize isotope dilution.

## Introduction

In the last two decades the development of method for estimating bacterial production has changed our view of the role of bacteria in the carbon cycle of planktonic ecosystems. Bacteria are recognized as important producers of particulate organic carbon which, through bacterial grazing, is transported to higher trophic levels [36]. Among the new methods for assessing bacterial production, radiolabeled

thymidine incorporation into DNA [11] and leucine incorporation into protein [20] are the most commonly used in both limnetic and marine environments. Some methodological problems still occur, especially in converting thymidine or leucine incorporation to equivalent bacterial biomass. The conversion factors have often been determined empirically in laboratory experiments by comparing the incorporation rate of tracer to bacterial cell production. In different studies the factor can vary by an order of magnitude, depending on the various environmental and experimental conditions [2, 16]. The advantage in the leucine method is that bacterial production can be calculated directly from protein synthesis, which can then be converted to carbon, assuming constant protein/dry weight and carbon/dry weight ratios for aquatic bacteria [35]. Several comparisons made recently have found good agreement between the two methods [8, 24, 31], and, in general, these methods offer a simple and so far the most reliable approach for estimating bacterial production in aquatic environments. However, the methods have been used mostly in marine environments and eutrophic freshwaters, but seldom in oligotrophic or mesotrophic lakes or in humic lakes, typical of the boreal zone.

In brown-colored, humic lakes the production rates of bacterioplankton are poorly investigated, although much recent research has been conducted on the quantitative significance of bacterioplankton and its role in the carbon cycle in poly- and mesohumic waters [14, 33, 39]. Heterotrophic bacteria can make up a large biomass and thus represent a carbon source for higher trophic levels comparable to primary production. In humic lakes, the large carbon pool consists mostly of allochthonous matter, and the production of autochthonous carbon is usually limited because of the weak penetration of light into the water. However, the high dissolved organic carbon (DOC) pool consists mostly of refractory humic substances, which are less available to bacteria [28]. Thus, DOC released from phytoplankton may be at least occasionally as important an energy source for bacteria in humic lakes as in clear water lakes.

The aim of this study was to estimate bacterioplankton production in a mesohumic lake using the [$^{14}$C]leucine method and to evaluate factors regulating the production at different times of the year. The conversion factor of leucine incorporation to biomass is empirically determined, since the special characteristics of humic lakes may have an impact upon the uptake kinetics of leucine. As any conversion factor from bacterial biomass to carbon has not been published from humic freshwaters, this factor was also empirically determined in a laboratory experiment.

## Methods

### *Sampling*

Water samples were taken in 1991–1992 from a bay of Lake Pääjärvi, an oligotrophic, mesohumic lake in southern Finland (maximum depth 87 m, area 13.4 km$^2$). The lake water is brown-colored (80–100 mg Pt liter$^{-1}$) due to the high concentration of dissolved organic matter. The depth of the euphotic zone is about 3.0–3.5 m, and in summer the lake is thermally stratified. All samples were taken with a tube-sampler (volume 2.0 liter), usually from three depths (0–1, 1–2, and 2–3 m), and in winter from two depths (0–1 and 1–2 m). Sampling was carried out every third day during periods of two weeks in late spring (25 May 1991–12 June 1991), in summer (16 July 1991 and 30 July 1991–14 August 1991),

in autumn (24 September 1991–9 October 1991), and once in November. In winter, samples were usually taken every third week between December 1991 and May 1992. Laboratory experiments and production measurements were performed immediately after sampling.

## *Incorporation of [$^{14}$C]Leucine*

Triplicate samples of 5 ml were incubated in the laboratory at simulated in situ temperature and light (approximately 500 $\mu$mol m$^{-2}$ s$^{-1}$) for 60 min, with 30 nM of L-[$^{14}$C]leucine (specific activity 342 mCi mmol$^{-1}$). In winter, samples were incubated for 120 min at 3.5 ± 0.5°C and at simulated light intensities. Glutardialdehyde-treated (approximately 4%) controls were run in parallel. In all experiments, combusted (450°C) 20-ml glass vials were used for incubations. Incubations were ended by cooling sample bottles in ice cold water for 1 min and then adding 0.5 ml of 50% trichloroacetic acid (TCA) to reach a final concentration of 5%. Samples were then cooled for an additional 15 min and filtered through 0.2-$\mu$m pore-size cellulose nitrate filters (Sartorius). The filters were rinsed twice with 1 ml of ice cold 5% TCA and once with distilled water and dissolved in 0.2 ml of ethylenglycol-monomethylether together with 9 ml scintillation liquid (OptiPhase 3). They were stored for 24 h at room temperature before counting. The total activity of added [$^{14}$C]-leucine in experiments was counted from a 0.5 ml subsample into which 0.5 ml absorption liquid (1:7 ethanolamin/ethanol), 6 ml distilled water, and 9 ml scintillation liquid were added. Radioactivity was counted with a Wallac Ultrobeta 1210 liquid scintillation counter. Quench correction was made by external standardization.

## *Cell Counts and Cell Volume*

Bacterial samples were preserved with 0.2-$\mu$m filtered glutardialdehyde at a final concentration of 2%. Subsamples for cell counting (1.0 ml) were filtered onto black 0.2 $\mu$m Nuclepore filters and stained with particle-free 10 nM acriflavine solution [6]. Cells were counted and sized with a Nikon Optiphot epifluorescence microscope (×1200 magnification). For each sample at least 20 fields were counted or fields were counted until the standard error of counted fields was <10%. Cell length and width were measured from 100 cells by comparison to the globes of a calibrated eyepiece graticule (Patterson Globe and Circle, GI, Eyepiece Graticules Ltd.).

