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Bacterioplankton in a small polyhumic lake with an anoxic hypolimnion

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Abstract

Bacterioplankton biomass and dark fixation of inorganic carbon were measured in the highly humic (water colour up to 550 mg Pt l^{-1}) and acidic lake, Mekkojärvi. Strong thermal and chemical stratification developed in the water column early in spring and led rapidly to anoxia in the hypolimnion, which extended to less than 1.0 m from the surface. In the epilimnion only small bacteria were abundant. In the anoxic zone both the abundance and the mean size of bacteria were considerably higher than in the epilimnion. These differences are thought to be the result of different grazing pressure from zooplankton in the two zones. In late summer a high concentration of bacteriochlorophyll *d* in the upper hypolimnion indicated a high density of photosynthetic bacteria. Bacterial biomass was similar to that of phytoplankton in the epilimnion, but 23 times higher in the whole water column. In August, dark fixation of inorganic radiocarbon in the anaerobic zone was 51% of the total ^{14}C-incorporation and the contribution of light fixation was only 5.4%. In the polyhumic Mekkojärvi, bacterioplankton was evidently a potentially significant carbon source for higher trophic levels, but bacterioplankton production could not be supported by phytoplankton alone. Allochthonous inputs of dissolved organic matter probably support most of the bacterial production.

Introduction

A high concentration of dissolved organic matter (DOM) is typical of small, sheltered lakes in the northern boreal zone. Humic compounds have physical and chemical characteristics which directly or indirectly affect aquatic organisms. One of the most important factors is the strong absorption of light (Eloranta, 1978; Jones & Arvola, 1984). In spring, after melting of the ice, the absorption of light in a thin water layer results in rapid development of steep thermal and chemical stratification of the water column. In small lakes with sufficient depth this leads to incomplete vernal overturn (e.g. Salonen *et al.*, 1984). In humic lakes the euphotic layer is shallow (Jackson & Hecky, 1980; Arvola & Rask, 1984) and phytoplankton production may be restricted by light availability. However, utilization of the high concentration of DOM by decomposers means that bacterial biomass and total plankton respiration can be high in relation to phytoplankton biomass and production (e.g. Rodina, 1967; Salonen, 1981a). As a result of high decomposition, an anoxic hypolimnion is regularly found in small humic lakes (Salonen *et al.*, 1984).

This work was part of the ecosystem study of a small, sheltered, polyhumic headwater lake, Mekkojärvi. Its aim was to assess the potential role of bacteria for the food chains of Mekkojärvi.

Special attention was paid to the likely consequences for bacterioplankton of the steep stratification of the water column.

Study site

Mekkojärvi (Fig. 1) is a small polyhumic (colour 300–550 mg $Pt\,l^{-1}$), naturally acidic (pH 4.6–6.2) lake in southern Finland and with sometimes incomplete spring mixing (Salonen *et al.*, 1984). The lake is surrounded by a floating Spaghnum mat and therefore has no shallow littoral. In summer the water column is vertically steeply stratified (Table 1).

Material and methods

Sampling was performed weekly between May and August, 1984 at the middle of the lake (Fig. 1). Water samples were taken from the surface to 2.5 m depth at 0.25 m intervals using a plastic tube connected to a bottle to which vacuum was applied with a hand pump. Oxygen and temperature were measured in the field using a combined probe (Yellow Springs Instruments). Water colour was determined from the absorbance at 420 nm with a spectrophotometer (Hitachi 101). The attenuation of light in the water column was calculated (Hutchinson, 1957) as

$$I_z = I_0\, e^{-n'z},$$

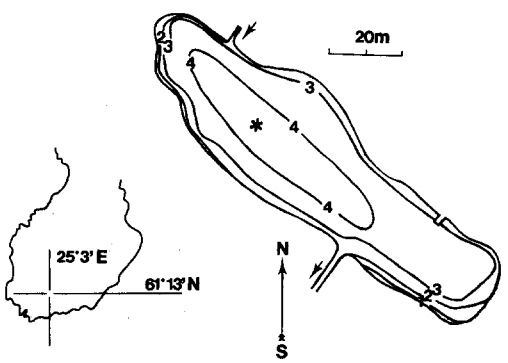

Fig. 1. Location and bathymetric map of Mekkojärvi. * – the sampling point.

