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Net autotrophy in an oligotrophic lake rich in dissolved organic carbon and with high benthic primary production

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ABSTRACT: Biomass and production of microbiota—primary producers as well as heterotrophic bacteria—were studied both in the pelagial and in the benthic habitat over 2 yr in the shallow oligotrophic Lake Eckarfjärden, Sweden. Both biomass and production of microbiota were concentrated in the benthic habitat. Despite a high dissolved organic carbon (DOC) concentration of about 25 mg C l^{-1} in the water, the total bacterial production was lower than the total primary production. Moreover, measurements of DOC concentrations in the in- and outflow, and CO_2-saturation measurements, indicate that the system is net autotrophic. Generally, low-productive systems (<100 µg C l^{-1} d^{-1}) tend to be net heterotrophic. In contrast, we found a low-productive (55 µg C l^{-1} d^{-1}) but net autotrophic system, the conditions of which were largely influenced by benthic production. Many lakes in the world are shallow and may provide substantial benthic areas suitable for primary production. Hence, it is important to include this habitat when evaluating whether lakes are autotrophic or heterotrophic systems.

KEY WORDS: Primary production · Bacterial production · Microphytobenthos · Phytoplankton · Bacteria · Benthic habitat · Pelagial habitat

INTRODUCTION

About 90% of the published studies on bacterial and primary production in lakes concern pelagic environments (Vadeboncoeur et al. 2002). However, less frequently performed benthic studies show that benthic microbiota may contribute significantly to the total production in lakes (e.g. Doremus & Clesceri 1982, Wetzel 1996). There are strong couplings between the benthic and pelagic zone, and resource competition may occur between organisms in and between these zones. In shallow oligotrophic lakes, a large part of primary production is often focused in the benthic zone. In these lakes, phytoplankton are limited by nutrients whereas microphytobenthos can utilise nutrients from the sediment (Carlton & Wetzel 1988, Hagerthey & Kerfoot 1998). In deeper and/or nutrient-rich lakes, on the other hand, the primary production may be allocated mainly to the pelagic habitat. If nutrient concentrations are high enough to allow a large biomass of phytoplankton, they may decrease light availability, and thereby biomass and production of microphytobenthos (Hansson 1992, Havens et al. 2001).

In clearwater lakes, bacterioplankton utilise extracellular organic carbon originating from phytoplankton, and bacterioplankton production may be coupled to the primary production (e.g. Bell 1983). Similarly, benthic bacteria may utilise exudates from microphytobenthos (Goto et al. 2001). Bacteria are also capable of utilising organic humic substances, and in brownwater systems bacterioplankton production is not dependent on exudates from primary production. Instead, brownwater lakes are often dominated by bacterioplankton production (e.g. Tranvik 1989, del Giorgio & Peters 1994).

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In general, clearwater lakes are considered as net autotrophic, i.e. the primary production exceeds bacterial respiration (Schindler et al. 1972, Ochs et al. 1995). However, recent studies have shown that many lakes are net heterotrophic, with bacterioplankton respiration exceeding primary production (del Giorgio & Peters 1994, del Giorgio et al. 1997, Jansson et al. 2000). As a consequence, many lakes are also supersaturated with CO_2 (Kling et al. 1992, Sobek et al. 2003). Jansson et al. (2000) compared production data from lakes with different dissolved organic carbon (DOC) concentrations, and found net heterotrophic conditions down to a concentration of 5 mg DOC l^{-1}. This includes a large number of lakes, especially in northern temperate regions. Blomqvist et al. (2001) performed an enrichment experiment demonstrating that it is the DOC per se and not other effects of humic substances (e.g. reduced light availability) that determines the differences between humic and clearwater lakes.

The shallow oligotrophic hardwater lakes along the central Swedish coast belong to the clearwater lakes. Phytoplankton biomasses are low, and light penetrates down to large areas of the lake bottom. There, a thick (10 to 15 cm) benthic microbial mat develops, consisting of heterotrophic bacteria as well as microphytobenthos (Brunberg et al. 2002). Thus, the light availability might sustain a substantial primary production by microphytobenthos. However, DOC concentrations in the lakes are high, over 20 mg DOC l^{-1}, which is enough to sustain a large heterotrophic bacterial production and a net heterotrophic system. In this study, we followed the benthic and pelagic microbial biomass and production over a period of 2 yr in one of these lakes. The purpose was to determine the distribution of primary production and heterotrophic bacterial production between the benthic and pelagic habitat and to identify relationships between primary production and heterotrophic bacterial production. Our aim was also to elucidate whether these oligotrophic clearwater lakes are net autotrophic or net heterotrophic systems.

