

Dynamics of heterotrophic bacteria attached to *Microcystis* spp. (Cyanobacteria)

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ABSTRACT: We studied the ecology of heterotrophic bacteria attached to the mucilaginous colonies of *Microcystis* spp. (Cyanobacteria) in the eutrophic lake Frederiksborg Slotssø, Denmark. The succession in bacterial abundance, production and potential aminopeptidase activity in 20 µm fractionated samples was followed during periods in which *Microcystis* dominated the phytoplankton. We operationally defined that nets of 20 µm mesh-width segregated bacteria associated with *Microcystis* (*Microcystis*-associated bacteria, MB; >20 µm size fraction) from the mainly free-living bacteria in the filtrate (FB, <20 µm size fraction). According to this definition, the contribution of MB during summer 1995 and autumn 1994, respectively, averaged 10 ± 4 (\pm standard deviation) and $37 \pm 12\%$ of total bacterial biomass and 25 ± 13 and $43 \pm 16\%$ of total bacterial production, as estimated from thymidine (TdR) incorporation. During summer, MB further contributed $55 \pm 18\%$ of total leucine incorporation measured at 600 nM leucine and $53 \pm 12\%$ of total potential aminopeptidase activity. Although 20 µm mesh-width nets also retained particles other than *Microcystis*, our results indicate that *Microcystis* was a 'hotspot' for bacterial activity, comparable to larger aggregates known as marine or lake snow. During summer, growth rate and specific aminopeptidase activity of MB generally exceeded those of FB, which points to diversified microenvironments or species compositions. In order to balance gain and loss rates within the community of MB, we hypothesize that a large fraction of MB produced were exported from *Microcystis* to the surrounding water, only modified by the loss due to viral lysis. This idea arose from reported low loss rates of *Microcystis* and continuous measures indicating that a surplus of more than 70% of MB production (TdR) was not reflected as biomass increases within the community of MB. According to this hypothesis *Microcystis* may be considered as a bacterial 'incubator' for the surrounding water.

KEY WORDS: Attached bacteria · *Microcystis* · Eutrophic lake · Microhabitat

INTRODUCTION

The cyanobacterium *Microcystis* is a common genus in eutrophic lakes and often dominates the phytoplankton during summer and autumn. *Microcystis* forms large (>100 µm) mucilaginous colonies usually colonized by (heterotrophic) bacteria (Whitton 1973). Bacteria associated with *Microcystis* are hypothesized to live in a microhabitat distinct from the surrounding water, because the microscale physicochemical environment is influenced by the particulate nature of *Microcystis* and the metabolism of the autotrophic and heterotrophic organisms present.

In general, large particles in aquatic systems are colonized by bacteria at densities exceeding the surrounding water on a per volume basis; however, attached bacteria usually contribute less than 10% of total bacterial abundance (Kirchman 1993). Particles are argued to be favourable microenvironments compared to the surrounding water, because availability of potential substrates to bacterial growth is facilitated by organic components of particles, adsorbed matter, and, possibly, an increased flux of dissolved nutrients during sinking (reviewed by Fletcher 1991, Kirchman 1993). Further, Herndl (1988) suggested that short-circuit nutrient cycles may form when autotrophic and heterotrophic organisms are closely associated in particulate matter (marine snow), thus increasing the

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regenerative power and, potentially, the overall microbial activity.

Microcystis is a distinct bacterial microhabitat compared to a detrital particle, because the attached bacteria are closely associated with the principal producer of organic carbon for bacterial growth. The potential for microenvironmental conditions is demonstrated as elevated pH within the mucilage of axenic photosynthesizing *Microcystis* colonies (Richardson & Stolzenbach 1995). Respiring bacteria counteract the chemical changes in O₂, CO₂ and pH induced by photosynthesis and a mutualistic relationship between cyanobacteria and associated (heterotrophic) bacteria has frequently been suggested (Whitton 1973), but to our knowledge no studies have yet evaluated the diffusive regime of *Microcystis* including attached bacteria. Intracellular gas vacuoles enable *Microcystis* to ascend the water column at speeds up to 3.2 m d⁻¹ (Reynolds 1973, Reynolds & Rogers 1976), which prevents sedimentation and increases the exchange of nutrients with the water surrounding the colonies. The buoyancy is counterbalanced by accumulated carbohydrates during photosynthesis, and to some extent the diel light cycle drives vertical up and down migrations (Thomas & Walsby 1986). Loss of *Microcystis* from the water column is also reduced, because grazing by zooplankton is inhibited by the colony size and a potential toxicity from intracellular microcystin (DeMott & Dhawale 1995). Similarly, mesozooplankton has been shown not to ingest bacteria attached to *Microcystis* (Bern 1987) and bacteria embedded in mucilage may be protected from protozoan ingestion (Jürgens & Güde 1994). Gradually during autumn, however, *Microcystis* disappears from the pelagic waters and sinks to the sediment (Boström et al. 1989), because low temperature inhibits the ability to regain buoyancy (Thomas & Walsby 1986).

A few studies have estimated the relative contribution of bacteria attached to *Microcystis* in eutrophic lakes. Bacteria attached to *Microcystis* accounted for 6 to 40% of total bacterial abundance during 3 sampling days and from 7 to 30% of total bacterial production during a diel study in Lake Vallentjunsjön, Sweden (Brunberg 1993). Less specific for *Microcystis*-associated bacteria, 15 to 33% of the bacterioplankton was retained in the >5 µm pore-size fraction during a 2 yr study in a Japanese pond (Konda 1984) and >50% of bacterial production was associated with >3 µm pore-size fractions in Lake Norrviken, Sweden (Bell et al. 1983). Thus, bacteria attached to *Microcystis* may contribute significantly to pelagic bacterial carbon dynamics.