Table 1. Some physical and chemical characteristics of Mekkojäri at two depths. The values are means of the results obtained between May and August, 1984.

	0 m	2.5 m
Colour (mg $Pt\,l^{-1}$)	458	334
pH	5.1	6.1
Conductivity (mS m^{-1} 20 °C)	4.2	7.1
Alkalinity (meq l^{-1})	0.025	0.420
$NO_2 + NO_3 - N$ ($\mu g\,l^{-1}$)	4.2	3.0
NH_4-N ($\mu g\,l^{-1}$)	21	518
Total-N ($\mu g\,l^{-1}$)	853	1378
PO_4-P ($\mu g\,l^{-1}$)	11	160
Total-P ($\mu g\,l^{-1}$)	32	192

where I_z = incident light at depth z; I_o = light at the surface; n' = extinction coefficient of light, which was corrected by the water colour at each depth (Jones & Arvola, 1984).

Inorganic nutrients were determined with an AKEA autoanalyzer immediately after return to the laboratory. For phosphate we used the molybdate method (Murphy & Riley, 1962) and $NO_3 + NO_2$-nitrogen was determined as nitrite after the reduction in a cadmium-copper column (Wood *et al.*, 1967). NH_4-nitrogen was determined with an ammonium electrode (Orion Model 95–10). Total nitrogen and phosphorus of unfiltered water samples were determined after persulphate digestion (Koroleff, 1979).

For determination of chlorophyll, 1 liter of water was filtered through a Whatman GF/C filter and stored at −18 °C. The chlorophylls were extracted with 94 or 99.9% ethanol at +6 °C for 24 h (Arvola, 1981), and measured with a Hitachi 101 or Shimadzu UV-240 spectrophotometer using wavelengths of 665 and 750 nm for chlorophyll *a*, and 654 nm for bacteriochlorophyll *d* (Stanier & Smith, 1960; Takahashi & Ichimura, 1970).

Phytoplankton primary production and inorganic carbon dark fixation were measured with a ^{14}C-technique (Vollenweider, 1969; Sorokin & Kadota, 1972; Overbeck, 1979). Close interval ^{14}C-incorporation measurements were made using a sampler (Fig. 2) designed to complete all steps required in the determination *in situ* at the

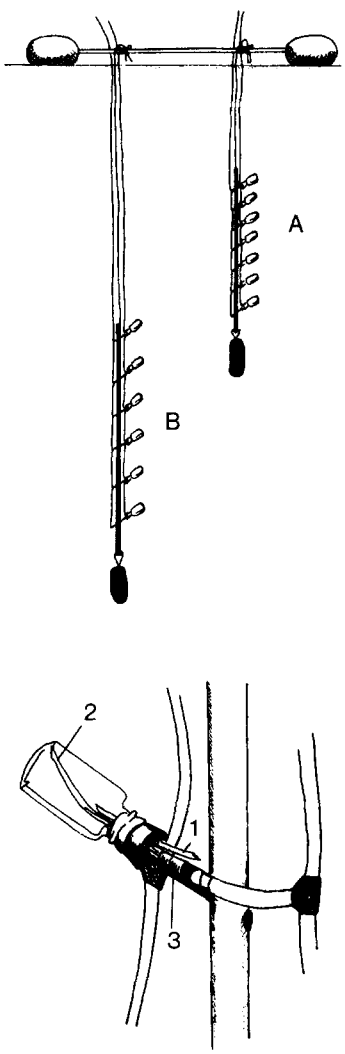

Fig. 2. Design of the device for making inorganic carbon uptake experiments in the hypolimnion. Upper panel – the two devices suspended in the water; lower panel – detailed drawing of the incubation bottle. 1 – Water inlet; 2 – air and water outlet; 3 – syringe for radiocarbon solution.