MATERIALS AND METHODS

Study area. The study was performed in Lake Eckarfjärden, an oligotrophic clearwater lake situated along the coast of central Sweden (60° 22′ N, 18° 12′ E). The lake has 1 outlet and 2 small inlets that are dried out during parts of the year. The catchment is dominated by coniferous forest (73% of total area). Although the lake is small (0.23 km^2) and shallow (mean depth 1.5 m), the theoretical water retention time is long (383 d). The lake water is characterised by very low phosphorus concentrations (average total-P, 12 ± 10 µg P l^{-1}) and high nitrogen concentrations (average total-N, 1132 ± 261 µg N l^{-1}), resulting in a high N:P quotient. Hard water conditions are indicated by high alkalinity (average 2.5 ± 0.3 mEq l^{-1}), conductivity (average 26 ± 4 mS m^{-1}) and pH (average 8.1 ± 0.3). The lake has an unusual combination of high DOC concentration (>20 mg C l^{-1}) together with moderate water colour (mean absorbance at 420 nm [$A_{420\,nm}$] 0.147 ± 0.043 cm^{-1}), which indicates that much of the DOC might originate from within the lake. The biomass of phytoplankton and heterotrophic bacterioplankton is low whereas the benthic habitat is covered by a thick (10 to 15 cm) microbial mat, consisting mainly of cyanobacteria and heterotrophic bacteria (Brunberg et al. 2002). Approximately half of the benthic area also hosts the macroalgae *Chara* sp. (Fig. 1) (Blomqvist et al. 2002). Zooplankton biomass in the lake is low during summer and is probably controlled by high grazing pressure by zooplankton-feeding fish (Andersson et al. 2003).

Sampling. Water samples for analyses of oxygen and water temperature were taken with a 0.3 m tube sampler in the surface waters and from close to the bottom at the deepest location of the lake (2.5 m). In winter, the surface samples were taken from just below the ice. Temperature was measured with a thermometer within the sampler and oxygen reagents were added directly in the field. Water samples for all other analyses were taken with a 1.5 m long tube sampler at 15

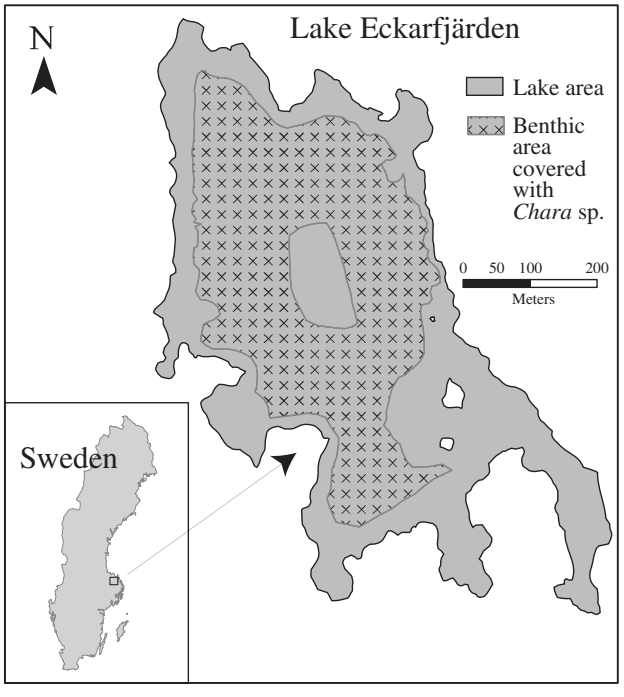

Fig. 1. Map of Lake Eckarfjärden showing its location along the Baltic coast of Sweden, and the distribution of *Chara* sp.

locations in the lake and pooled into a bucket, from which water sub-samples were drawn. Samples for biomass of phytoplankton and heterotrophic bacterioplankton were preserved in the field. Sediments for analysis of biomass of microphytobenthos and biomass and production of benthic heterotrophic bacteria were taken with a Willner core sampler at 1.5 m water depth. The sampler was carefully lowered in between stands of the macroalgae *Chara* sp. (i.e. no *Chara* sp. included in the samples). The top 5 cm of the microbial mat were transferred to plastic jars, from which subsamples for determination of heterotrophic bacterial production were drawn in the field and the rest were brought back to the laboratory for preservation of biomass samples. Purple sulphur bacteria (which thrives on the boarder between oxic and anoxic conditions) were often found at 5 cm depth, and hence, the top 5 cm was chosen as representative of the oxic layer of the sediment. Five samples for production of microphytobenthos were randomly taken with Jönsson cores (Jönsson 1991) from sediments at 1.5 m depth. Water samples from the 2 inlets and the outlet were taken on 1 occasion in April 2002, and analysed for DOC and total organic carbon (TOC) concentrations.

Water chemistry and biomass of microbiota. The water chemistry was measured 20 times during 2001 and 2002. When no other reference is given, analyses were performed according to European or Swedish standard methods (Swedish University of Agricultural Sciences [SLU] 2004; available online at http://info1.ma.slu.se/ma/www_ma.acgi$Analysis?ID=AnalysisList). Temperature, pH, water colour, conductivity, alkalinity, molybdate-reactive P (MRP), ammonium-N, dissolved oxygen, DOC, TOC and chlorophyll *a* were measured within 24 h after sampling. Water for analysis of total-P, total-N and (nitrite+nitrate)-N were frozen for later analysis. Water colour was measured spectrophotometrically at 420 nm in a 5 cm cuvette. Ammonium-N was analysed according to Chaney & Marbach (1962). DOC (water filtered through preignited Whatman GF/F filters) and TOC were analysed by combustion and subsequent IR detection of the resulting carbon dioxide, using a Shimadzu TOC 5000 Carbon Analyser. Concentrations of total-P and (nitrite+nitrate)-N were analysed according to Menzel & Corwin (1965) and Wood et al. (1967), respectively. Chlorophyll *a* was measured according to ISO (1992), with a sample of 1 ml wet sediment.