## *Primary Production, Nutrients, and DOC*

Production of phytoplankton was estimated by the $^{14}$C method [34]. The methods and results will be published in detail by L. Arvola, P. Kankaala, and T. Tulonen (ice-free period), and T. Tulonen, P. Kankaala, A. Ojala, and L. Arvola (winter).

## *Maximum Incorporation of [$^{14}$C]Leucine*

For the determination of the maximum incorporation of [$^{14}$C]leucine, four dilution experiments were carried out using lake water from various times and depths. One experiment was performed in June 1991, with unfiltered lake water, immediately after sampling. In dilution experiments, lake water was filtered through a 1.0-$\mu$m pore-size Nuclepore filter and diluted 1:2 or 1:4 with filter-sterilized (0.1-$\mu$m Nuclepore cartridge) lake water into acid washed and preignited bottles (0.5 or 2.0 liter). These were incubated for 3–5 days at a simulated in situ temperature (except in April 1991, when incubated at 15 and 20°C) and light intensities. Subsamples were removed from the cultures on the third or fourth day when the bacterial growth was supposed to be in the exponential growth phase. Various amounts of [$^{14}$C]leucine (5–120 nM) were added to triplicate 5-ml subsamples, and the leucine incorporation rates were determined.

### *Conversion Factor Experiments*

The factor for converting the leucine incorporation rate to bacterial cell or biomass production was estimated with dilution experiments using the same experimental procedure explained above. Subsamples were removed from the cultures at various times to determine rates of [$^{14}$C]leucine incorporation and bacterial abundance and biovolume.

Conversion factors were calculated using two methods. In the cumulative method [5] the factor for conversion from leucine incorporation to biovolume production rate was estimated for each experiment as the slope of the regression between cumulative incorporated leucine and biovolume. In the derivative method [19] the following equation was used:

$$C\ (\text{cells or } \mu m^3\ mol^{-1}) = \frac{\mu N_t}{v_t} \quad (1)$$

where N = the bacterial cell number (cells $ml^{-1}$) or biovolume ($mm^3$ $liter^{-1}$) at time t ($h^{-1}$), v = the incorporation rate (pM $h^{-1}$) at time t, $\mu$ = growth rate. Growth rate was calculated from the following equation

$$\mu\ (h^{-1}) = \ln(N_2/N_1) \times 1/[t \times \ln(2)] \quad (2)$$

where subscripts denote values at two times and ln indicates natural logarithms.

For the factor converting bacterial biomass to carbon, a similar dilution experiment was used, but the samples were incubated for 4 days in 200-ml bottles. Sample bottles were removed daily to determine particulate organic carbon (POC) and bacterial biovolume. POC was determined from duplicate samples filtered onto precombusted (450°C, 4 h) 47 mm Whatman GF/F filters, which were stored in a desiccator. Five to eight subsamples were punched out from the filters, and POC was determined by high temperature combustion [32]. Filters moistened with 0.5 ml of sample water were used as blanks.

The factor for converting biovolume to carbon biomass was calculated by comparing the increase in POC ($\mu g$ $liter^{-1}$) with the increase in bacterial biovolume, Bv ($mm^3$ $liter^{-1}$), from the following equation:

$$C\ (pg\ C\ \mu m^{-3}) = \frac{\Delta POC}{\Delta Bv} \quad (3)$$

## Results

### *Maximum Incorporation of [$^{14}$C]Leucine*

In all experiments, additions of >30 nM gave maximum incorporation of [$^{14}$C]leucine (Fig. 1). This was especially clear in experiments in which the dilution procedure was used to obtain exponentially growing bacteria. In experiments where leucine was added directly to unfiltered lake water or, where the bacterial activity stayed lower in spite of the dilution, the incorporation of leucine increased again at concentrations >60 nM (Fig. 1B).

### *Conversion Factors*

In all conversion factor experiments, both cell numbers and incorporation of leucine increased at the same rate after a short lag phase (Table 1, Fig. 2A). Conversion factors calculated by the derivative method were high during the first day, but decreased as the bacteria started to grow (Fig. 3). The average conversion factor for