depth of sampling. Thus the organisms were not influenced by abnormal light intensity, and contamination of anoxic water by oxygen could be avoided. The dark ^{14}C-fixation in the hypolimnion was measured one day later, because the close interval sampling/incubation system was available for only one vertical series. Washed and precombusted (4 h at + 500 °C) scintillation vials (20 ml) were attached to the rubber caps, and 0.5–1.0 ml $NaH^{14}CO_3$-solution was added into the injection syringes. After estimation of the depth at which the water became anoxic (using YSI oxygen meter), two samplers were slowly lowered into the anaerobic hypolimnion and the samplers were tied to a buoy. On the lower sampler the vials were located at 0.2 m and on the upper one at 0.1 m intervals (Fig. 2). The vials were first rinsed ca. 5 min by applying vacuum so that lake water flowed through the vials. Then $NaH^{14}CO_3$-solution was injected into the vials by applying pressure in the tubes attached to the syringes. The incubation was started at ca. 11.00 and continued for 24 h. After the incubation, the samplers were removed and immediately protected from direct sunlight. The scintillation vials were detached from the samplers and 25% glutaraldehyde added (final conc. 0.25%). After mixing, the vials were wrapped in aluminum foil and put in crushed ice. In the laboratory 0.5 ml water was injected under the surface of 0.5 ml of a carbon dioxide absorption solution (1 part ethanolamine, 7 parts ethyleneglycol monomethyl ether; Burnison & Perez, 1974) to determine the amount of added radioactivity. Finally the liquid volume in the vial was filled to 8 ml with deionised water. After this, inorganic carbon was measured with a carbon analyser (Salonen, 1981b) with an exhaust tube led outdoors to dispose of radioactive carbon dioxide. The incorporated radioactivity was measured after the addition of HCl into an 8 ml subsample and bubbling for 20 minutes (Schindler *et al.*, 1972). The radioactivities were measured with an LKB-Wallac Ultrobeta 1210 Liquid Scintillation Counter using Lumagel (Lumac) as scintillant.

Subsamples for the microscopic counting of bacteria were taken from the vials immediately after the determination of dissolved inorganic carbon and prepared according to Bergström *et al.* (1986), using acriflavine stain. One preparation from each sample was made the day after sampling and the slides were stored at room temperature protected from light. Earlier experience had shown that the fluorescence of acriflavine lasts for months under such conditions. Bacteria were enumerated within one week of sampling using a Nikon Optiphot epifluorescence micro-

scope (light source HBO 100 mercury lamp, excitation 430–490 nm, objective UV-F100 N.A. 1.30 and final magnification 1250×). Bacteria were counted from 10 randomly chosen rectangular fields (until June 12, the number of fields was 10–40) from the center of each filter. Bacterial aggregates were sometimes observed, but they were not discriminated in the counts. The total number of cells counted on each filter was 100–300.

The linear dimensions of one hundred cells on each filter were measured with a calibrated ocular micrometer. We chose an estimate of 0.25 pg C μm^{-3} (range in literature from 0.11 (Nagata, 1986) to 0.56 (Bratbak, 1985), respectively) to convert bacterial volume to carbon. Phytoplankton carbon biomasses were estimated from chlorophyll using a carbon to chlorophyll ratios 27 and 67, which is the range given by Riemann *et al.* (1989).

Results and discussion

Physical and chemical properties

Mekkojärvi lost its ice cover at the beginning of May and thermal stratification then developed rapidly (Fig. 3). During summer, the temperature fluctuated between 12.3 and 19.6 °C at the surface and between 3.0 and 7.3 °C below 1.5 m. Due to the shallow epilimnion, diel changes in the surface temperature of Mekkojärvi can be 10 °C in extreme cases in midsummer. Therefore the epilimnetic temperatures reported here are nearer to the minimum temperature, which occurs early in the morning, than the maximum temperature, which is generally observed at the end of the afternoon.

After ice melt, oxygen in the hypolimnion was rapidly depleted and the euphotic zone also remained undersaturated. Even during the May primary production maximum, the oxygen saturation at the surface varied between 60 and 80%. In June, July and August the respective values were 55–83, 52–80 and 48–65% indicating continuous dominance of heterotrophic processes. The anoxic layer reached its highest position in August (Fig. 3).