We aimed to study the ecology of *Microcystis*-associated bacteria (MB) based on successive and physiological parameters in comparison with the rest of the pelagic bacterial community (mainly free-living bacte-

ria, FB). We operationally defined that 20 µm mesh-width nets separated MB (>20 µm) and FB (<20 µm) to make it feasible to obtain continuous measures of bacterial abundance and activity. Based upon this, the microhabitat of MB is characterized as a 'hotspot' for bacterial activity with overall significance in bacterial carbon processing and population dynamics during blooms of *Microcystis*.

MATERIALS AND METHODS

Study site. The study was carried out in Frederiksborg Slotssø, Denmark, during autumn 1994 and summer 1995. The lake (mean depth: 3.1 m) is highly eutrophic with a dense bloom of colony forming cyanobacteria during summer and autumn, when chlorophyll concentrations may exceed 100 µg l⁻¹ (Andersen & Jacobsen 1979, Christoffersen et al. 1990).

Sampling. Water from 0 and 0.5 m depth was taken from the middle of the lake and mixed in equal volumes either at noon (autumn) or in the morning (summer).

Subsamples of 10 ml for bacterial abundance and activity were fractionated with 20 µm mesh-width 25 mm nets (Nitex). We operationally defined that bacteria attached to *Microcystis* (MB) dominated the >20 µm size fraction in abundance and activity and that bacteria in the filtrate (FB, <20 µm size fraction) mainly originated from the water phase. The 20 µm mesh-width net retained 98% of the *Microcystis* colonies. No further attempts were made to quantify detachment of MB during fractionation and the contribution of bacteria attached to other particles than *Microcystis* in the >20 µm size fraction.

Bacterial biomass. MB collected on 20 µm mesh-width nets were transferred to 0.2 µm prefiltered Milli-Q water and processed as the filtrate. Samples were fixed with glutaraldehyde (1.2% final concentration) and stored at 4°C for 3 to 4 mo (autumn) or 0.5 to 2 mo (summer). Recent studies have demonstrated that storage for several weeks can reduce bacterial number and cell volume distribution (Turley & Hughes 1992, Gundersen et al. 1996). Effects of storage were not accounted for in the present study. Bacteria associated with particles and the walls of storage containers were detached by 2 min of sonication with a Branson Sonifier 250 adjusted to 50% duty cycle and 306 µm amplitude (25 W) (Velji & Albright 1993). No effect of sonication was observed on bacterial abundance in 3 µm pore-size filtrates (data not shown). Bacteria were quantified by direct microscopic counting of DAPI-stained (4',6-diamidino-2-phenylindole, Sigma; final concentration 0.1 µg ml⁻¹) bacteria collected on 0.2 µm pore-size black polycarbonate filters (Porter & Feig

1980). Bacterial cell volumes were calculated from measured length/widths or diameters from enlarged micrographs. Cell volumes were converted to cell carbon by the factor $105 \text{ fg C } \mu\text{m}^{-3}$ (Theil-Nielsen & Søndergaard in press), as the bacterial volumes used to calculate this factor were estimated with similar equipment and methods.

Bacterial production. Bacterial production was estimated from the incorporation of [^3H]-thymidine (TdR) into DNA (Fuhrman & Azam 1980) and during summer was also from [^3H]-leucine (Leu) incorporation into proteins (Kirchman et al. 1985). Incorporation rates were measured at substrate saturation concentrations estimated from kinetic experiments before each sampling for TdR incorporation and 4 times during summer for Leu incorporation.

Triplicate samples and 1 blank were incubated at *in situ* conditions for 1 h with [*methyl*- ^3H]-thymidine (Amersham) diluted to 20 nM (10 Ci mmol^{-1}) and L-[4,5- ^3H]-leucine (Amersham) diluted to 600 nM (1 Ci mmol^{-1}) final concentrations. Incorporation was stopped by adding 100% trichloroacetic acid (TCA) to a final concentration of 5%. TCA was added to the blanks prior to the incubations. Radiolabeled macromolecules were extracted for 1 h at 4°C before the samples were fractionated with $20 \mu\text{m}$ mesh-width nets. The $20 \mu\text{m}$ mesh-width nets, retaining ^3H incorporated by MB, were placed on $0.4 \mu\text{m}$ pore-size cellulose-nitrate membrane filters (Micro Filtration System), similar to the filters used to collect bacteria from the filtrates. Filters were rinsed with cold 5% TCA ($\times 4$) and 80% ethanol ($\times 2$) (Wicks & Roberts 1988, Hollibaugh & Wong 1992) and radioactivity was quantified with an LKB Wallac 1219 Rackbeta liquid scintillation counter.

Thymidine incorporation was converted to cell production with the factor $2 \times 10^{18} \text{ cells mol}^{-1}$, which has previously been estimated for Frederiksborg Slotssø (Smits & Riemann 1988) and another eutrophic lake (Bell et al. 1983). Carbon production was derived from cell production by the average cell carbon specified for each fraction. Leucine incorporation was converted to carbon production using the factor $3.1 \text{ kg C mol}^{-1}$ (Simon & Azam 1989). Exponential growth rates were calculated to relate bacterial production and biomass.