The biomasses of phytoplankton, microphytobenthos, heterotrophic bacterioplankton and benthic heterotrophic bacteria were determined 20 times during 2001 and 2002. Phytoplankton samples were preserved with Lugol's solution and microphytobenthos with formaldehyde (final concentration 2%). Species composition and biovolume of phytoplankton were determined using an inverted phase-contrast microscope, after overnight sedimentation of the organisms in 10 ml of water. Carbon biomass was calculated assuming a wet weight of 1 g cm^{-3} and conversion factors for carbon content of different taxa of phytoplankton (Olrik et al. 1998). The different taxa of phytoplankton were divided into 3 groups: autotrophic flagellates, autotrophic non-flagellates and mixotrophic flagellates according to Isaksson et al. (1999). Species composition and carbon biomass of microphytobenthos were determined in the same way as for phytoplankton, with the exception that sediment samples were diluted 200 times with water (1:1 of deionised water:tap water) and counted after sedimentation in 1 ml chambers.

Heterotrophic bacterioplankton and benthic bacteria were preserved with formaldehyde to a final concentration of 4%. The bacteria were counted and measured with an epifluorescence microscope. Sediment samples were first sonicated with a Rapidis ultrasonic disintegrator (1 min, 100 W); the other samples were directly stained with acridine orange and filtered through 0.2 µm black polycarbonate filters. A total of at least 200 cells were counted for each sample, and the total heterotrophic bacterial biovolume was estimated by measuring at least 100 cells. Bacterial dry weight (DW) and carbon content was calculated according to Loferer-Krössbacher et al. (1998), using the formula $DW = 435 V^{0.86}$, where V = volume, and assuming that 50% of the DW was carbon.

All biomass data were calculated on an areal basis, i.e. per m^2 within a water column of 1.5 m depth (mean depth of the lake) for pelagic microbiota, and within the surface sediment layer of 5 cm for the benthic microbiota.

Production of microbiota. Primary production of phytoplankton and microphytobenthos was measured *in situ* with ^{14}C-incorporations on 14 and 17 occasions, respectively. The primary production in the pelagic zone was measured with duplicates at 5, 25, 50, 100 and 150 cm depth in 60 ml bottles. Dark incubations were made at 5 and 150 cm depth. Sodium[^{14}C]bicarbonate was added to reach a concentration of 1.44 µmol l^{-1} in the bottles, which were incubated for 4 h around noon. The total dissolved inorganic carbon concentrations varied between 2.0 and 3.1 mmol l^{-1}. The incubations were stopped with 0.5 ml 37% formaldehyde. The samples were transported to the laboratory, where sub-samples of 3 ml were bubbled with hydrochloric acid (HCl) overnight, treated with scintillation cocktail (Perkin Elmer, Optiphase Hisafe 2) and counted in a scintillation counter.

Sediments for analysis of benthic primary production were sampled randomly within an area situated at 1.5 m water depth (mean depth of the lake) and then incubated at the same depth in Jönsson cores, where the radio-

active tracer was percolated down into the sediment (Jönsson 1991). The incubation depth was chosen as representative of the assumed average benthic production in the lake, after test measurements at 0.5, 1.0 and 1.5 m depth (Andersson et al. 2003). Sodium[^{14}C] bicarbonate was added to reach a concentration of 2.22 µmol l^{-1} in each core. Three light incubations and 2 dark incubations were made for 2 h around noon. At the end of the incubation a sub-sample of the top cm was taken from each core. The sub-sample was thoroughly mixed, after which triplicate samples of 0.2 ml were taken and treated with HCl. The samples were then brought back to the laboratory and dried in an oven at 40°C for 2 h and then treated with organic solvent Biolute-S. After approximately 24 h a scintillation cocktail (Perkin Elmer, Optiphase Hisafe 2) was added and the samples were measured in the scintillation counter.

The primary production h^{-1} was re-calculated to daily primary production by assuming direct proportionality of primary production to light (Wetzel & Likens 1991). Light measurements were taken from a continuous monitoring at the nearby field station of Lake Erken (K. Pettersson, Erken Laboratory, unpubl. data). Monthly production was also calculated using the same assumptions. If more than 1 sampling was performed in 1 mo, an average of the primary production values was used. For missing months in the sampling programme, the primary production per light was assumed to be mean values from the month before and after. In this way, a calculated yearly primary production was achieved.

Heterotrophic bacterial production in the water column was measured according to Bell (1993) on 17 occasions (the same dates as the primary production of phytoplankton). Incubations were started within 1 h after sampling around noon. A total of 5 ml of water was incubated *in situ* with [methyl-^{3}H]-thymidine (final concentration 30 nmol l^{-1}) for 0.5 to 2 h, depending on season and temperature. Adding 0.5 ml of 37% formaldehyde stopped the incubations. The samples were then transported to the laboratory, where they were kept at 4°C until further analysis.