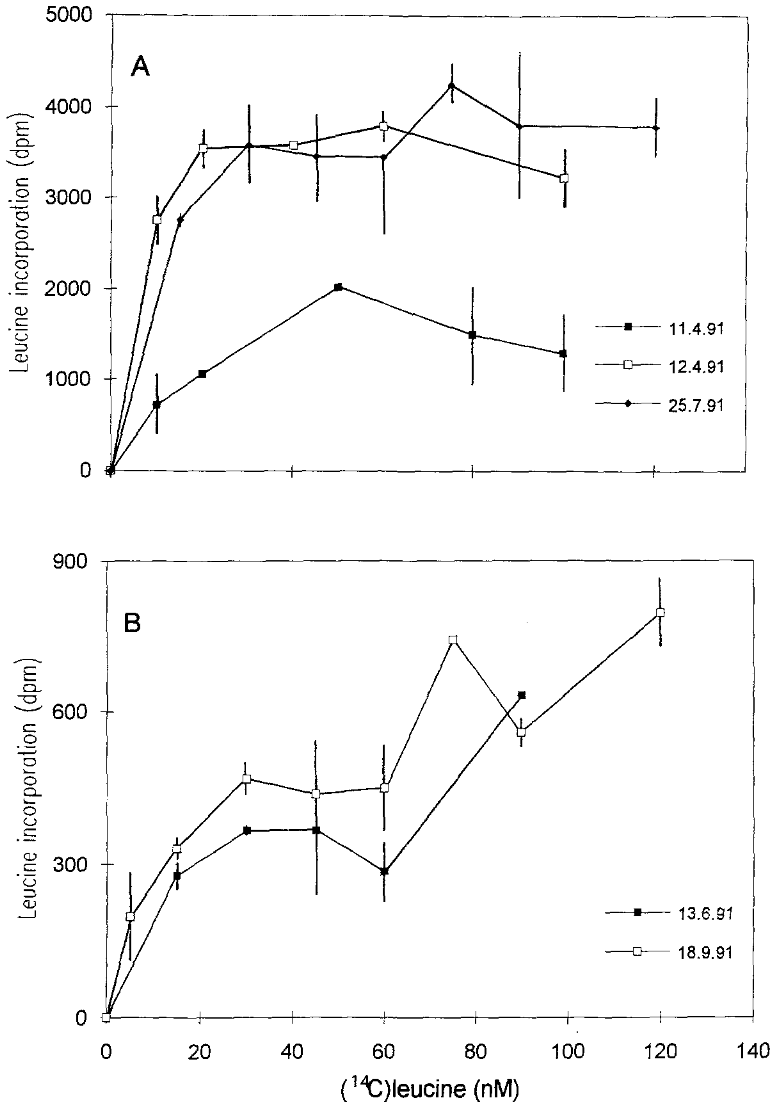


**Fig. 1.** [$^{14}$C]Leucine incorporation (mean ± SD) at different added [$^{14}$C]leucine concentrations. **(A)** Experiments with bacteria growing exponentially. In experiment 25 July 1991 correct units for leucine incorporation are tenfold. **(B)** Experiments with low bacterial activity.

leucine was $7.09 \times 10^{16}$ cells mol$^{-1}$ and $7.71 \times 10^{15}$ μm$^3$ mol$^{-1}$ if the high values of the first day were excluded (Table 2). Using the cumulative method, the conversion factor from leucine incorporation to biovolume was $5.38 \times 10^{15}$ μm$^3$ mol$^{-1}$. Some data points were excluded in the regression analyses if the biovolume or incorporation of leucine started to decrease. The biovolume of bacteria and POC also increased simultaneously during the incubation (Fig. 2B), but no clear lag phase was found. The average carbon to biovolume ratio was 0.361 pg C μm$^{-3}$

**Table 1.** Summary of data used to calculate the factor for converting leucine incorporation to bacterial cell and biomass production

| Date | Depth | Time ($h^{-1}$) | Cell number ($10^6$ cells $ml^{-1}$) | Cell volume ($\mu m^3$) | Biovolume ($mm^3$ $liter^{-1}$) | Leucine uptake (pM $h^{-1}$) |
|---|---|---|---|---|---|---|
| 16 April | 0–1 m | 0 | 1.38 | 0.045 | 0.062 | 25 |
| | | 26 | 1.66 | 0.107 | 0.178 | 90 |
| | | 46 | 2.60 | 0.092 | 0.239 | 816 |
| | | 68 | 3.86 | 0.103 | 0.398 | 1090 |
| | | 94 | 4.36 | 0.109 | 0.475 | 1307 |
| 4 June | 0–1 m | 0 | 0.83 | 0.020 | 0.017 | 8 |
| | | 24 | 0.90 | 0.024 | 0.021 | 10 |
| | | 48 | 1.27 | 0.035 | 0.044 | 230 |
| | | 72 | 1.64 | 0.078 | 0.128 | 743 |
| | | 120 | 2.05 | 0.063 | 0.129 | 977 |
| | 1–2 m | 0 | 0.65 | 0.018 | 0.012 | 6 |
| | | 24 | 0.85 | 0.019 | 0.016 | 13 |
| | | 48 | 1.01 | 0.024 | 0.024 | 59 |
| | | 72 | 1.43 | 0.029 | 0.042 | 200 |
| | | 120 | 2.20 | 0.041 | 0.090 | 393 |
| | 2–3 m | 0 | 0.73 | 0.021 | 0.015 | 30 |
| | | 24 | 0.86 | 0.027 | 0.023 | 33 |
| | | 48 | 1.26 | 0.043 | 0.054 | 189 |
| | | 72 | 1.66 | 0.046 | 0.076 | 406 |
| | | 120 | 2.12 | 0.076 | 0.161 | 415 |
| 22 July | 0–1 m | 0 | 0.70 | 0.017 | 0.012 | 185 |
| | | 24 | 0.92 | 0.022 | 0.020 | 379 |
| | | 48 | 2.83 | 0.095 | 0.278 | 8689 |
| | | 72 | 2.83 | 0.105 | 0.296 | 7353 |
| 16 September | 0–1 m | 0 | 0.92 | 0.017 | 0.016 | 25 |
| | | 24 | 1.00 | 0.016 | 0.016 | 56 |
| | | 48 | 1.26 | 0.031 | 0.039 | 170 |
| | | 72 | 1.93 | 0.073 | 0.141 | 1893 |
| | | 96 | 2.64 | 0.096 | 0.251 | 2249 |

(SD = 0.097), and the average carbon content of bacterial cells was 33.1 fg C $cell^{-1}$ (SD = 6.3) (Table 3).