Amino peptidase activity. The potential amino peptidase activity was measured with the fluorogenic model substrate L-leucine-4-methyl-coumarinylamid hydrochloride (Leu-MCA) according to Hoppe (1993).

MB collected on $20 \mu\text{m}$ mesh-width nets were resuspended in $0.2 \mu\text{m}$ sterile filtered lake water and processed as the filtrate. Triplicate samples were incubated with Leu-MCA (Sigma) at 0.2 mM final concentration (saturation level) in the dark at *in situ* temperature. After 1 h incubation, Leu-MCA was added to blank samples and fluorescing hydrolysates were

quantified subsequently with a spectrofluorophotometer (Shimadzu RF-5001PC). From August 3 to 17 samples had to be stored until later quantification (less than 3 wk at -18°C). After incubation and Leu-MCA addition to the blanks, samples were filter sterilized ($0.45 \mu\text{m}$ cellulose-nitrate membrane filters, Micro Filtration System) to minimize the hydrolysis from particle bound enzymes during freezing and thawing and stored at -18°C . Two later experiments showed that sterile filtration upon incubation reduced fluorescence by 26 and 62%. Stored samples are therefore corrected by an average factor of 1.99 and excluded from statistical analysis and other concluding calculations.

Chlorophyll, POC, DOC. Chlorophyll was collected on Whatman GF/C filters, extracted with 96% ethanol overnight and measured spectrophotometrically (Jespersen & Christoffersen 1987). Particulate organic carbon (POC) and dissolved organic carbon (DOC) were separated by filtration with precombusted (550°C) 13 mm GF/F filters. POC was measured as CO_2 emitted from combustion of filters (600°C) by use of an infrared gas analyser (Søndergaard & Middelboe 1993) and DOC was measured by a Pt-catalytic carbon analyser (Shimadzu TOC-5000).

Statistical analysis. Equivalence of MB and FB in growth rates and specific aminopeptidase activity were tested nonparametrically with the Wilcoxon signed rank test. Spearman rank correlation coefficients (r_s) were calculated to test patterns among variables, unless other calculations are mentioned. In the kinetic experiments, leucine incorporations at the respective concentrations were fitted to the Michaelis-Menten equation of enzyme kinetics by the least squares estimation of the parameters V_{max} and K_m . Randomness of data points relative to fitted lines were confirmed with Runs test and Sign test and equivalence of K_m values were compared with *t*-tests. The level of significance was 0.05.

RESULTS

Kinetic experiments showed that thymidine (TdR) incorporation was saturated at 20 nM, whereas leucine (Leu) incorporation never saturated below 200 nM. The Michaelis-Menten equation of enzyme kinetics was fitted to the saturation curves of Leu incorporation with values of r^2 between 0.78 and 0.99. All values of K_m were higher in the $>20 \mu\text{m}$ size fraction compared to the $<20 \mu\text{m}$ size fraction, although this difference was statistically significant in only 2 of 3 comparisons, i.e. on July 26 and August 1. Leu incorporation in the $>20 \mu\text{m}$ size fraction, assigned to MB, therefore showed less affinity and saturated at higher concentrations compared to the $<20 \mu\text{m}$ size fraction, domi-

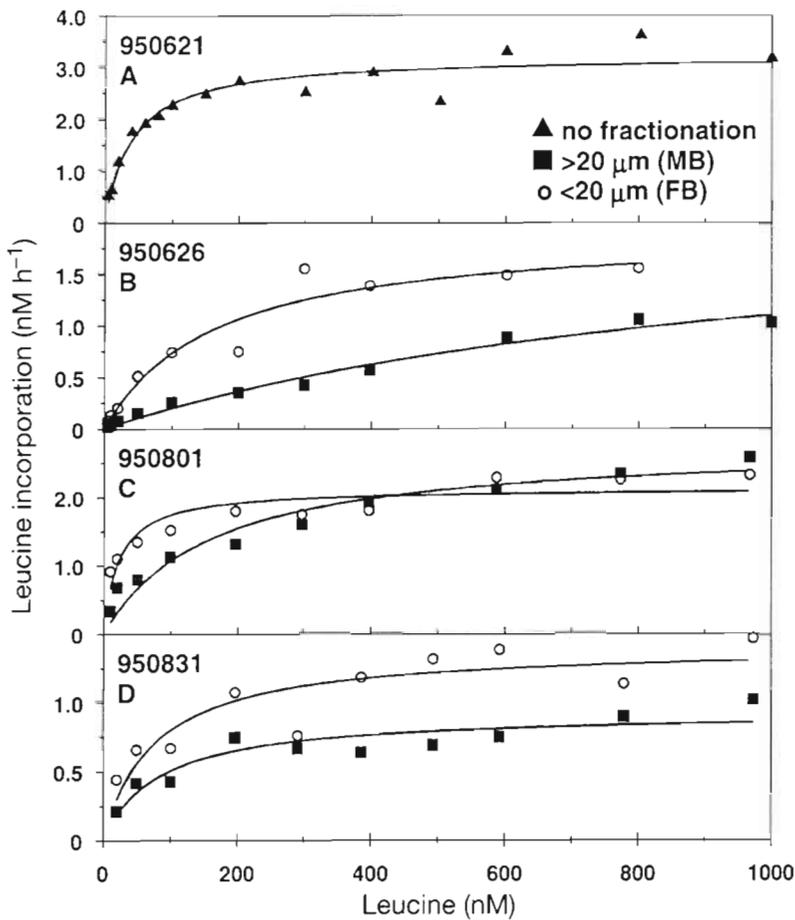


Fig. 1. Kinetics of leucine incorporation fitted to the Michaelis-Menten equation of enzyme kinetics with values of r^2 from 0.78 to 0.99. (A) No fractionation, (B–D) MB designates $>20 \mu\text{m}$ size fractions, presumed to be dominated by *Microcystis*-associated bacteria, and FB designates $<20 \mu\text{m}$ size fractions dominated by mainly free-living bacteria

