

Abundance, biomass and size structure of the microbial assemblage in the high mountain lake Gossenköllesee (Tyrol, Austria) during the ice-free period

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ABSTRACT

The abundance, biomass and morphology of the microbial components (picocyanobacteria, heterotrophic bacteria, heterotrophic and autotrophic nanoflagellates, ciliates) of the pelagic food web of Gossenköllesee were investigated over two summer periods. The density of bacteria remained relatively stable not only over time but also in vertical profiles ($2.5 - 5.5 \times 10^5$ cells mL^{-1}). Bacterial biomass ranged between 35 to 63 mgC m^{-2} ($5.4 - 15.3 \mu\text{gC l}^{-1}$). Small rod shaped bacteria with mean cell volumes of $\sim 0.05 \mu\text{m}^3$ dominated numerically but filamentous forms (longer than 10 μm), mainly found in the upper water layers, amounting to more than 65% of the total bacterial biomass, increased the mean cell volume up to $0.27 \mu\text{m}^3$ ($\text{SD}=0.88$). Bacterial biomass represented between 48 and 86% of the total microbial biomass ($40 - 90 \text{ mgC m}^{-2}$), however at 8 m depth the biomass of heterotrophic flagellates (HNF) reached up to 26 mgC m^{-2} ($2,852 \text{ cells mL}^{-1}$). From 0-4 m depth small spherical HNF species with cell volumes $< 10 \mu\text{m}^3$ were dominant, whereas in deeper water layers large flagellates with cell volumes $> 50 \mu\text{m}^3$ dominated throughout the sampling period. Ciliate abundance was low in the upper part of the water column. Only *Askenasia chlorelligera* and *Urotricha* cf. *pelagica* occurred with numbers of up to 1,500 cells l^{-1} . At 8 m depth, *Balanion planctonicum* was the dominant species throughout the study period reaching numbers of up to 16,000 cells l^{-1} . Ciliate abundance was significantly correlated with chlorophyll-a concentrations ($r_s=0.55$, $p < 0.01$) confirming the algivory of the dominant species. No significant correlation was found between the parameters of chlorophyll-a, bacteria and HNF but the abundance of ciliates was negatively correlated with the length of bacteria ($r_s = -0.41$, $p < 0.05$).

Key words: bacterioplankton, heterotrophic flagellates, ciliates, microbial biomass, alpine lake, high mountain lake

1. INTRODUCTION

High mountain lakes have been of interest to biologists since the beginning of this century (Zschokke 1900, Steinböck 1938, 1955). These lakes, situated above the timberline, have relatively small catchment areas with little or no vegetation and are, consequently very oligotrophic.

One of the first and basic information about living assemblages and biological processes of these ecosystems is given by Eppacher (1966), Pechlaner (1969) and Tilzer (1973). Compared to more eutrophic systems, the pelagic food webs of these oligotrophic lakes seem to be less complex (Fott *et al.* 1992) and the microbial loop might play an important role in recycling nutrients for higher trophic levels (Weisse & Stockner 1993). Straškrabová & Šimek (1993) suggested that the significance of the pelagic microbial assemblage increases with oligotrophication and acidification of the lake water.

Up to now little information is available on the temporal and spatial evolution of microbial pelagic food webs of high mountain lakes (e.g., Felip 1997), while many recent papers focus on more specific topics like the effect of UVB radiation on bacterivory (Sommaruga *et al.* 1996), bacterial diversity and activity (Alfreider *et al.* 1996; Posch *et al.* 1997; Pernthaler *et al.* 1998) and palaeolimnology (Koinig *et al.* 1998). Other authors describe the impact of the melting winter

cover on microbial dynamics in the pelagic zone (Pernthaler *et al.* 1997; Andreatta 1998) or compare the microbial standing stock of various high mountain lakes (Sattler 1998).

This study was carried out within the Mountain Lakes Research (MOLAR) Program of the European Community (Patrick *et al.* 1998) to test the hypothesis that the importance of pelagic microbial food web increases with acidification. In this report we show the composition, biomass, size, and relationships between the members of the microbial food web (autotrophic picoplankton, heterotrophic bacteria, autotrophic / heterotrophic nanoflagellates, and ciliates) of Gossenköllesee throughout two ice-free summer seasons.