During the summer inorganic nutrients accumulated in the anaerobic hypolimnion (Fig. 4) where nitrates and nitrites were probably partly reduced to ammonium. The concentration of $NO_2 + NO_3$ nitrogen varied from 0 to 20 $\mu g\,l^{-1}$ (mean 3 $\mu g\,l^{-1}$), ammonium from 7 to 810 $\mu g\,l^{-1}$ (mean 193 $\mu g\,l^{-1}$) and inorganic phosphorus from 7 to 240 $\mu g\,l^{-1}$ (mean 64 $\mu g\,l^{-1}$) in the

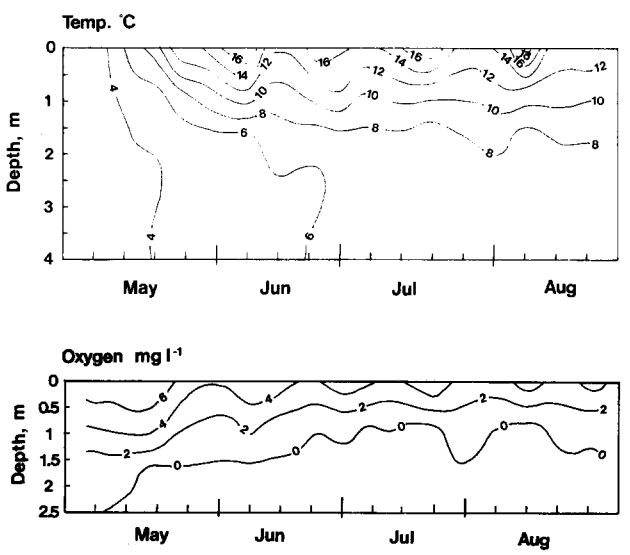

Fig. 3. Isopleths for water temperature and oxygen.

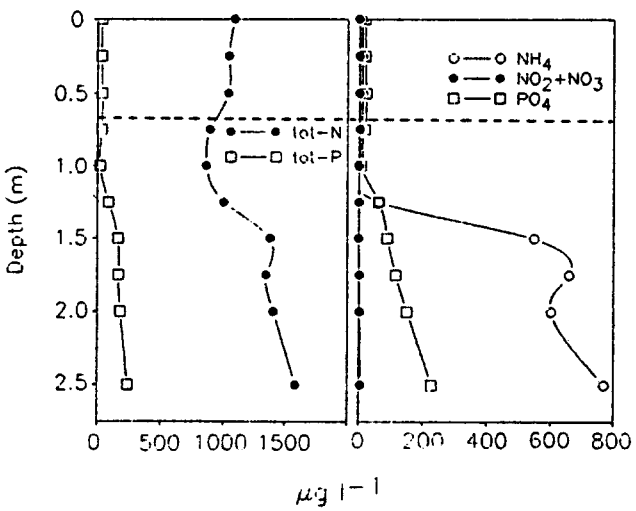

Fig. 4. Example of the vertical distribution of nitrogen (as N) and phosphorus (as P) on 14 August 1984. The oxycline is indicated by the dashed line.

water column during the summer. In the aerobic epilimnion the concentrations of $NO_2 + NO_3$ nitrogen were 0 to 18 $\mu g\,l^{-1}$ (mean 6 $\mu g\,l^{-1}$), NH_4 nitrogen 10 to 74 $\mu g\,l^{-1}$ (mean 28 $\mu g\,l^{-1}$) and phosphate phosphorus 5 to 36 $\mu g\,l^{-1}$ (mean 15 $\mu g\,l^{-1}$). Also total nitrogen (range 720–1300 μg $N\,l^{-1}$) and phosphorus (20–250 μg $P\,l^{-1}$, respectively) concentrations increased towards the bottom by the end of the summer. However, it is noticeable that low concentration of inorganic nutrients extended well below the epilimnion (Fig. 4).

The increase in water colour (Fig. 5) towards the end of the summer indicated increased input of DOM from the catchment area. Consequently the attenuation of light in the water column was high (Fig. 5) limiting the 1% level of surface light to a depth of 0.75–1.0 m.