nated by FB. Leu incorporation at 600 nM, the concentration used to estimate bacterial production, tended to saturate (Fig. 1). Most estimates of K_m ranged from 21 to 164 nM, corresponding to 97 and 79% saturation at 600 nM according to the Michaelis-Menten equation. The exception was MB on June 26, where $K_m = 1016 \text{ nM}$, corresponding to only 37% saturation.

Results from summer 1995 and autumn 1994 are presented in succession according to seasons rather than chronology, because general patterns of bacterial growth dynamics are assumed to be reflected (Fig. 2). Chlorophyll and POC were positively correlated ($r_s > 0.75$, $p < 0.007$) and *Microcystis* spp. dominated the phytoplankton during both seasons.

MB increased in biomass from $8 \mu\text{g C l}^{-1}$ in early July to a maximum value of $15.9 \mu\text{g C l}^{-1}$ on August 7 and afterwards declined gradually to $5.3 \mu\text{g C l}^{-1}$ (Fig. 2). In parallel, MB production (TdR) increased from ca 0.25 to $3.77 \mu\text{g C l}^{-1} \text{ h}^{-1}$ on August 3 and decreased steadily to $0.79 \mu\text{g C l}^{-1} \text{ h}^{-1}$ ($r_s = 0.80$, $p = 0.003$, $n = 15$). Similar trends were measured from leucine incorporation at 600 nM ($r_s = 0.73$, $p = 0.008$, $n = 14$) and converted to production (Leu) the estimates were 4-fold higher than calculated from the TdR method. Production also corre-

Table 1. Spearman rank correlation coefficients (r_s) between parameters for *Microcystis*-associated bacteria (MB, size fraction $>20 \mu\text{m}$) and bacteria living in the surrounding water (FB, size fraction $<20 \mu\text{m}$). Levels of significance are indicated with asterisks: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. –: $p > 0.05$

	MB versus FB		Temperature	Chlorophyll	POC	DOC
Summer 1995						
Bacterial biomass	–	MB	0.65*	–	–	–
		FB	–	–	–	–
Bacterial cell production (thymidine method)	0.65*	MB	0.80***	0.59*	0.63*	–
		FB	0.56*	0.65*	–	–
Bacterial biomass production (leucine method)	–	MB	0.81***	–	–	–
		FB	–	–	–	–
Amino-peptidase activity ^a	–	MB	–	0.89**	0.81*	–
		FB	0.86*	–	–	–
Autumn 1994						
Bacterial biomass	0.60*	MB	0.90***	0.86**	0.94***	–
		FB	0.60*	–	0.59*	–
Bacterial production (thymidine method)	0.59*	MB	0.69*	0.55*	0.83**	–
		FB	0.55*	–	0.56*	–

^aData from August 3 to 17 are excluded ($n = 10$)