### *Bacterial Production*

The production rates of bacterioplankton in lake Pääjärvi were calculated from [$^{14}C$]leucine incorporation using a conversion factor of $7.71 \times 10^{15}$ $\mu m^3$ $mol^{-1}$. This factor was supposed to be the most reliable as it was calculated by using only actively growing bacteria (see Discussion). Bacterial production was highest during the spring and summer experiments (Fig. 4A), on average 8.0 and 8.7 $\mu g$ C $liter^{-1}$ $day^{-1}$. The production rate varied greatly between the sampling dates (range 3.9–13.2 $\mu g$ C $liter^{-1}$ $day^{-1}$). The highest production always occurred after or simultaneously with the phytoplankton production peak. On average, bacterial production in spring was 25% and in summer 19% of the primary production of the

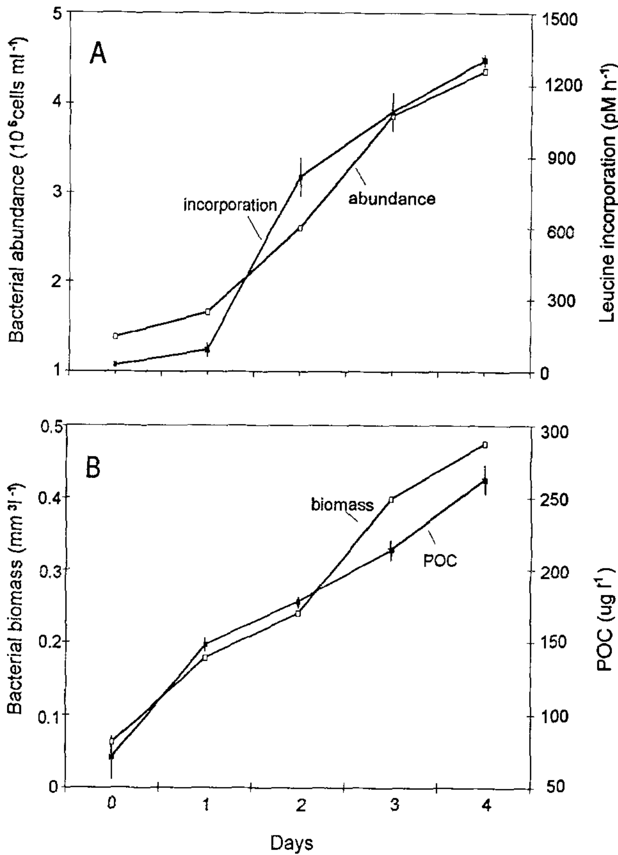


**Fig. 2.** Growth experiment in April 1991 to estimate conversion factor for leucine method. **(A)** Bacterial abundance and [$^{14}$C]leucine incorporation rate (mean ± SD). **(B)** Bacterial biomass and particulate organic carbon (POC). Vertical bars indicate ranges of duplicate POC determinations.

euphotic zone (0–3 m) (Table 4). Also, the average abundance and biomass of bacteria were at their highest levels in spring and summer (Fig. 4B).

In autumn, the bacterial production was on average 3.4 μg C liter$^{-1}$ day$^{-1}$, and both the density and biomass of bacteria clearly decreased during the experiment. The average bacterial production to biomass ratio was low (0.17) compared with that of the summer experiment (0.28). The average production of phytoplankton was only 10.5 mg C m$^3$ day$^{-1}$, and thus the ratio of bacterial production to phytoplankton production increased from the summer value of 0.19 to 0.32 in the autumn.

During winter and early spring, when the water temperature varied from 0.6 to 5.4°C, bacterial production was low, on average 1.3 μg C liter$^{-1}$ day$^{-1}$, and biomass and cell numbers were also at their lowest levels (Fig. 5). In winter, phytoplankton production was close to zero due mainly to the minimal light penetration through the snow-covered ice. In the epilimnion, primary production had already started to increase in April, but the most pronounced increase was measured