Vertical distribution of chlorophylls

The concentration of chlorophyll *a* in 0–2.0 m water column decreased during May from 19–31 $\mu g\,l^{-1}$ to 1.1–5.5 $\mu g\,l^{-1}$ in the epilimnion (Fig. 6). After the depletion of oxygen in the 1.5–2 m water layer (Fig. 3), bacteriochlorophyll *d* developed in the hypolimnion (Fig. 6) indicating the existence of green sulphur bacteria. The maximum concentration of bacteriochlorophyll *d* developed at 1.5–1.75 m where the concentration reached 800–1000 $\mu g\,l^{-1}$. Light intensity at that depth was <0.03% of the surface light. At the lower boundary of the ca. 1 m thick maximum of green sulphur bacteria, the cells were probably limited by light, while the upper boundary was possibly controlled by the availability of H_2S and cladoceran grazing. The occurrence of green sulphur bacteria rather than other types in Mekkojärvi is probably due to the strong predominance of red light (a result of the very brown colour of the water) in the anoxic hypolimnion, low light intensity and low concentration of hydrogen sulphide (e.g. Bergstein *et al.*, 1979; Parkin & Brock, 1980; 1981a; 1981b; Mazumder & Dickman, 1989). Parkin & Brock (1980a) found only bacteriochlorophyll *d* in humic Mary lake, where the proportion of surface light available for bacterial photosynthesis was of the same order (<0.02%) as in Mekkojärvi. Compared with the concentrations of bacteriochlorophyll *d* observed in Mary lake and in the other 5 lakes studied by Parkin & Brock (1980), the concentrations in Mekkojärvi were high. Our bacteriochlorophyll *d* values may be in error because we applied the formula given for acetone extraction (Takahashi & Ichimura, 1970) although we used ethanol as a solvent. However, Takahashi & Ichimura (1968) in Jap-

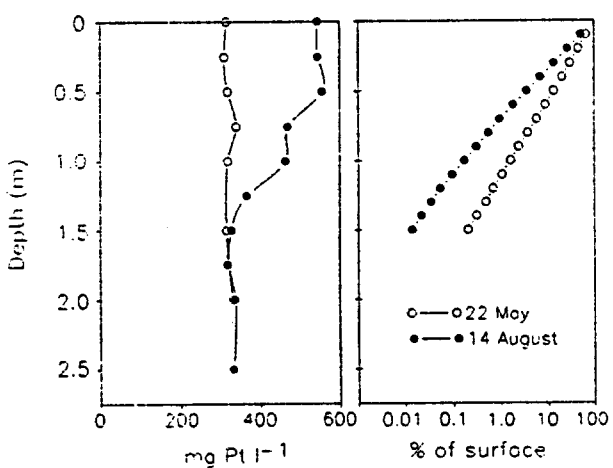

Fig. 5. Water colour and light penetration early and late in summer.

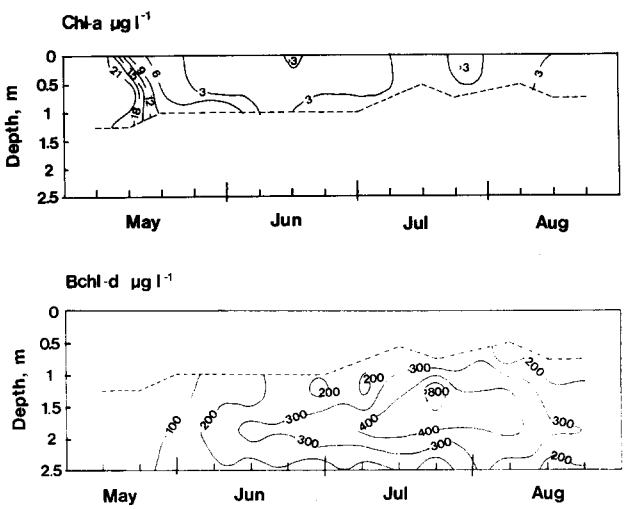

Fig. 6. Temporal variation of chlorophyll *a* and bacteriochlorophyll *b*. The oxycline is indicated by the dashed line.

anese lakes and Lawrence *et al.* (1978) in a meromictic salt lake observed similarly high concentrations of bacteriochlorophyll *d*. Small bacterial biomass in relation to bacteriochlorophyll *d* concentration may also suggest overestimation of pigment, but this kind of comparison is so dependent on the volume to carbon conversion factor as to be of little real value. In general there was no clear relationship between the bacterial biomasses and the concentrations of bacteriochlorophyll *d*.