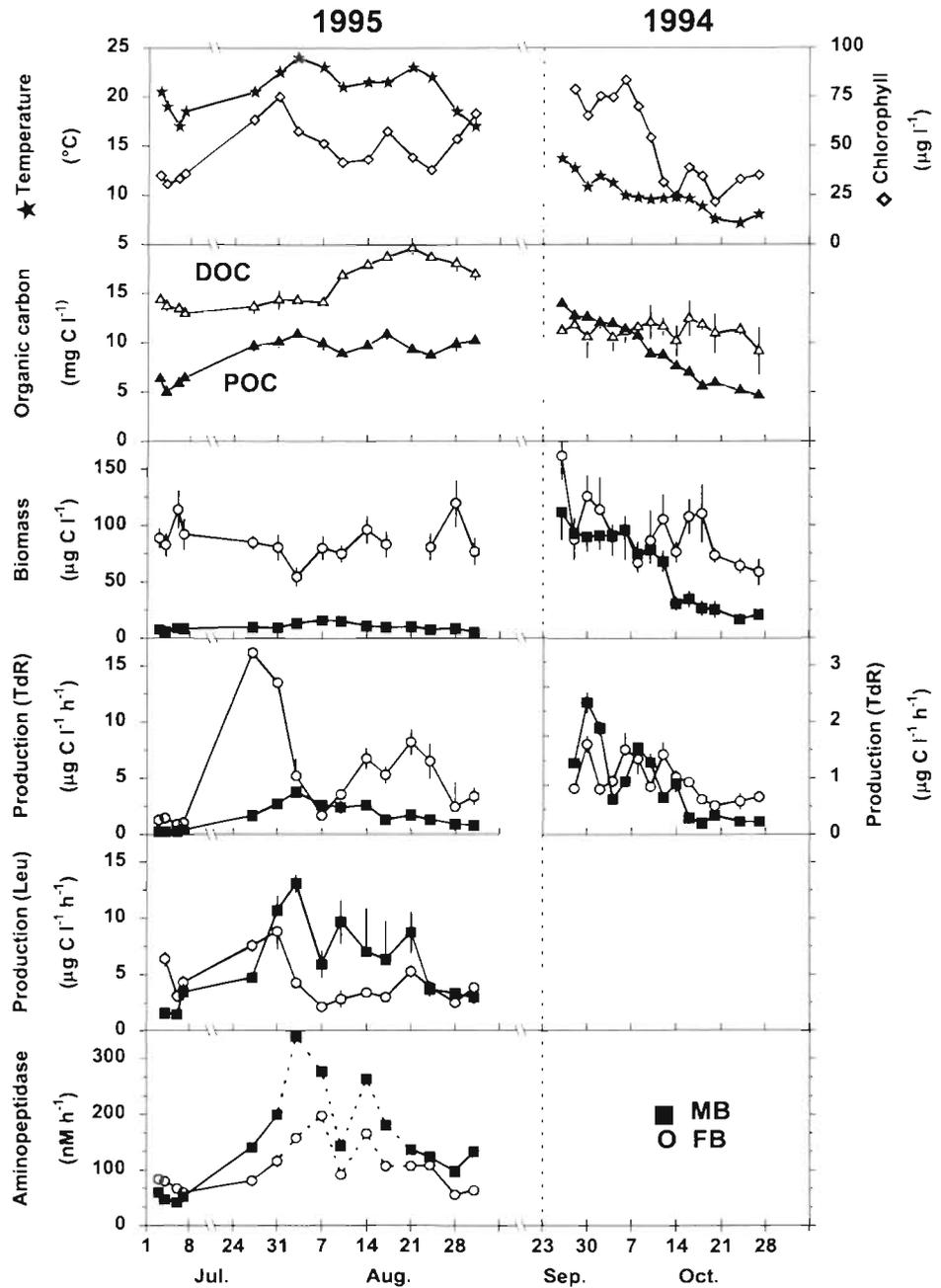


Fig. 2. Continuous measures of abiotic, algal and bacterial parameters, Frederiksborg Slotssø, summer 1995 and autumn 1994. (■) *Microcystis*-associated bacteria (MB, >20 µm mesh-width size fraction) and (○) bacteria in the surrounding water (FB, <20 µm mesh-width size fraction). Error bars indicate standard deviation of triplicate samples of dissolved organic carbon (DOC), particulate organic carbon (POC), bacterial production and aminopeptidase activity. Aminopeptidase from August 3 to 17 are multiplied by 1.99 to correct for fluorescence lost during sterile filtration

lated significantly with aminopeptidase activity (TdR: $r_s = 0.89$, $p = 0.008$, $n = 10$; Leu: $r_s = 0.87$, $p = 0.014$, $n = 9$). During summer neither biomass nor production of MB correlated significantly with chlorophyll, whereas the decline in chlorophyll during autumn correlated significantly with decreases in biomass and production of MB (Table 1). From late September to late October, MB biomass was reduced from about 100 to 25 $\mu\text{g C l}^{-1}$ and production (TdR) decreased from a level between 0.71 and 2.68 $\mu\text{g C l}^{-1} \text{h}^{-1}$ to ca 0.25 $\mu\text{g C l}^{-1} \text{h}^{-1}$ ($r_s = 0.73$, $p = 0.009$, $n = 14$).

FB biomass was rather constant and ranged mostly between 75 and 125 $\mu\text{g C l}^{-1}$ during both seasons, although it tended to decrease during autumn ($r_s = -0.57$, $p = 0.040$, $n = 14$) (Fig. 2). During summer FB production (TdR) increased from ca 1 $\mu\text{g C l}^{-1} \text{h}^{-1}$ in early July with 2 peak values on July 27 at 16.2 $\mu\text{g C l}^{-1} \text{h}^{-1}$ and on August 21 at 8.2 $\mu\text{g C l}^{-1} \text{h}^{-1}$, but declined afterwards to about 3 $\mu\text{g C l}^{-1} \text{h}^{-1}$. The latter peak of production followed the dynamics of DOC from August 7. During autumn FB production (TdR) fluctuated between 0.91 and 1.82, but decreased to 0.6 $\mu\text{g C l}^{-1} \text{h}^{-1}$.

Table 2. Relative contribution of *Microcystis*-associated bacteria (MB, size fraction >20 μm) in Frederiksborg Slotssø during summer 1995 and autumn 1994. Values are calculated as $\text{MB}/(\text{MB}+\text{FB}) \times 100\%$ and listed as average \pm standard deviation with the range in parentheses

Period	Abundance (%)	Biomass (%)	Production		Potential aminopeptidase activity (%) ^a
			Thymidine (%)	Leucine (%)	
Summer 1995	8 \pm 3 (5–15)	10 \pm 4 (6–19)	25 \pm 13 (9–61)	55 \pm 18 (20–78)	53 \pm 12 (37–68)
Autumn 1994	32 \pm 11 (15–46)	37 \pm 12 (19–52)	43 \pm 16 (24–70)		

^aData from August 3 to 17 are excluded

$\text{C l}^{-1} \text{ h}^{-1}$ in late October, significantly correlated with the production of MB (Table 1).