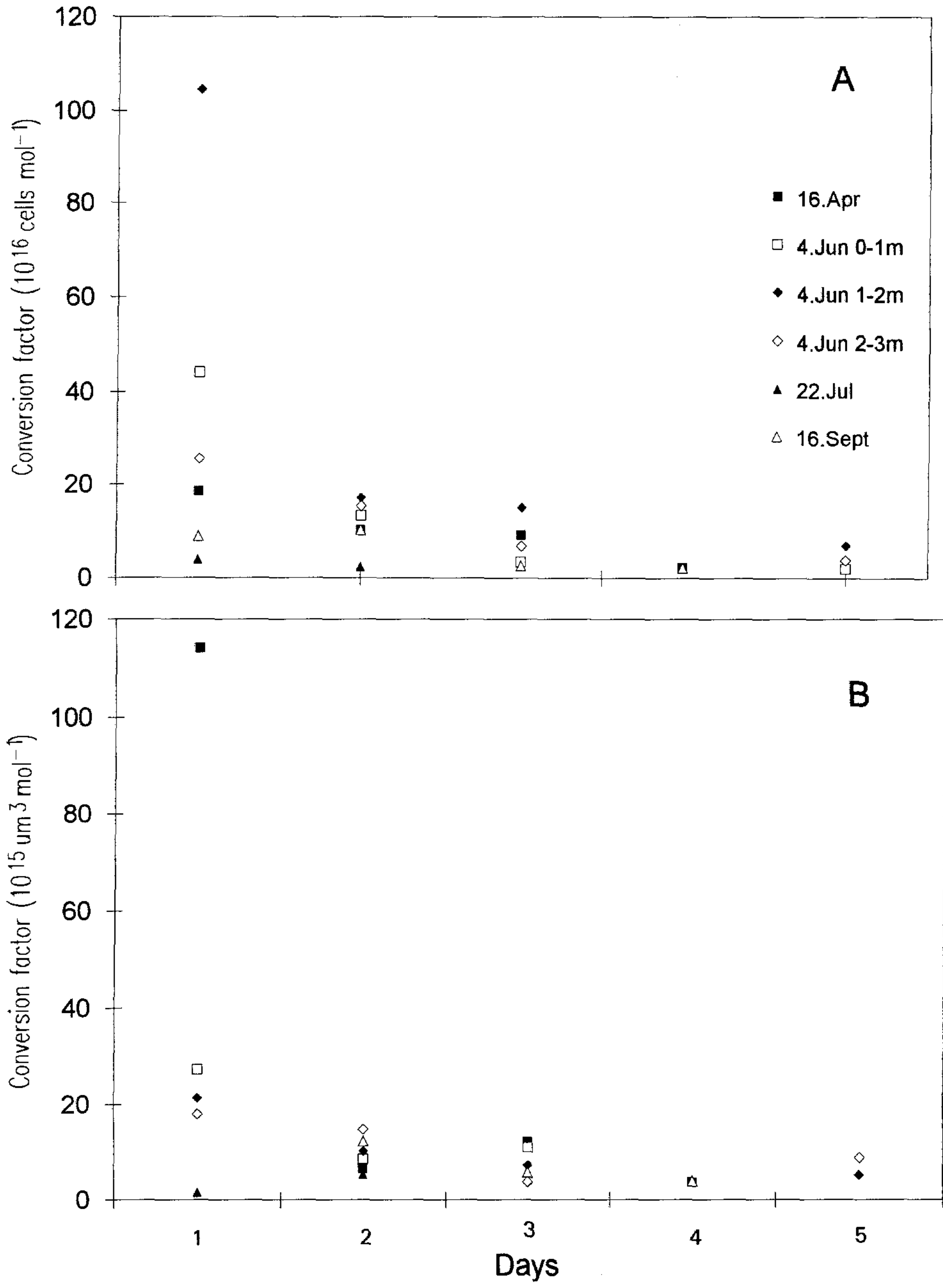


**Fig. 3.** Conversion factors from growth experiments as calculated by the derivative method. **(A)** $10^{16}$ cells $mol^{-1}$, **(B)** $10^{15}$ $\mu^{-3}$ $mol^{-1}$.

**Table 2.** Summary of conversion factor experiments in Lake Pääjävi

| Experiment | Depth ($m^{-1}$) | CF[a] ($10^{16}$ cells $mol^{-1}$) | CF[a] ($10^{15}$ $\mu m^3$ $mol^{-1}$) | CF[b] ($10^{15}$ $\mu m^3$ $mol^{-1}$) | (n) | ($r^2$) |
|---|---|---|---|---|---|---|
| 16 April | 0–1 | 7.25 | 7.38 | 7.14 | 5 | 91.2 |
| 4 June | 0–1 | 6.23 | 9.64 | 7.41 | 4 | 99.9 |
| | 1–2 | 13.03 | 7.44 | 6.80 | 4 | 97.9 |
| | 2–3 | 8.61 | 9.08 | 5.56 | 4 | 89.5 |
| 22 July | 0–1 | 2.37 | 5.42 | 2.33 | 3 | 99.9 |
| 16 September | 0–1 | 5.04 | 7.29 | 3.03 | 5 | 97.1 |
| Mean | | 7.09 | 7.71 | 5.38 | | |
| (SD) | | (3.60) | (1.50) | (2.20) | | |

[a]Conversion factor calculated by derivative method. Average values from each experiment, after the high initial values (<24 h) were excluded
[b]Conversion factors calculated by cumulative method; (n) is the number of data points and ($r^2$) the regression coefficients

**Table 3.** Increments of bacterial cell number, biovolume and carbon biomass and calculated carbon/cell (C/n) and carbon/biovolume (C/Bv) ratios in the growth experiment in April 1991

| Time $h^{-1}$ | Abundance (cells ($10^6$) $ml^{-1}$) | Biovolume ($mm^3$ $liter^{-1}$) | Carbon ($\mu g$ C $liter^{-1}$) | C/n ratio (fg C $cell^{-1}$) | C/Bv ratio (pg C $\mu m^3$) |
|---|---|---|---|---|---|
| 46 | 0.94 | 0.061 | 29.7 | 31.6 | 0.487 |
| 68 | 2.20 | 0.220 | 65.8 | 29.9 | 0.299 |
| 74 | 2.66 | 0.280 | 76.2 | 28.8 | 0.272 |
| 94 | 2.70 | 0.297 | 114.6 | 42.4 | 0.386 |
| Avg. | | | | 33.1 | 0.361 |
| SD | | | | 6.3 | 0.097 |

after the ice breakup at the beginning of May (Fig. 5). Bacterial production began to increase slightly earlier than the primary production, although in bacterial cell numbers and biomasses there was not such an obvious increase.

The standard regression analysis revealed that for all the data (log transformed), primary production predicted 52% ($r^2 = 0.52$, $n = 80$, $P < 0.001$) and water temperature 54% ($r^2 = 0.54$, $n = 80$, $P < 0.001$) of the bacterial production. The regression calculated on data collected at low temperatures (<7.0°C), resulted in an even stronger relationship ($r^2 = 0.77$, $n = 30$, $P < 0.001$) between water temperature and bacterial production (Fig. 6). No significant relationship between POC, DOC, or nutrient concentrations and bacterial production was found.