Bacterial density and biomass

In Mekkojärvi the number of bacteria varied between 2.3×10^6 and 3.9×10^7 cells ml^{-1} and the highest numbers were observed in the anoxic hypolimnion. These densities are higher than in acidic lakes with somewhat lower humic content (Salonen, 1981a; Andersson, 1983; Johansson, 1983). Bacterial abundance in lake water seems to correlate positively with humic concentration (Hessen, 1985b; Tranvik, 1988) and this might explain the high density of bacteria in Mekkojärvi.

The mean volume of bacteria varied from 0.089 to 0.295 μm^3 being within the range reported from humic lakes (e.g. Salonen, 1981a: 0.09–0.25 μm^3; Johansson, 1983: 0.10–0.35 μm^3; Hessen, 1985b: 0.076–0.144 μm^3). Small cells dominated in the aerobic epilimnion while in the anaerobic hypolimnion the proportion of large bacteria increased (Fig. 7). In spite of their high density, the contribution of small cells to the total bacterial biomass was low. In the anoxic hypolimnion, where bacteria were larger and more abundant, bacterial biomasses were several times higher than in the epilimnion (Fig. 8). Consequently epilimnetic bacteria contributed only 7–31% to the bacterial biomass of the whole water column. In the middle

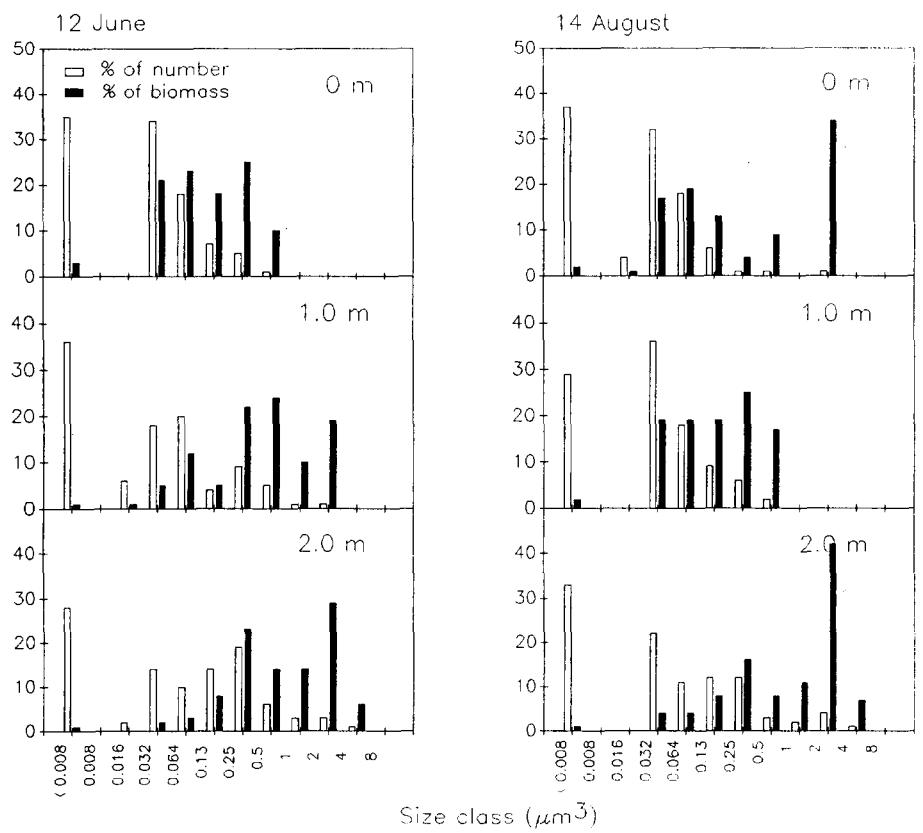

Fig. 7. Size distributions of bacteria at two dates.