2. MATERIAL AND METHODS

2.1. The study site

Gossenköllesee, a small (1.7 ha) and shallow ($z_{\text{max}}=9.9$ m, $z_{\text{mean}}=4.7$ m) lake is situated in a silicious catchment area in the Austrian Alps at the altitude of 2,417 m a.s.l. Further information about the most important chemical (Tab. 1) and hydrological parameters is given elsewhere (e.g., Felip *et al.* 1995; Sommaruga & Psenner 1997; Thies *et al.* 1999).

2.2. Sampling

Samples were taken at the deepest point of the lake during the icefree period in summer 1996 and 1997.

We took samples at 1, 2, 4, 6 and 8 m depth with a 5-L Schindler-Patalas sampler. Subsamples for all investigated organisms - bacterioplankton (BAC), heterotrophic nanoflagellates (HNF), autotrophic nanoflagellates (ANF; only in 1997), ciliates (CIL) and picocyanobacteria (PICY) - were collected from the same sampler.

2.2.1. Enumeration, abundance and biomass

Picocyanobacteria (PICY) – 100 ml subsamples were fixed with NaCacodylate solution. 25 ml were filtered onto black membrane filters and enumerated by epifluorescence microscopy using phycoerythrin excitation. A minimum of 200 counting grids were inspected.

2.2.2. BAC, HNF and ANF

Subsamples of 100 ml were fixed with 36% formaldehyde (final conc. 2% v/v) and stored at 4 °C until processing within 14 days. For enumeration, small volumes (10 - 25 ml) were filtered onto black membrane filters (Poretics 0.2 µm for BAC and 1 µm for HNF/ANF, respectively), stained with the fluorochrome DAPI (4',6'-diamidino-2-phenylindole) and counted by epifluorescence microscopy (Zeiss Axio-plan) according to Porter & Feig (1980). A minimum of 400 bacteria were counted in at least 10 counting grids. We inspected a minimum of 200 counting grids for the estimation of flagellate numbers. Each flagellate was checked for autofluorescence by using green excitation.

Bacterial cell volumes and shapes were determined with an image analysis system (LUCIA, Laboratory Imaging, Prague, CZ) as described by Loferer-Krößbacher *et al.* (1998). Cell volumes (V) were converted to bacterial biomass (C) using the equation given by Norland (1993)

$$C \text{ (fg)} = 120 \times V^{0.76} \quad (1)$$

For estimating HNF and ANF biomass, cell lengths (L) and widths (w) of 50 individuals were measured. We considered flagellates as prolate spheroids and calculated therefore the cell volumes as

$$V = (\pi/6 \times L) \times w^2 \quad (2)$$

Cell volumes were converted to carbon units using the factor of 220 fg C per µm³ biovolume (Borsheim & Bratback 1987).

For ciliates, subsamples of 500 ml lake water were fixed with acid Lugol's solution. In case of longer storage they were postfixated with 50 ml of filtered formaldehyde 36%. Ciliates were counted under an inverse microscope (Zeiss Axiovert) after sedimentation of a 60 ml subsample into Utermöhl chambers (500 ml when CIL were very scarce). The size of at least 30-50 ciliates was determined by using an eyepiece micrometer. Biovolumes were corrected by a shrinking

factor of 1.4 (Müller & Geller 1993) and converted to carbon units using the factor of 140 fgC per µm³ biovolume (Putt & Stoecker 1989). For a more detailed description of sampling and elaboration procedures see Straškrabová *et al.* (1999, this issue).

Physical and chemical background data were collected by applying the methods described in the methodical manual of the MOLAR project (Wathne 1996) and by Thies *et al.* (1999).

3. RESULTS

3.1. Temperature, chlorophyll-*a* and nutrients in 1997

Spring thermal mixis began already during the melting phase of the lake ice-cover by the end of June. One week after ice break-up (July 3rd) the lake was completely stratified. The epilimnion expanded down to 4 m depth with temperatures between 11 and 13.1 °C (August – September). At 8 m depth, the temperatures remained relatively low (6-8 °C) throughout the summer season (Fig. 1a). With ongoing stratification the chlorophyll-*a* maximum shifted down to the deepest layer and remained there until autumnal mixis (Tab. 1). Except in September the whole water column was well oxygenated (oxygen saturation: 97-105%). Maximum values were observed in August at 6 and 8 m depth with up to 125% saturation (Fig 1b). Total dissolved phosphorous (TDP) (Fig. 1d) accounted for 15 to 70% of total phosphorous and reached the maximum value of 1.8 µg l⁻¹ at 8 m depth in July and September. Dissolved organic carbon (DOC) was between 0.31 and 0.54 mg l⁻¹ and tended to increase during summer (Fig 1c), it was highest at the surface during the autumnal mixis in October (Tab. 1).