Heterotrophic bacterial production in the sediment was measured on 14 occasions (the same dates and times as for primary production of microphytobenthos). A saturation experiment was performed to determine the amount of thymidine needed for measuring bacterial production in the sediment. First, portions of 0.2 ml sediment were incubated with 1.04, 2.08, 3.13, and 4.17 µmol l^{-1} [methyl-^{3}H]-thymidine. The results showed that 2.08 µmol l^{-1} [methyl-^{3}H]-thymidine gave a high record from the scintillation counter but was not enough to shut off the de novo pathway. Following this, a dilution curve with non-radioactive thymidine was performed with 0.5, 1, 2, 3, and 5 times dilution of the [methyl-^{3}H]-thymidine. This test showed that dilution with equal amount of non-radioactive thymidine shut off the de novo pathway. Analyses of benthic heterotrophic bacterial production were then performed according to Bell & Ahlgren (1987), within 1 h after sampling. In short, triplicates were incubated *in situ* with 0.2 ml sediment and 2.08 µmol l^{-1} [methyl-^{3}H]-thymidine and an equal amount of cold thymidine (total thymidine concentration, 4.16 µmol l^{-1}). A blank was also incubated after addition of 5 ml of 80% ethanol. The incubations were run for 0.5 to 2 h, depending on temperature and season. The incubations were stopped with 5 ml of 80% ethanol and the samples were transported to the laboratory where they were kept at 4°C until further analysis. DNA was extracted and the samples were measured in the scintillation counter according to Bell & Ahlgren (1987). The empirical conversion factor of 2×10^{18} cells mol^{-1} incorporated thymidine was used to calculated the production of heterotrophic bacteria (Bell 1993). Carbon biomass was calculated according to Loferer-Krössbacher et al. (1998), using the formula DW = $435 V^{0.86}$, and assuming the same biovolume of the bacteria as in biomass samples from the same date and assuming that 50% of the DW was carbon.

The daily bacterial production was calculated by multiplying the production h^{-1} by 24. Monthly bacterial production was calculated based on an average from the sampling dates within a month. The yearly bacterial production was achieved by summing up the monthly productions.

CO_2-saturation. On 2 occasions, at noon on 16 July and 3 October 2002, the CO_2-concentration was measured using a portable infrared gas analyser (EGM-3, PP Systems) and CO_2-saturation for Lake Eckarfjärden was calculated according to Sobek et al. (2003).

RESULTS

Water chemistry

The water chemistry in Lake Eckarfjärden showed clear seasonal patterns that were in accordance with earlier studies in the lake (cf. Brunberg et al. 2002, Andersson et al. 2003). Temperature ranged between 0.5 and 20°C. During winter, the lake was ice-covered (maximum thickness approximately 50 cm). Conductivity and alkalinity were high (Table 1) and showed pronounced seasonality, with high winter values and somewhat lower summer values. pH values were also high (Table 1) but showed the opposite seasonal variation, with minimum values in winter below the ice. Oxygen measurements revealed the same pattern in both years, with over-saturation in summer and undersaturation in the winter (range 0.2 to 19.8 mg O_2 l^{-1}).

Table 1. Water chemistry of Lake Eckarfjärden, measured on 20 occasions during 2001 and 2002. A_{450}: absorbance at 450 nm; Cond.: conductivity; Alk.; alkalinity; MRP: molybdate-reactive P; TP: total-P: TN: total-N; DOC: dissolved organic carbon

	pH	Water colour (A_{450})	Cond. (mS m^{-1})	Alk. (mEq l^{-1})	MRP (µg l^{-1})	TP (µg l^{-1})	(NO_3+NO_2)-N (µg l^{-1})	NH_4-N (µg l^{-1})	TN	TN:TP (µg l^{-1})	DOC (mg l^{-1})
Mean	8.1	0.149	26	2.5	2	12	91	254	1132	126	24.6
SD	0.2	0.044	4	0.3	5	10	223	226	261	87	2.8
Min.	7.7	0.080	19	2.0	0	3	3	28	820	35	20.2
Max.	8.6	0.227	33	3.1	20	48	984	636	1741	441	31.1

In March 2001, at the end of the ice-covered season, anoxic conditions were reached near the bottom.

Nitrogen concentrations were high whereas the concentrations of phosphorus were low, resulting in a high N:P ratio (average 126). Phosphorus concentrations and total nitrogen concentrations did not show any pronounced seasonality. Ammonium nitrogen, however, showed high winter values and low summer values. The maximum total phosphorus concentrations coincided with oxygen depletion in the bottom waters in March 2001. The TOC was almost exclusively made up by DOC and was very high (average 25 mg C l^{-1}) without any seasonal variation. The water colour was moderate (mean $A_{420\ nm}$ = 0.149), which is considered unusually low in combination with such high concentrations of DOC when compared to other Swedish lakes (Brunberg et al. 2002). Chlorophyll *a* measurements revealed maximum values in summer and minimum values in winter (range 0 to 6.4 µg chl *a* l^{-1} in lake water and 2.4 to 24.6 µg chl *a* g^{-1} wet wt in microbial mat).