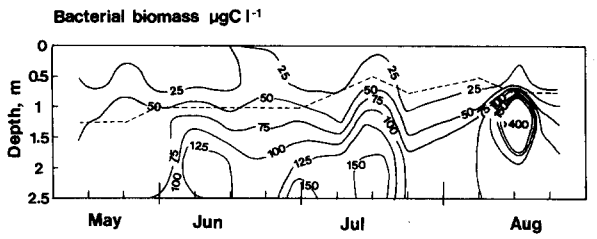

Fig. 8. Temporal variation of bacterial biomass in the water column. The oxycline is indicated by the dashed line.

of August bacterial biomass increased remarkably at the depth of maximum concentration of bacteriochlorophyll *d*.

^{14}C-incorporation

In August, the inorganic carbon uptake at the surface of the aerobic epilimnion was below 100 mg C m^{-3} d^{-1} in light and reduced rapidly, along with decreasing light, in deeper water (Fig. 9). The measured phytoplankton primary production was within the range of values observed in other humic lakes of the same area (Arvola, 1983; Rask *et al.*, 1986). In the anaerobic hypolimnion the maximum values were similar, up to 90 mg C m^{-3} d^{-1} in darkness and 63 mg

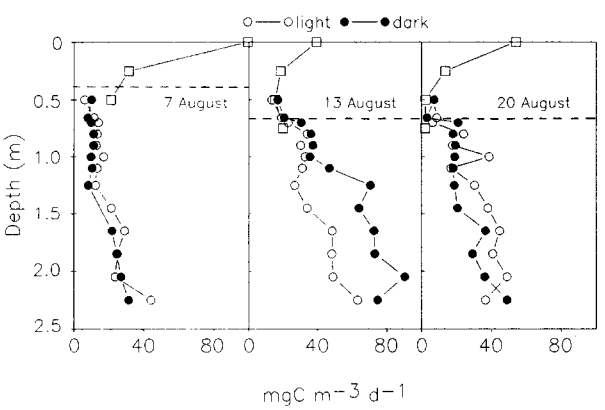

Fig. 9. Light and dark fixation of inorganic carbon in August. The oxycline is indicated by the dashed line. Light fixation determined in the epilimnion (open squares) using a more traditional vacuum sampling through a tube and incubation in glass bottles is shown for comparison.

C m^{-3} d^{-1} in light. In the hypolimnion both dark and light incorporation of inorganic carbon showed similar vertical distributions. In the second experiment dark results were consistently higher than light results, possibly because the light and dark incubations were not simultaneous. The differences between light and dark ^{14}C-incorporation and between different dates suggest that, in spite of the rather low temperature in the hypolimnion, the activity of the anaerobic microbial community can exhibit appreciable fluctuations. This aspect should be investigated in view of possible diel periodicity of bacterial photosynthesis. Bacterial photosynthesis was so low, and dark inorganic carbon uptake so fluctuating that it could not have been estimated by successive incubations.

High incorporation in the hypolimnion reflects various chemosynthetic and anaerobic processes (Sorokin, 1965). In general, incorporation of ^{14}C in the hypolimnion followed the same pattern as the bacterial biomass. During summer, the plankton of Mekkojärvi became increasingly 'heterotrophic', as the illuminated and aerobic epilimnion became shallower. Because of the much higher depth of the anaerobic hypolimnion than that of the epilimnion, areal dark fixation of inorganic carbon in anoxic water was quantitatively of the same order as, or higher than, the primary production of epilimnetic phytoplankton. In different bacterial groups the proportion of cell carbon originating from inorganic carbon in water varies according to metabolic type. For aerobic bacteria the range is typically only 3–5% (Sorokin, 1965; Overbeck, 1979), whereas for chemoautotrophic and photosynthetic bacteria it can be the sole carbon source (Sorokin, 1965; Hansen, 1983). Thus it is impossible to convert dark inorganic carbon uptake in the anoxic hypolimnion to values directly comparable with phytoplankton primary production. In the literature, estimates of the photosynthetic bacterial contribution to total planktonic primary production in humic and eutrophic lakes range from 0.26–6.3% (Parkin & Brock, 1980; 1981a; Steenbergen & Korthals, 1982). In meromictic clear water lakes bacterial photosynthesis may contrib-

ute as much as 50–80% of primary production (e.g. Lawrence *et al.*, 1978; Biebl & Pfennig, 1979).