## Discussion

### *Conversion Factors*

The difficulties of the [$^3$H]- and [$^{14}$C]leucine methods are similar to that of the [$^3$H]thymidine method, i.e., the determination of empirical conversion factors. The great variability in conversion factors both for thymidine and leucine are most likely due to the differences in experimental procedures [2, 16]. In growth experi-

**Fig. 4.** Production of bacteria and phytoplankton (**A**) and abundance and biomass of bacteria (**B**) during the ice free period in 1991 at the surface water (0–3 m) of Lake Pääjärvi. (Primary production data from Arvola et al., unpublished).

ments, problems may also arise from isotope dilution due to the exogenous substrate pool or the de novo synthesis of leucine [9]. Thus, special care should be paid to using the appropriate amount of tracer to ascertain the maximum uptake by bacteria. Jørgensen [16] suggested that, especially in freshwaters with high concentrations of DOC, high amounts of leucine (as high as 100 nM of [$^3$H]leucine) must be added to ensure the maximal incorporation. If the amount of added leucine is not appropriate, the conversion factor will be too high. However, Heinänen and Kuparinen [12] have concluded that the conversion factor values were not affected by additions of nutrients and carbon (sucrose) into cultures of brackish water

**Table 4.** The average values for investigated parameters in different seasons at the surface water of Lake Pääjävi. Bn, bacterial abundance ($10^6$ cells $ml^{-1}$); BB, bacterial biomass (μg C $liter^{-1}$); BP, bacterial production (mg C $m^3$ $day^{-1}$); and PP, phytoplankton production (mg C $m^3$ $day^{-1}$)

| Experiment period | Bn | BB | BP | BP/BB ratio | PP | BP/PP ratio | Temp. °C |
|---|---|---|---|---|---|---|---|
| Spring | 2.94 | 30.1 | 8.0 | 0.27 | 32.2 | 0.25 | 9.2 |
| Summer | 3.68 | 30.7 | 8.7 | 0.28 | 46.4 | 0.19 | 19.7 |
| Autumn | 2.11 | 20.1 | 3.4 | 0.17 | 10.5 | 0.32 | 9.3 |
| Winter | 1.41 | 11.5 | 1.3 | 0.11 | 3.9 | 0.33 | 1.8 |
| All | 2.54 | 23.1 | 5.3 | 0.23 | 23.3 | 0.24 | 10 |

bacteria, and thus their calculated average value could be applied to a variety of substrate conditions.

Problems may arise if part of the added leucine is taken up by organisms other than bacteria, or the bacterial growth is stimulated by the tracer. This might have been the case in my experiments in which the incorporation of leucine increased at a concentration >60 nM. The dissolved leucine concentration in lake water was measured occasionally, revealing concentrations between 4.1–10.1 nM (U. Münster, unpublished). This indicates that isotope dilution due to the ambient leucine concentration could not have been more than approximately 20% [9].

Using the derivative method, high conversion factors were measured during the first day of incubation. Kirchman and Hoch [19] noticed a similar pattern, and they suggested that isotope dilution was the most likely cause for high initial conversion factors at the time of lag phase in bacterial growth. This means that the conversion factors should be calculated using only exponentially growing bacteria, which is, however, difficult if the microbial activity is low. In this study, the bacterial growth model after the lag phase varied between experiments, being more often linear than exponential. The cumulative method may be more suitable for conditions in which bacterial growth is clearly linear [5], but in this method the lag phase in bacterial growth and the later decrease of production may change the slope of the regression.

The conversion factor calculated by the derivative method ($7.09 \times 10^{16}$ cells $mol^{-1}$), which was used in this study to estimate bacterial production in humic water, was quite similar to those derived from eutrophic lakes ($7.0 \times 10^{16}$ cells $mol^{-1}$ [31] and $6.4 \times 10^{16}$ cells $mol^{-1}$ [16]). More variable values have been derived from marine environments. Chin-Leo and Benner [7] found a factor of $4.46 \times 10^{16}$ cells $mol^{-1}$, which is clearly lower than in this study. A higher value was derived from marine subarctic waters ($10.8 \times 10^{16}$ cells $mol^{-1}$ [18]) but most consistent values were found from estuarine waters ($3.1–7.8 \times 10^{16}$ cells $mol^{-1}$ [19]). Although leucine is incorporated mainly into proteins and is therefore closely related to biomass production, the calculations have usually been based on the increase in bacterial cell numbers. Only in a few studies has the leucine incorporation rate been compared to the increments in bacterial biovolumes. Bjørnsen and Kuparinen [5] obtained a factor of $7.5 \times 10^{15}$ $\mu m^3$ $mol^{-1}$ for Antarctic marine bacteria, and Heinänen and Kuparinen [12] obtained a factor of $6.8 \times 10^{15}$ $\mu m^3$ $mol^{-1}$ for brackish bacteria, both of which were calculated using the cumulative method. These values were slightly higher than the value of $5.38 \times 10^{15}$ $\mu m^3$