Consistently, MB comprised a smaller fraction of total abundance or biomass as compared to total production and potential aminopeptidase activity, which corresponds to higher specific activities of MB (Table 2). During summer 1995 cell growth rates of MB were significantly higher than those of FB ($p < 0.002$), in contrast to autumn 1994 where averages (\pm standard deviation) of 0.40 ± 0.19 and $0.30 \pm 0.10 \text{ d}^{-1}$ for MB and FB, respectively, were not statistically different ($p = 0.069$). Further, specific aminopeptidase activity of MB was significantly higher than that of FB when tested nonparametrically (TdR: $p = 0.006$, $n = 10$; Leu: $p = 0.018$, $n = 9$) and values of MB and FB averaged 1.60 ± 0.67 and $0.41 \pm 0.32 \text{ mol Leu-MCA hydrolysed per mol C produced (TdR)}$, respectively, and 0.027 ± 0.010 and $0.018 \pm 0.006 \text{ mol Leu-MCA hydrolysed per mol C produced (Leu)}$, respectively, assuming negligible algal leucine incorporation.

Production derived from the thymidine and leucine methods correlated significantly for MB ($r_s = 0.90$, $p = 0.001$, $n = 14$), as did summed rates (MB + FB), representing total production from each method ($r_s = 0.78$, $p = 0.005$, $n = 14$) (Fig. 3). The molar ratios of incorporated Leu and TdR (Leu:TdR ratio) were consistently larger for MB than for FB and averaged 23.2 and 3.81, respectively, which reflects that MB produce more biomass per cell division than FB. This result is qualitatively in accordance with the higher cell volumes measured for MB (average: $0.095 \mu\text{m}^3 \text{ cell}^{-1}$) as compared to FB ($0.071 \mu\text{m}^3 \text{ cell}^{-1}$), but the small difference in volumes is insufficient to explain the larger difference in Leu:TdR molar ratios based on reported linear or exponential relationships between cell volume and cell carbon (Simon & Azam 1989, Theil-Nielsen & Søndergaard in press). In 2 later experiments, we tested whether this difference reflected that samples were extracted for 1 h with 5% TCA before fractionation. It was the hypothesis that macromolecules labelled with either $^3\text{H-Leu}$ or $^3\text{H-TdR}$ distributed differently upon precipitation with TCA. Less radioactivity was mea-

sured in the <20 μm size fraction after TCA extraction and decreases amounted to 6.5 and 7.7% for TdR and 2.1 and 6.7% for Leu, but within the limits of the standard deviations. The measures are biased because recovered radiolabel averaged only $75 \pm 14.5\%$ (range 58 to 92%) of unfractionated totals (Fig. 4). Anyway, Leu:TdR ratios in <20 μm size fractions were unaffected by the order of TCA-extraction and fractionation (Expt 1: 15.8 ± 0.34 ; Expt 2: 10.8 ± 0.34), whereas Leu:TdR ratios in >20 μm size fractions were higher and more variable without any consistent trends. On this basis we conclude that the relative disagreement between Leu:TdR ratios and cell volumes measured during summer are robust to methodological errors. Taken at face values, calculated growth rates indicate that the leucine method overestimated the MB produc-

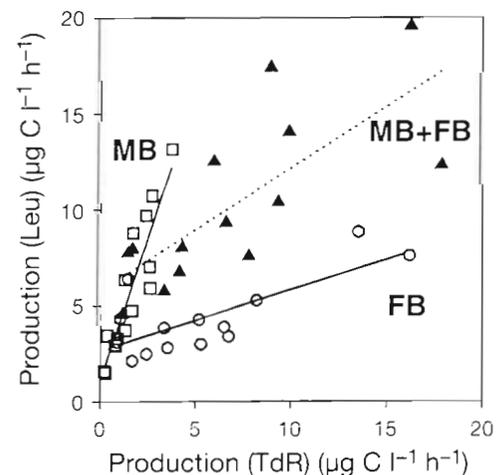


Fig. 3. Relationship between bacterial production of biomass estimated with the thymidine (TdR) and leucine (Leu) methods, Frederiksborg Slotssø, summer 1995. (□) Bacteria attached to *Microcystis* (MB, >20 μm mesh-width size fraction), (○) mainly free-living bacteria (FB, <20 μm mesh-width size fraction) and (▲) summed rates (MB + FB), corresponding to unfractionated samples. Lines are fitted with linear regressions: MB, $\text{Leu} = \text{TdR} \times 2.992 + 1.052$ ($r^2 = 0.81$); FB, $\text{Leu} = \text{TdR} \times 0.315 + 2.646$ ($r^2 = 0.53$); and (MB + FB), $\text{Leu} = \text{TdR} \times 0.641 + 5.735$ ($r^2 = 0.56$)