The seasonal variations for all chemistry parameters were similar between the 2 years, except for total-P, which was lower during 2002 than during 2001. Further details of water chemistry are available from Brunberg et al. (2002) and Andersson et al. (2003). The DOC concentrations in the inlets, 15.5 and 18.5 mg C l^{-1}, respectively (measured in April 2002), were lower than the DOC concentrations in the lake and in the outlet at the same time (20.0 mg C l^{-1} at both sites).

Biomass

The biomass of microbiota was concentrated in the microbial mat (Fig. 2). Calculated on an areal basis, the biomass of benthic heterotrophic bacteria and microphytobenthos was on average 70 and 110 times higher, respectively, than the biomass of heterotrophic bacterioplankton and phytoplankton. The biomasses of heterotrophic bacterioplankton and phytoplankton were similar (Fig. 2a). The heterotrophic bacterioplankton biomass ranged between 15 and 245 mg C m^{-2} (mean 73 mg C m^{-2}, mean cell volume 0.20 µm^3) and showed a seasonal pattern, with summer maxima and winter minima. The biomass was significantly lower (Student's paired *t*-test, $p < 0.05$) during 2002 than 2001. The biomass of phytoplankton ranged between 5 and 180 mg C m^{-2} (mean 69 mg C m^{-2}) with the same seasonal pattern as heterotrophic bacterioplankton. However, in contrast to the heterotrophic bacterioplankton the phytoplankton showed a higher biomass during 2002 than 2001. During both years phytoplankton were dominated by Chrysophyceae, mainly *Ochromonas* spp., and approximately half of the phytoplankton were identified as potentially mixotrophic species according to Isaksson et al. (1999).

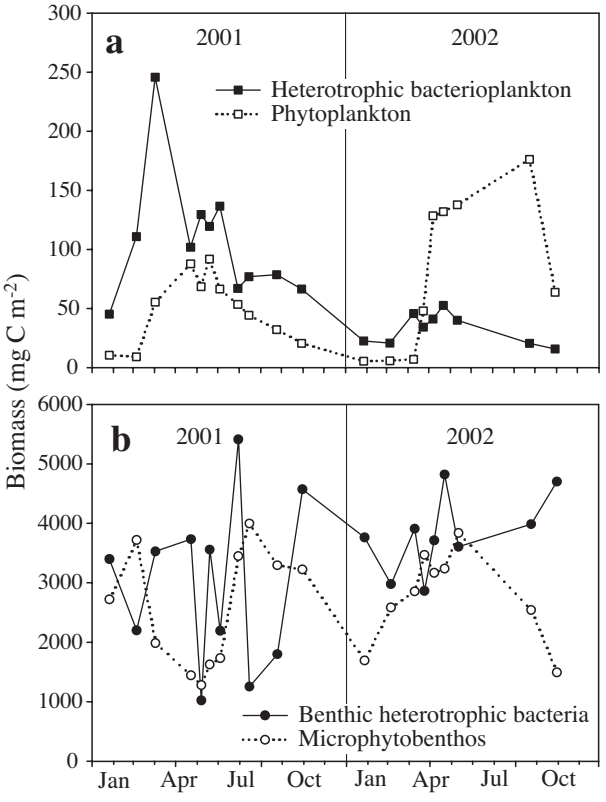

Fig. 2. (a) Biomass of phytoplankton and heterotrophic bacterioplankton in a water column of 1.5 m depth (mean depth of the lake) and (b) biomass of microphytobenthos and heterotrophic benthic bacteria in the top 5 cm of the microbial mat of Lake Eckarfjärden. Note the different scales of biomass in (a) and (b)

In the benthic habitat, the biomass of heterotrophic bacteria (Fig. 2b, mean 3350 mg C m^{-2} mean cell volume 0.20 µm^3) was significantly higher (paired *t*-test, $p < 0.05$) than the biomass of microphytobenthos (mean 2670 mg C m^{-2}). Large fluctuations between the sampling dates were found in the benthic heterotrophic bacterial biomass, while the biomass of microphytobenthos showed a seasonal pattern with maximum during summer in both years. However, following the anoxic period in spring 2001, very low biomasses were recorded during late spring and early summer during that year. The microphytobenthos was mainly cyanobacteria and purple sulphur bacteria and to a lesser extent pennate diatoms.

Production

The production by microbiota (Fig. 3) was higher in the benthic than in the pelagic habitat (paired *t*-test, $p < 0.05$). Phytoplankton and heterotrophic bacterioplankton contributed equally to the pelagic production, with 24 and 25 g C m^{-2} yr^{-1}, respectively. The benthic production by microbiota was about twice as high, with microphytobenthos producing 56 g C m^{-2} yr^{-1} and benthic heterotrophic bacteria 44 g C m^{-2} yr^{-1}. The production of all microbiota showed a clear seasonality, with high summer values and low winter values.