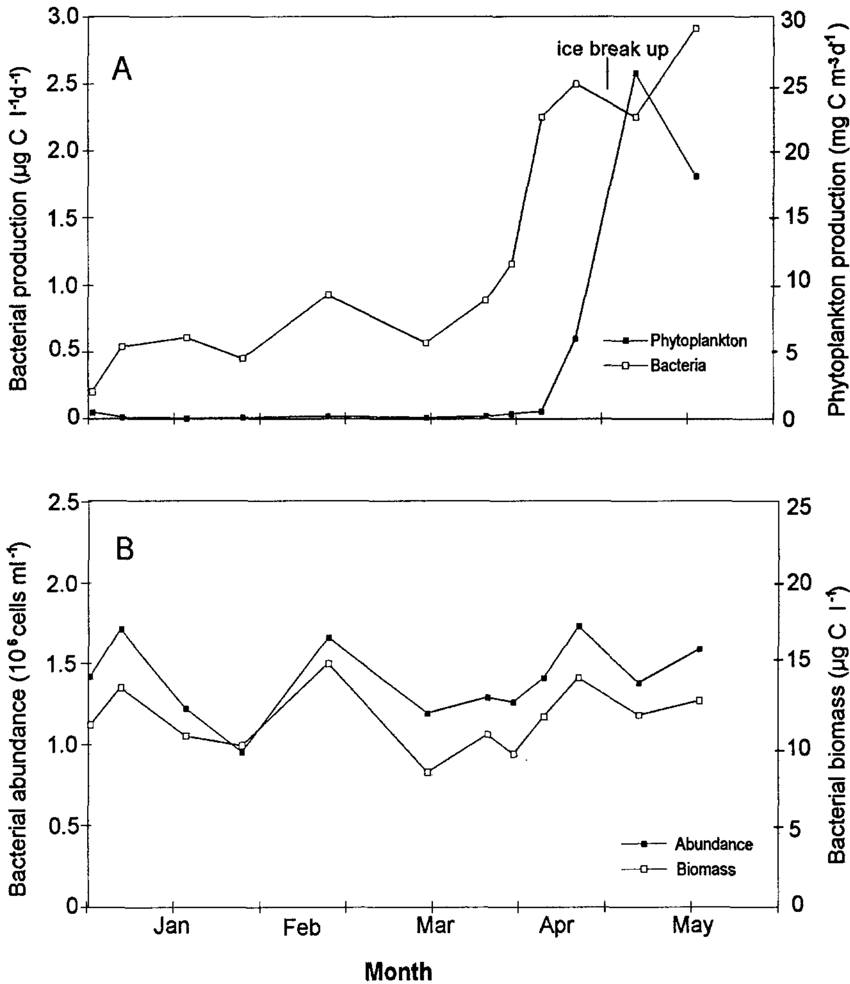


**Fig. 5.** Production of bacteria and phytoplankton (**A**) and abundance and biomass of bacteria (**B**) in winter and spring in 1992 in Lake Pääjärvi. (Primary production data from Tulonen et al., unpublished).

$mol^{-1}$ obtained in this study, but agreed well with the factor $7.71 \times 10^{15}$ $\mu m^3$ $mol^{-1}$ calculated using the derivative method.

The question is whether to use empirically determined conversion factors or calculate the production directly via protein synthesis. Simon and Azam [35] proposed that production can be calculated for aquatic bacteria assuming constant mole percent leucine in protein, protein/dry weight, and carbon/dry weight ratios. However, this can underestimate the actual production if an intracellular or extracellular isotope dilution occurs. Simon and Azam [35] suggested that a twofold dilution could be used in all environments.

The disadvantage of using empirical conversion factors is that the biomass has to be converted to carbon biomass. In this study, the carbon to biovolume conversion

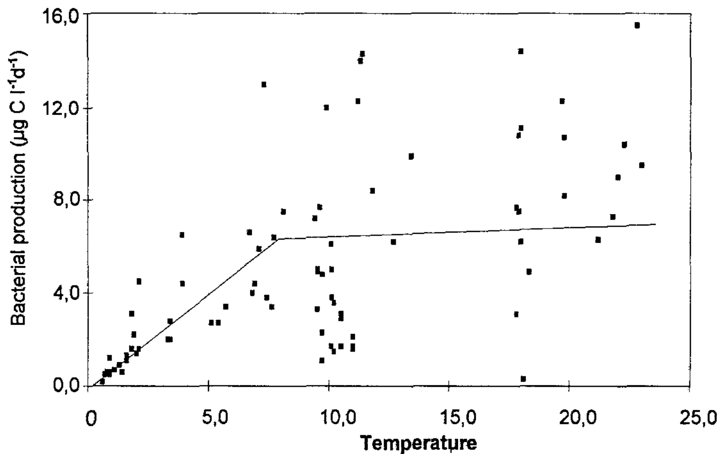


**Fig. 6.** The relationship between water temperature and bacterial production (data points from each sampling depth) in Lake Pääjärvi. Line drawn by eye.

factor (0.36 pg C $\mu m^3$) determined for humic lake water bacteria agreed well with the values obtained for marine (0.38 pg C $\mu m^3$ [26]) and for estuarine and freshwater (0.35 pg C $\mu m^3$ [4]) bacterioplankton. They are all about three times higher than the theoretically calculated and widely used conversion factor of 1.21 pg C $\mu m^3$ [41]. With the exception of the results of Nagata and Watanabe [29], and Kogure and Koike [21], all of whom have obtained low conversion values (0.14–0.21 pg C $\mu m^3$), the empirical calibrations of carbon-to-volume ratio seemed to raise the ratio. In many studies with high conversion factors, the average cell size of bacteria is relatively small ([23, 26], this study), which supports the suggestion that small cells have relatively more carbon and dry matter than large cells [26, 35].