Tab. 1. Range of the main chemical parameters during the 1997 sampling period.

		mean	min	max
Temperature	(°C)	8.4	4.6	13.1
pH		7.0	6.7	7.2
Conductivity 25 °C	(µS cm ⁻¹)	20.7	19.6	23.9
Alkalinity	(µeq l ⁻¹)	96.6	89.0	112.0
O ₂	(mg l ⁻¹)	9.0	7.6	11.1
Total Phosphorous	(µg l ⁻¹)	2.7	1.5	6.2
Tot. Dissolved Phosph.	(µg l ⁻¹)	1.0	0.6	1.8
DOC	(mg l ⁻¹)	0.4	0.3	0.5
Dissolved Nitrogen	(mg l ⁻¹)	0.3	0.3	0.4
Chlorophyll- <i>a</i>	(µg l ⁻¹)	1.8	0.7	3.7

3.2. Abundances

Picocyanobacteria occurred at very small densities ranging from 0 to 696 cells ml⁻¹ throughout the two sampling periods. Therefore, no cell-volumes were estimated. Most cells were attached to detritus aggregates and no specific pattern of distribution could be observed (data not shown).

Bacterial cell numbers ranged between 2.5 and 5.5×10⁵ ml⁻¹ (Tab. 2). In both summer seasons the bacterial abundance slightly increased towards autumn.

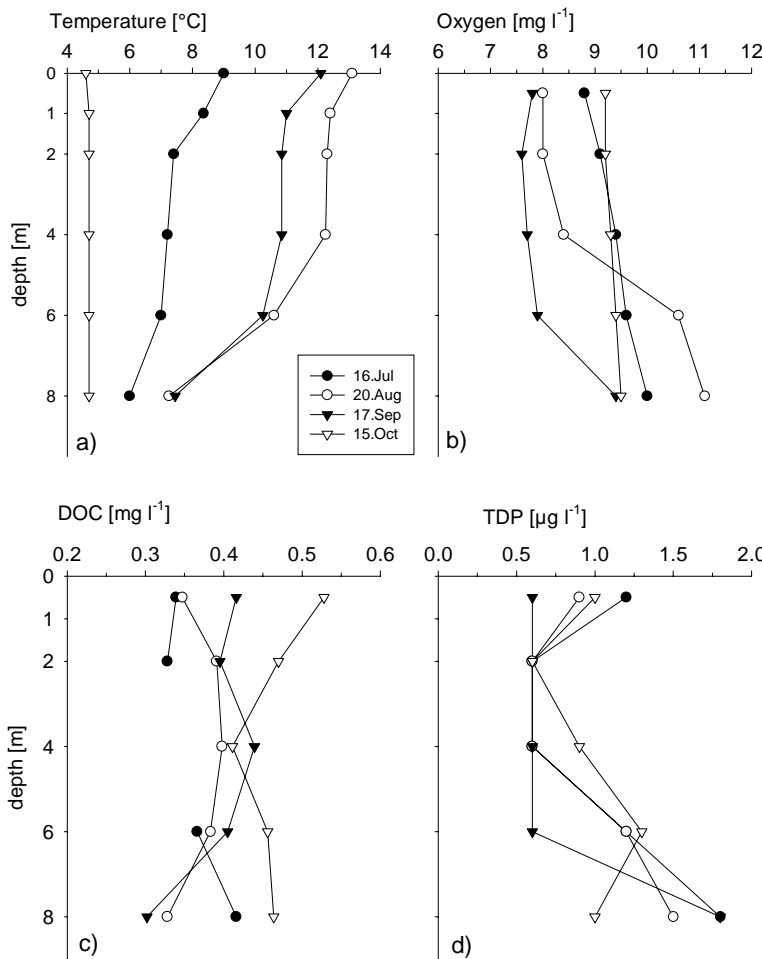


Fig. 1. Vertical profile of a) Temperature (T°C), b) dissolved oxygen (O₂), c) dissolved organic carbon (DOC), and d) total dissolved phosphorous (TDP) during the ice-free period 1997.