General discussion

High bacterial biomass compared to primary production (e.g. Rodina, 1969; Salonen, 1981a; Hessen, 1985b) and phytoplankton biomass (Johansson, 1983) is typical of humic lakes. In the whole water column, bacterial biomass exceeded phytoplankton biomass on average 23-fold, whereas in the oxic epilimnion there was practically no difference (respective ratio ca. 2). In Mekkojärvi, where the coloured water attenuates light rapidly, algae might be adapted to low light intensity and hence may have developed higher chlorophyll concentrations than in those lakes from which the chlorophyll to carbon ratio used in this study (Riemann *et al.*, 1989) was obtained. If so, the difference between the estimated bacterial and algal biomasses would have been even higher.

The mortality of bacteria (Servais *et al.*, 1985) can be a consequence of spontaneous cell lysis, infection by bacteriophages (Sherr, 1989; Børsheim *et al.*, 1990), or grazing. In the first two cases, autotrophic bacteria would be indifferent, but heterotrophic bacteria would act as a 'sink' in the energy transfer of the system (Ducklow *et al.*, 1986). In Mekkojärvi the small size of epilimnetic bacteria most probably results from size selective grazing by heterotrophic nanoflagellates (e.g. Andersson *et al.*, 1983) and daphnids (Peterson *et al.*, 1978; Kankaala, 1988). Large cell size in the anaerobic layer is probably due to different metabolic bacterial groups, high concentration of inorganic and organic nutrients and low grazing pressure. Grazing by phagotrophic nanoflagellates has been suggested to be the predominant loss mechanism for bacterioplankton (e.g. Fenchel, 1982; Sanders *et al.*, 1989; Tranvik, 1989). Salonen & Jokinen (1988) studied grazing by heterotrophic nanoflagellates in the epilimnion of Mekkojärvi using latex beads as tracers and found phagotrophic nanoflagellates to be major consumers of epilimnetic bacteria, clearing daily ca. 22% of the water column. Their estimated clearance may, however, be an underestimate caused by the methods (Sieracki *et al.*, 1987).

Cladocerans are also able to feed on bacterioplankton in lakes (Riemann & Bosselmann, 1984). The dominant species in Mekkojärvi, *Daphnia longispina*, is a very efficient filter feeder which, particularly when young, is also able to collect small bacterial size particles (Brendelberger & Geller, 1985; Hessen, 1985a). According to Kankaala (1988), *D. longispina* consumed 3–48% of bacterial biomass daily in the epilimnion of Mekkojärvi; she concluded, however, that the pathway via bacterivorous flagellates (e.g. Sanders & Porter, 1990) is a more important link to zooplankton than direct grazing on bacteria. This conclusion is sensitive to the size of the factor used to convert bacterial volumes to carbon. Kankaala used a conservative conversion factor of 0.121 pg μm^{-3} and the use of a higher factor (e.g. 0.35, Bjørnsen, 1986) would have made direct utilization of bacteria by zooplankton seem much more important. Irrespective of the pathway by which bacterial production enters zooplankton, the extremely high biomass of *D. longispina* in Mekkojärvi (Salonen & Lehtovaara, 1991) suggests that phytoplankton primary production is insufficient to support zooplankton growth and hence that bacteria play an important role in the planktonic food chains of Mekkojärvi.

Direct grazing estimates for protozoans in anaerobic waters have not generally been performed (see Sanders & Porter, 1986). Pigments of photosynthetic bacteria have sometimes been observed in the guts of zooplankters (Takahashi & Ichimura, 1968; Sorokin, 1970) and Mazumder & Dickman (1989) demonstrated that *Daphnia* can control the upper boundary of phototrophic bacterial population in a meromictic lake. In Mekkojärvi, the dense population of *D. longispina* typically stays near to the oxycline, and may dive into the upper anoxic hypolimnion (Salonen & Lehtovaara, 1991) to feed on bacterioplankton. Taking into account the proportionally high volume of anaerobic water in small humic lakes with shallow stratification, hypolimnetic production

and its utilization may well prove important for the productivity of such lakes.

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