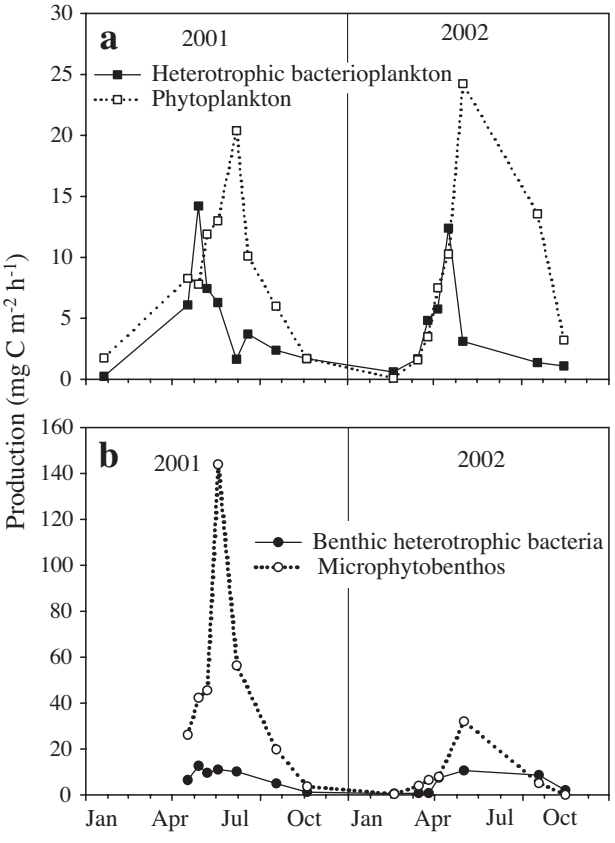

Fig. 3. Primary production and heterotrophic bacterial production (a) in a 1.5 m water column in the pelagic habitat and (b) in the top 5 cm of the microbial mat of Lake Eckarfjärden. Note the different scales of production in (a) and (b)

The total primary production (pelagic and benthic) was higher than the total heterotrophic bacterial production (paired *t*-test, $p < 0.05$). On an annual basis, the primary production was 80 g C m^{-2}, while bacterial production was 69 g C m^{-2}. In the benthic habitat, the daily production by microphytobenthos was significantly higher than the benthic heterotrophic bacterial production (paired *t*-test, $p < 0.05$). Correspondingly, phytoplankton production was significantly higher than the heterotrophic bacterioplankton production when comparing the measured values h^{-1} (Fig. 3a). However, when calculated on a daily basis, including adjustment to varying light intensities for primary production, no significant differences were found.

The measured primary production values ranged between 0.1 and 24 mg C m^{-2} h^{-1} for phytoplankton, and between 0.03 and 144 mg C m^{-2} h^{-1} for microphytobenthos (Fig. 3). In general, the production of microphytobenthos was higher during 2001 than 2002. The benthic heterotrophic bacterial production ranged between 0.6 and 17 mg C m^{-2} h^{-1} and the heterotrophic bacterioplankton production between 0.2 and 14 mg C m^{-2} h^{-1} (Fig. 3).

Biomass vs. production

Strong correlations were found between biomass and production in the pelagic habitat (Table 2). The biomass heterotrophic bacterioplankton was positively correlated to the heterotrophic bacterioplankton production ($R^2 = 0.734$, $p < 0.01$) as was phytoplankton biomass to phytoplankton production ($R^2 = 0.629$, $p < 0.05$). In the benthic habitat, no correlations were found between the biomass of the microbiota and their production.

The production per biomass (specific production) of microbiota was generally much higher in the pelagic habitat than in the sediment (paired *t*-test, $p < 0.001$). The average specific production for phytoplankton and microphytobenthos was 0.14 and 0.013 g C g C^{-1} h^{-1}, respectively. Heterotrophic bacterioplankton and benthic heterotrophic bacteria had a specific production of 0.13 and 0.003 g C g C^{-1} h^{-1}, respectively. A pronounced seasonality in specific production was found for heterotrophic bacterioplankton, with summer maxima and winter minima. This corresponds well to the observed fluctuations in biomass. In contrast, in the sediment, the specific benthic heterotrophic bacterial production showed an opposite fluctuation to the bio-

Table 2. Correlation matrix for biomass and production of microbiota in Lake Eckarfjärden during 2001 and 2002 (Pearson correlation test). Correlations are indicated with r^2 values when $p < 0.05$. –: no significant correlation. B: biomass; BP: bacterial production; PPH: primary production h^{-1}; PPD: primary production d^{-1}; bact. pl: heterotrophic bacterioplankton; bent. bact.: heterotrophic benthic bacteria; phytopl.: phytoplankton; microphytob.: microphytobenthos

	B bact. pl.	B bent. bact.	B phytopl.	B microphytob.	BP bact. pl.	BP bent. bact.	PPH phytopl.	PPH microphytob.	PPD phytopl.
PPD microphytob.	0.67	–	–	–	–	0.58	–	0.98	–
PPD phytopl.	–	–	–	–	–	0.78	0.97	0.54	
PPH microphytob.	0.73	–	–	–	–	0.64	–		
PPH phytopl.	–	–	0.63	–	–	0.79			
BP bent. bact.	0.59	–	0.61	–	0.59				
BP bact. pl.	0.59	0.64	–	–					
B microphytob.	–	–	–						
B phytopl.	–	–							
B bent. bact.	–								

mass, i.e. when the biomass was low, the specific production was high and vice versa.