Tab. 2. Mean abundance of the microbial components (minimum and maximum values are in brackets). Mean values are weighted over the water column; n = 5, except for ciliates (n = 3). n.d. not determined.

Date	ANF (cells ml ⁻¹)	PICY (cells ml ⁻¹)	HNF (cells ml ⁻¹)	BAC (cells × 10 ⁵ ml ⁻¹)	CIL (cells ml ⁻¹)
23. Jul 1996	n.d.	318 (267 - 493)	1156 (598 - 2260)	3.77 (3.28 - 4.19)	2672 (333 - 16483)
06. Aug 1996	n.d.	491 (477 - 696)	920 (567 - 2852)	3.21 (2.96 - 3.26)	2458 (716 - 13383)
16. Aug 1996	n.d.	385 (145 - 473)	739 (610 - 1108)	3.55 (2.53 - 3.83)	2969 (616 - 16666)
03. Sep 1996	n.d.	247 (236 - 267)	562 (418 - 1050)	4.48 (3.36 - 4.96)	282 (233 - 516)
17. Jul 1997	634 (482 - 1123)	99 (0 - 205)	335 (321 - 431)	2.82 (2.80 - 3.16)	2054 (112 - 12771)
21. Aug 1997	867 (624 - 3363)	252 (0 - 357)	681 (482 - 2299)	3.83 (3.44 - 5.45)	1179 (861 - 2078)
17. Sep 1997	1343 (1040 - 2100)	136 (124 - 171)	474 (283 - 808)	3.37 (2.90 - 5.25)	1091 (250 - 6116)
15. Oct 1997	634 (406 - 917)	269 (141 - 393)	343 (227 - 539)	4.82 (4.41 - 5.35)	3270 (2888 - 3700)

The vertical distribution of bacterial abundance was rather homogeneous in 1996 when lowest values were always found at 8 m and the difference to the other depths never exceeded 52%. Contrastingly, in 1997 the 6 and 8 m water-layers showed highest abundances and differed from the other depths by up to 125% (Fig. 2).

With the exception of 8 m depth, HNF abundance in 1996 (5.6×10^2 to 1.5×10^3 cells ml⁻¹) was about twice as high as in 1997 (2.3 to 8.1×10^2 cells ml⁻¹) (Tab. 2). Highest cell numbers of up to 2.85×10^3 cells ml⁻¹ were found between July and August at 8 m depth near the lake bottom in both years (Fig. 2). The abundance of