### *Bacterial Production*

The spring and summer production values of bacterioplankton (3.9–13.2 $\mu g$ C liter$^{-1}$ day$^{-1}$) measured in the epilimnion of Lake Pääjärvi were higher than the values estimated from the same lake with the thymidine method (1.2–4.1 $\mu g$ C liter$^{-1}$ day$^{-1}$ [17]). However, if the same carbon-to-biomass factor is used (multiplying the value by three) the bacterial production rates are very similar to bacterial production in these two studies. In two Swedish humic lakes, Sundh and Bell [38] have also measured similar values with the thymidine method (5.5 and 18.8 $\mu g$ C liter$^{-1}$ day$^{-1}$). Much higher production values for bacterioplankton have been measured in a Norwegian humic lake (32 $\mu g$ C liter$^{-1}$ day$^{-1}$ [13]) and in mesocosm experiments performed with polyhumic water (40–70 $\mu g$ C liter$^{-1}$ day$^{-1}$ [33]), but in these studies the bacterial production was estimated using prefiltered lakewater and the increase in cell numbers and biomass during incubation. The bacterial production values of Lake Pääjärvi can be considered quite low compared with eutrophic lakes ([3] and references therein) or to studies in which large amounts of data from various environments have been collected together [10].

The importance of allochthonously produced and phytoplankton-derived organic matter to bacterial production can be estimated by evaluating the carbon demand of bacteria. Based on an assumption of 50% growth efficiency for bacteria [30] in spring and summer in the euphotic zone, the bacterial production could have been sustained by phytoplankton production. However, if the whole water column is considered, the ratio of bacterial production to primary production clearly becomes lower. In humic lakes the primary production is restricted to the uppermost few meters, but bacterial production can occur at all depths. Because the measurements obtained in 1992 at the sampling site of this study showed that the bacterial production was quite constant in the whole water column, 0–5 m (unpubl. results), the carbon demand of bacteria must be approximately twofold that in the uppermost 3 m water layer. Thus, if the growth efficiency of bacteria was less than 50%, as recently proposed in many studies [4, 22], bacterioplankton must have used allochthonous carbon as their energy source.

The positive correlations between bacterial and phytoplankton production are reported from both marine and freshwaters [10], although in environments receiving high inputs of organic matter, a poor correlation may exist and the bacteria/phytoplankton ratio is typically high [14, 33]. In this study, particularly in spring and summer, increases in bacterial production and primary production coincided. This indicates that the labile organic substrates released by phytoplankton might support bacterioplankton production in Pääjärvi. The extracellular release of dissolved organic matter (DOM) from phytoplankton is estimated to be approximately 25% of primary production [37]. Sundh and Bell [38] reported that the heterotrophic bacteria assimilated the labile photosynthetically produced DOM at high rates both in humic and clearwater lakes. Münster [28] has shown that the labile DOM substrates are rapidly oscillating in lake water due to their simultaneous release and utilization, whereas polymeric substrates vary over longer time intervals. In autumn and winter, when primary production was low, the amount of carbon needed to sustain bacterial production was clearly higher than was available from phytoplankton production. Thus, the bacterioplankton then used more allochthonous organic matter than in spring and summer. The bacterial production rates in autumn were, however, clearly lower than in summer, which indicates that the components of DOM in the lake water were utilized at lower rates.

Recent studies have emphasized that not only the amount of available DOC, but also the proper C:N:P ratio in the water might be a key element in regulating bacterial production [25]. Tulonen et al. [40] have shown that additions of nutrients can increase the availability of allochthonous DOM for bacteria. As phosphate phosphorus concentrations in Pääjärvi are very low ($<5.0$ μg $PO_4$-P $liter^{-1}$) and the N:P ratio very high, phosphorus is the limiting nutrient for phytoplankton production and probably also for bacteria during the most of the year [1]. In summer, protozoans and mesozooplankton can be important regenerators of inorganic nutrients in the pelagial zone [15], but in winter their role is less significant. The hydrolysis of dissolved organic phosphorus compounds and microbial enzyme activity may also enhance the availability of nutrients for bacteria [28]. On the other hand, humic substances can result in the formation of enzyme complexes and inhibition of enzyme activities, which in turn contributes to phosphorus limitation in humic lakes.

In winter, temperature can limit the use of organic matter and thus bacterial

production. Wikner and Hagström [43] reported that bacterial growth was dependent on water temperature, especially at less than 6°C, but other factors, such as grazing, regulated bacterioplankton populations at higher temperatures. Also in Pääjärvi, the high correlation between temperature and bacterial production was calculated at temperatures below 7.0°C. Although low water temperature can decrease bacterial growth rates, the grazers have recently been found to be important regulators of bacterial biomass and density in winter ([27], T. Tulonen unpublished). Wiebe et al. [42] have demonstrated that at low temperatures bacteria require more organic nutrients than at temperatures above 10°C. Thus, the increased primary production under the ice and formation of photosynthetically produced labile substrates could also have enhanced the bacterial production in April. The same phenomenon has been reported in the Baltic Sea [23], where the bacterial production showed an immediate response to development of phytoplankton under the ice cover.

In conclusion, bacterial production in the mesohumic Lake Pääjärvi was closely correlated to phytoplankton production, but bacterioplankton also needed allochthonous organic matter as an additional energy source, especially in autumn and winter. In winter, temperature can limit bacterial growth and the degradation rate of organic matter. The [$^{14}$C]leucine method proved to be a simple and useful method in evaluating bacterial production in humic lakes, but an appropriate amount of label is needed to ensure maximal uptake of leucine.

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