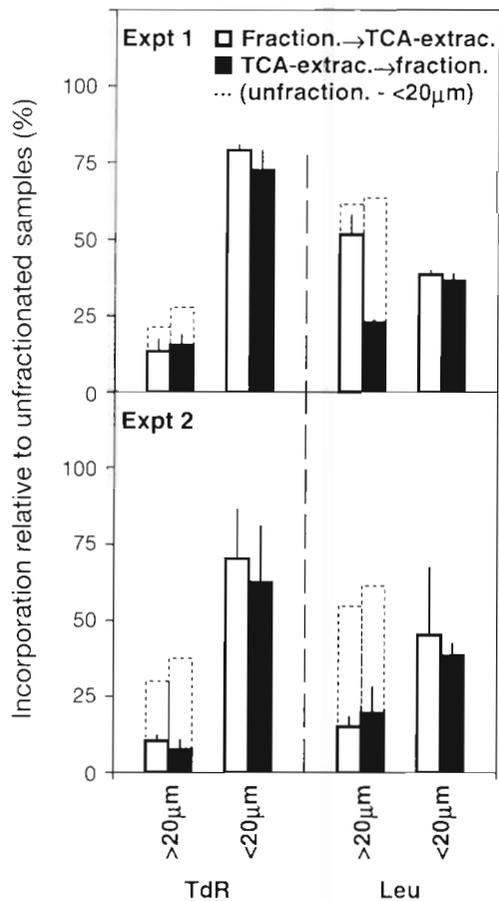


Fig. 4. Two experiments showing the distribution of TdR and Leu incorporation in samples fractionated with 20 µm mesh-width nets either before (open bars) or after (solid bars) 1 h extraction with 5% TCA. Unrecovered radiolabel (dotted bars) are added to bars of >20 µm size fractions

tion. Growth rates of MB averaged $3.2 \pm 1.8 \text{ d}^{-1}$ (TdR) and the unrealistically high value of $10.6 \pm 4.1 \text{ d}^{-1}$ (Leu), whereas estimates of FB agreed better, averaging 1.2 ± 0.62^{-1} (TdR) and $1.4 \pm 1.3 \text{ d}^{-1}$ (Leu). However, this conclusion is sensitive to both inaccuracies of the conversion factors used and storage effects on estimates of bacterial biomass and cell volume (Turley & Hughes 1992, Gundersen et al. 1996).

DISCUSSION

MB are not easily segregated from the FB. Manual collection of *Microcystis* colonies with micropipette is a specific but laborious method (Boström et al. 1989, Brunberg 1993). Our purpose, to study short-term dynamics of MB and FB in abundance, production and aminopeptidase activity, demanded a rapid segregation procedure, but the definition that 20 µm mesh-

width nets retained MB is not accurate: bacteria attached to large non-*Microcystis* particles were also included in the >20 µm size fraction and loss of MB to the filtrate during fractionation cannot be neglected (Bern 1987). We did not attempt to quantify these methodological errors, but point out that they work in opposite directions regarding the accuracy of MB in abundance and activity. We consider that our method gives a fair description of the dynamics of MB. In a parallel study during summer 1995, particles retained by 3 µm pore-size filters were predominated in number by transparent exopolymer particles (TEP, of maximum length 3.2 to 162.0 µm), colonized by 7% of the total bacterial community (Worm & Søndergaard in press). The relative contribution of MB was comparable (Table 2), but it is unlikely that TEP-bacteria dominated bacterial abundance in the >20 µm size fraction regularly, because TEP is deformable and less efficiently retained by 20 µm mesh-width nets compared to *Microcystis* colonies.

Leucine incorporation was included in the sampling programme as an independent supplement to the thymidine method to reflect bacterial growth in terms of both biomass (Leu) and cells (TdR). Previous studies with the leucine method have used maximally 100 nM leucine. We incubated at 600 nM leucine, because the kinetic experiments clearly demonstrated that more than 200 nM leucine was needed to approximate the saturation level, in agreement with previous kinetic studies in eutrophic systems (Jørgensen 1992, Riemann & Azam 1992, van Looij & Riemann 1993). At saturation level, intracellular isotope dilution of incorporated radiolabel is minimized and independent of affinity, which per se optimizes the coupling to bacterial production. However, high concentrations of leucine increase the risk that bacterial growth is stimulated and/or algae incorporate leucine. Indirect evidence of minor algal leucine incorporation at 600 nM includes that the kinetics of leucine incorporation was hyperbolically rather than linearly related to the respective concentrations, thus indicating the predominance of bacterial leucine incorporation by high affinity uptake systems rather than algal low affinity uptake systems and/or passive diffusion. In addition, incorporated leucine retained on 20 µm mesh-width nets (algal fraction) was highly significantly related to thymidine incorporation ($r_s = 0.90$, $p = 0.001$, $n = 14$) and thymidine at 20 nM is neither expected to stimulate bacterial growth nor to be taken up by algae (Bern 1985) (Fig. 3). The significant correlation between summed rates of thymidine and leucine derived production agrees with previous findings (Kirchman 1992, Servais 1992, Simon 1994). Based on these arguments bacterial production was calculated from leucine incorporation at 600 nM using a conversion factor, where

overall bacterial carbon production is calculated from the direct incorporation of exogenous radiolabelled leucine into bacterial proteins, a constant ratio between leucine in bacterial proteins and overall cell carbon and a 2-fold intracellular isotopic dilution (Simon & Azam 1989). Whether these presumptions are fulfilled for MB, including a quantification of algal leucine incorporation, are difficult to measure, because MB cells then have to be separated from cells and mucilage of *Microcystis*. The unrealistically high growth rates of MB, as calculated from leucine incorporation (mean 10.6 d^{-1}), indicate significant algal incorporation and speaks in favour of the thymidine method, but we emphasize that the conclusion is dependent on our choices of conversion factors. The volume to carbon conversion factor is in the lower range reported (Theil-Nielsen & Søndergaard in press) and lower leucine conversion factors of 0.900 and $1.080 \text{ kg C mol}^{-1}$ have been reported (Servais & Garnier 1993, Servais & Lavandier 1993). Increasing the volume to carbon conversion factor and decreasing that of leucine improves the balance between estimates of production for MB at the expense of FB. The conversion factor for thymidine incorporation was determined in the studied lake (Smits & Riemann 1988) and corresponds to values often found in other systems. Another possibility for biased growth rate calculations is that the storage of samples with glutaraldehyde for several weeks may have reduced the estimates of bacterial abundance and mean cell carbon (Turley & Hughes 1992, Gundersen et al. 1996), which leads to overestimated growth rates and underestimated carbon content and biomass of DAPI-stained bacteria. However, tight correlation between bacterial biomass and production indicate that effects due to storage were similarly distributed between the samples. Accordingly, we have no obvious reasons to reject relative differences in either bacterial biomass or specific activities, although both parameters are probably somewhat biased.