Correlations were also found between heterotrophic bacteria and primary producers (Table 2). The production of benthic heterotrophic bacteria was correlated to primary production of both phytoplankton ($R^2 = 0.794$, $p < 0.01$) and microphytobenthos ($R^2 = 0.642$, $p < 0.05$). There was also a relatively strong correlation between the biomass of heterotrophic bacterioplankton and the production of microphytobenthos ($R^2 = 0.667$, $p < 0.01$). However, no correlations were found between heterotrophic bacterioplankton production and primary production.

Couplings between microbiota and abiotic factors

All production by microbiota was positively correlated to temperature and thus showed seasonal variation (Table 3). In addition, heterotrophic bacterioplankton production was positively correlated to PO_4 concentrations ($R^2 = 0.667$, $p < 0.05$). The production of microphytobenthos d^{-1} was positively, albeit weakly, correlated to DOC concentrations in the water ($R^2 = 0.544$, $p < 0.05$). No microbial parameter showed any significant correlation with water colour, total-P, NO_3, total-N or TOC.

Table 3. Correlation matrix between the production of microbiota and abiotic factors in Lake Eckarfjärden during 2001 and 2002. Correlations are indicated with r^2 values when $p < 0.05$. –: no significant correlation. Abbreviations as in Table 1

	Temp.	pH	Cond.	Alk.	MRP	NH_4	DOC
BP bact. pl.	0.61	0.48	–	–	0.59	–	–
BP bent. bact.	0.89	–	–0.64	–0.66	–	–0.63	–
PPH phytopl.	0.75	–	0.75	–0.64	–	–0.62	–
PPH microphytob.	0.68	–	–	–0.71	–	–	–
PPD phytopl.	0.74	0.49	0.73	–0.66	–	–0.64	–
PPD microphytob.	0.61	–	–	–0.74	–	–	0.54

CO_2-saturation

On both occasions when CO_2-saturation was measured, Lake Eckarfjärden was under-saturated with respect to CO_2. In summer (17 July 2002) the CO_2-saturation was 92% and in autumn (3 October 2002) the saturation was 69%.

DISCUSSION

Three main results evolve from this study. (1) The microbiota were concentrated in the benthic habitat, and the total benthic production of microbiota was much higher than the total pelagic production of microbiota. (2) Despite the very high DOC concentrations in the water, the total primary production was higher than the total heterotrophic bacterial production. (3) The lake was most probably net autotrophic as indicated by CO_2 under-saturation and by the lower concentrations of DOC in the inlets than in the lake and the outlet.

Dominance of benthic primary production has also been shown for other shallow clearwater lakes (e.g. Wetzel 1996 and references therein). The dominance of mixotrophic species indicates that the pelagic primary production was limited by inorganic nutrients (Fagerbakke et al. 1996, Isaksson et al. 1999). For microphytobenthos, which have access to nutrients from the sediment, limitation by light would be expected. In Lake Eckarfjärden, however, light availability is high enough to enable a high benthic primary production and sustain a large biomass of microphytobenthos.

Although primary production and heterotrophic bacterial production were higher within the benthic habitat than in the pelagial, the specific growth rates were much

lower. A large fraction of the sediment bacteria is generally considered to be inactive or non-growing, resulting in low specific growth rates (Boström et al. 1989, Haglund et al. 2003). The low specific growth rate of microphytobenthos might be caused by light limitation. Mixing (resuspension, bioturbation, gas ebullition, etc.) of the unusually thick microbial mat of Lake Eckarfjärden may be essential for microphytobenthos to sustain and eventually reach layers where there is enough light for photosynthesis. Oxygen limitation may also restrict the growth rate (Peterson 1996). A large decrease in biomass of microphytobenthos was recorded in Lake Eckarfjärden following an anoxic period in the bottom waters in March 2001, indicating that microphytobenthos did suffer from oxygen stress during this last month of the ice-covered period. However, despite low specific growth rates in the benthic habitat, the production was still high enough to exceed the pelagic production. When instead comparing across the habitats, the total primary production of phytoplankton and microphytobenthos was found to exceed the total heterotrophic bacterial production.