During summer 1995, MB averaged only 10% of total bacterial biomass, but due to the high specific activity MB contributed on average either 25% (TdR) or 55% (Leu) of total bacterial production. MB also showed a high hydrolytic capacity and contributed on average 53% of the total potential aminopeptidase activity (Table 2). Specific aminopeptidase activity of MB also exceeded that of FB significantly. In autumn the colonization of *Microcystis* was more intense and peak values of MB biomass and production exceeded FB episodically. However, the relative contribution of MB declined to about 15 to 20% in late October, as *Microcystis* gradually disappeared from the surface water (Fig. 2). All measures of bacterial activity thus point to an important ecological role of MB in bacterial carbon

dynamics during blooms of *Microcystis* (Table 2), as evident from previous studies (Bell et al. 1983, Konda 1984, Brunberg 1993).

Obviously, *Microcystis* colonies occupied a negligible volume of the bulk water. Nevertheless, the abundance and activity of MB were indeed significant, which designates this microenvironment for heterotrophic bacteria as an intense 'hotspot'. Accordingly, bacterial activity of pelagic waters may be heterogeneously distributed on the macroscopic scale during blooms of *Microcystis*, as evident for larger particles known as marine or lake snow (Smith et al. 1992, Grossart & Simon 1993). If the measured growth rates reflect, at least, qualitative differences between actively growing cells, the higher growth rates of MB during summer indicate that the microenvironment of *Microcystis* was enriched with labile substrates relative to the surrounding water. In general, high substrate availability selects for low affinity/high capacity uptake systems (Søndergaard & Middelboe 1995). Consistently, such uptake systems characterized leucine incorporation of MB compared to FB (Fig. 1). The higher specific activity of MB may reflect the combined effects of (1) higher steady-state concentration of exudates within the mucilage of *Microcystis*, (2) increased exchange of nutrients with the surrounding water, as MB move through the water column driven by the density of *Microcystis* (Thomas & Walsby 1986), (3) adsorption of labile organic matter to the mucilage of *Microcystis* (Fletcher 1991), and (4) diversified species composition (Brunberg 1993). During autumn, however, growth conditions were rather similar according to the statistical similarity between measured growth rates. An opposite characterization of MB was given by Brunberg (1993), who found that thymidine incorporation per cell of MB was below that of FB in late summer and early autumn. Low mortality, nutrient limitation or accumulated algal metabolites were proposed to sustain MB abundance near 'carrying-capacity', generally characterized by relatively inactive bacteria, long turnover times and low specific growth rates. Most likely, the activity of MB may be inhibited, if the microenvironment of *Microcystis* becomes alkaline during photosynthesis, as evident from axenic colonies of *Microcystis* (Richardson & Stolzenbach 1995). However, no general pattern about the chemical microenvironment of *Microcystis* is obvious from the measured growth rates of DAPI-stained bacteria.

MB production (TdR) ranged from 0.23 to $3.77 \mu\text{g C l}^{-1} \text{ h}^{-1}$, whereas the calculated increases in biomass could be accounted for by a maximum of $0.067 \mu\text{g C l}^{-1} \text{ h}^{-1}$. Therefore, more than 70% of the MB production was not recovered as increased biomass, which indicates that most gain of MB, i.e. growth and colonization, balanced the loss from grazing, lysis, sedimenta-

tion and shedding (Pedrós-Alió & Brock 1983). We hypothesize that a large fraction of MB production was shedded to the surrounding water. This characterizes *Microcystis* as a bacterial 'incubator'. The idea is based on reported low loss rates of *Microcystis* from sedimentation and grazing during summer (Reynolds 1973, DeMott & Dhawale 1995). Further, we did not observe any protozoa grazing on MB, and Bern (1987) could not measure any grazing of MB by mesozooplankton. However, loss of MB due to viral lysis remains unsolved. In one marine study virus infection was detected in 2 to 37% of the bacteria associated with particles (Proctor & Fuhrman 1991). Applying this range to our results, however, shedding of MB is likely to be an important loss factor. Attached bacteria shedding daughter cells to the surrounding water is evident from studies of biofilm formation (Caldwell et al. 1992), and recognized from various types of natural particles including copepod fecal pellets (Jacobsen & Azam 1984), marine snow (Azam & Smith 1991) and diatom-mucus aggregates retained by 1 µm pore-size filters (Smith et al. 1995).

In conclusion, *Microcystis* offers attachment sites for a very dynamic community of heterotrophic bacteria, which contributes importantly to the carbon metabolism of pelagic bacteria. Up to half of total bacterial production was assigned to this microenvironment during summer and autumn. As *Microcystis* often dominates the phytoplankton of eutrophic lakes, the pelagic microenvironment of these systems may be considered heterogeneous with intense 'hotspots' of bacterial activity in association with the macroscopic colonies of *Microcystis*.

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