In a comparative study of 11 lakes, Jansson et al. (2000) concluded that bacterioplankton production exceeds phytoplankton primary production at DOC concentrations above 10 mg C l^{-1}. This is well below the 25 mg C l^{-1} that was found in Lake Eckarfjärden, but still the heterotrophic bacterioplankton production did not exceed, but was on the same level as, the phytoplankton primary production. Lakes with significant amounts of mixotrophs usually have higher biomass of heterotrophic bacteria than of phytoplankton (Simon & Azam 1992). This was also not found in Lake Eckarfjärden, where the biomass of heterotrophic bacterioplankton and phytoplankton was of similar size. This indicates that heterotrophic bacterial growth, despite the high DOC concentrations, was limited by carbon or at least co-limited by carbon and phosphorus. Carbon limitation would render a correlation between the primary production (i.e. newly released DOC exudates) and the heterotrophic bacterial production. This was also found in our dataset: the heterotrophic bacterioplankton biomass was correlated to the production of microphytobenthos, and the benthic heterotrophic bacterial production was correlated to the primary production of both phytoplankton and microphytobenthos. Another possible regulating factor for the heterotrophic bacterioplankton was the mixotrophic part of the phytoplankton, which during periods of intensive grazing might depress bacterial biomass and/or stimulate bacterial growth through regeneration of nutrients and carbon. A correlation was found between the biomass of mixotrophs and heterotrophic bacterial production ($r^2 = 0.59$, $p < 0.05$), but not between that of mixotrophs and heterotrophic bacteria. Hence P limitation cannot be excluded for the pelagic bacteria. However, summarising the available data we conclude that the heterotrophic bacteria are to a large extent regulated by the availability of carbon, despite the high DOC concentrations within the lake.

Traditionally, autochthonous carbon has been considered as a high quality carbon source for bacteria (Wetzel 1995) whereas a minor fraction of allochthonous carbon (10% according to Tranvik 1988) is available for bacterial growth. The low water colour in Lake Eckarfjärden, the differences in DOC concentrations between in- and outlet, and the fact that primary production by microphytobenthos was correlated to DOC imply that much of the dissolved carbon is produced within the lake. Hence, it should be a good carbon source for the bacteria. However, a relatively large amount of UV light may penetrate the shallow and clear lake water and transfer autochthonous carbon to forms that are less available for bacteria (Tranvik & Bertilsson 2001). Further characterisation of the DOC of Lake Eckarfjärden is thus needed to evaluate the role of carbon as a regulating factor for heterotrophic bacterial growth.

The measurements of CO_2-saturation and the fact that the DOC concentrations in the inlets were lower than in the lake and in the outlet indicate that Lake Eckarfjärden is net autotrophic. Monitoring of Lake Eckarfjärden over 3 yr (11 measurements during 2002) also showed that DOC concentrations were 'remarkably lower' in the inlet than in the outlet (Sonesten 2005). However, the calculated carbon produced by primary production of microbiota was not enough to sustain the bacterial production: assuming a growth efficiency of 25%, which is the mean bacterial growth efficiency found in lakes by del Giorgio et al. (1997), the primary production has to be 3 times as large as the heterotrophic bacterial production to achieve a net autotrophic system. Thus, other primary producers in the lake ecosystem must contribute substantially to the primary production. A good candidate for this role is the macroalgae *Chara* spp., forming meadows that cover approximately half of the lake area. The spatial distribution of *Chara* spp. in the lake has been determined using GPS and digital GPS (DGPS) equipment (Blomqvist et al. 2002, cf. Fig. 1). Studies performed in other *Chara* spp. lakes (reviewed in Kufel & Kufel 2002) report very high biomasses, ranging from 43 to 500 g DW m^{-2} and commonly above 200 g DW m^{-2}. Andersson & Kumblad (in press) used production values from Pereyra-Ramos (1981) and the mean biomass reported from Kufel & Kufel (2002) to calculate the production of *Chara* spp. in Lake Eckarfjärden. The result showed that *Chara* spp. contributes almost 140 g C m^{-2} yr^{-1} to the total primary production in the lake. This is in the lower range of reported production measure-

ments, e.g. 2 and 9 times lower than if calculated by production values from Hough & Putt (1988) and Kautsky (1995), respectively. However, this lower range of calculated *Chara* spp. production, together with the primary production of microbiota, is enough to render a total primary production that is higher than the bacterial respiration, and thus a net autotrophic system. Dark respiration by the primary producers during night hours is not included in the calculations. However, dark respiration is usually of minor importance compared to respiration during light; this is especially pronounced for cyanobacteria, the dominating benthic primary producer of Lake Eckarfjärden (Smith 1982, Geider & MacIntyre 2002).

Moreover, if the heterotrophic bacterial production is limited by carbon (as argued above), it is reasonable to assume a coupling to exudates from the primary production (Neely & Wetzel 1995, Espeland et al. 2001). The daily and yearly bacterial production in Lake Eckarfjärden thus might be overestimated in our study. If calculated only for the light hours, the bacterial respiration would be less than originally estimated, which further supports that net autotrophic conditions prevail.

Del Giorgio et al. (1997) found that bacterial respiration tends to exceed primary production in the pelagial in low-productive systems (pp <100 µg C l^{-1} d^{-1}). As a large part of the world's lakes are low-productive this would render that a majority of lakes are net heterotrophic. However, our study shows that even when the pelagic primary production is low (average 55 µg l^{-1} d^{-1}) the lake can be net autotrophic. The pelagial in Lake Eckarfjärden could be considered as net heterotrophic, but when including the benthic habitat the lake is net autotrophic.

In conclusion, our results clearly demonstrate the importance of including the benthic habitat when evaluating whether lakes are net autotrophic or heterotrophic. Despite very high DOC concentrations, the primary production of Lake Eckarfjärden was substantially higher than the bacterial production. This could also be the case in other lakes, especially in shallow and low-productive systems where the littoral habitat may contribute significantly to the total lake metabolism.

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