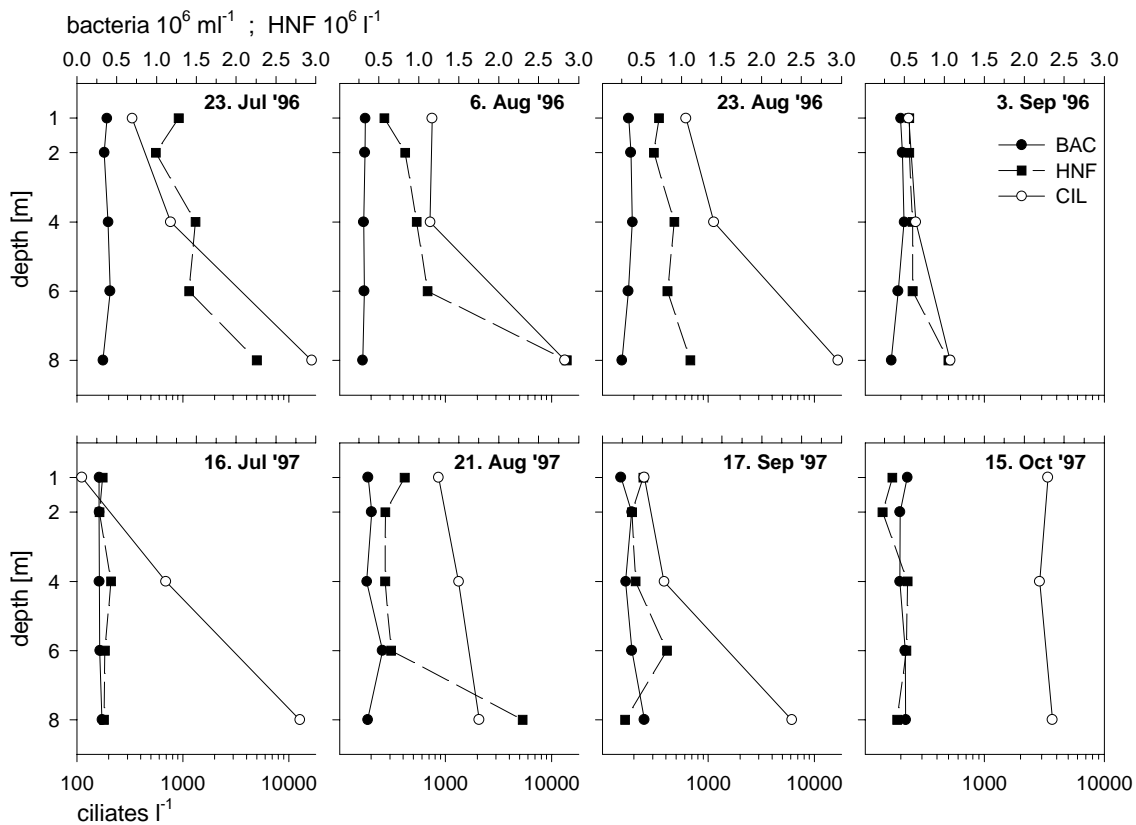


Fig. 2. Temporal and vertical distribution of the abundance of heterotrophic microorganisms during the ice-free season 1996 and 1997. HNF – heterotrophic nanoflagellates.

ANF, counted only in 1997, ranged between 4.83×10^2 and 3.36×10^3 cells ml^{-1} and showed the highest values at 8 m depth.

During both ice-free periods only three abundant ciliate species could be observed (Fig. 3). The cyclo-trich ciliate *Askenasia chlorelligera* was present in all samples with numbers between 40 and 1,500 cells l^{-1} . *Urotricha cf pelagica* occurred only at low abundances (17–133 cells l^{-1}). Another prostomatide ciliate, *Balanion planctonicum*, found at 6 and 8 m depth only, reached high densities of up to 16,300 ind l^{-1} . *Halteria grandinella* appeared only once with 17 cells l^{-1} . Additionally some single observations of *Lagynophrya acuminata* and *Paramecium cf putrinum* completed the species list of the ciliate assemblage.

3.3. Morphological diversity of bacterioplankton and heterotrophic nanoflagellates

Comparing the epilimnetic (1–4 m depth) and meta-hypolimnetic (6–8 m depth) layers, we could distinguish different morphotypes of the microbial assemblage during summer stratification. In both years a very similar pattern was observed (shown for 1996 in Fig. 4): bacterioplankton was always dominated by small rod-shaped forms with a cell volume of $0.05 \mu\text{m}^3$. Towards autumn the frequency of filamentous forms (longer than $50 \mu\text{m}$) increased in the upper water-lay-

ers, whereas in 8 m depth the abundance of threadlike bacteria remained small until fall overturn. Therefore, higher mean cell volumes were found at the surface (total range from 0.076 up to $0.265 \mu\text{m}^3$) than in deeper water-layers (total range 0.076 – $0.144 \mu\text{m}^3$).

Small HNF-species of up to $5 \mu\text{m}$ cell length, corresponding to a cell volume of 3 – $35 \mu\text{m}^3$, were dominant at the surface-layers but were less frequent near the bottom. Larger, 7 to $10 \mu\text{m}$ long flagellates with cell volumes $>50 \mu\text{m}^3$ occurred mainly at 8 m depth where they accounted for up to 85% of the total abundance (Fig. 4).

3.4. Biomass

Integrated microbial biomass ranged between 40 and 94 mgC m^{-2} (8.5 – $20 \mu\text{gC l}^{-1}$) showing a trend to increase towards the autumn. Except for one date, bacterial biomass accounted $>50\%$ (43 – 81%) of total heterotrophic microbial biomass, whereas HNF and CIL contributed 9 to 44% and 4 to 21% , respectively (Fig. 5).

The spatial range of heterotrophic biomass is listed in table 3. During thermal stratification maximum values of up to $39 \mu\text{gC l}^{-1}$ occurred at 8 m depth. Only there the proportion of the protists exceeded bacterial standing stock (Fig. 6).

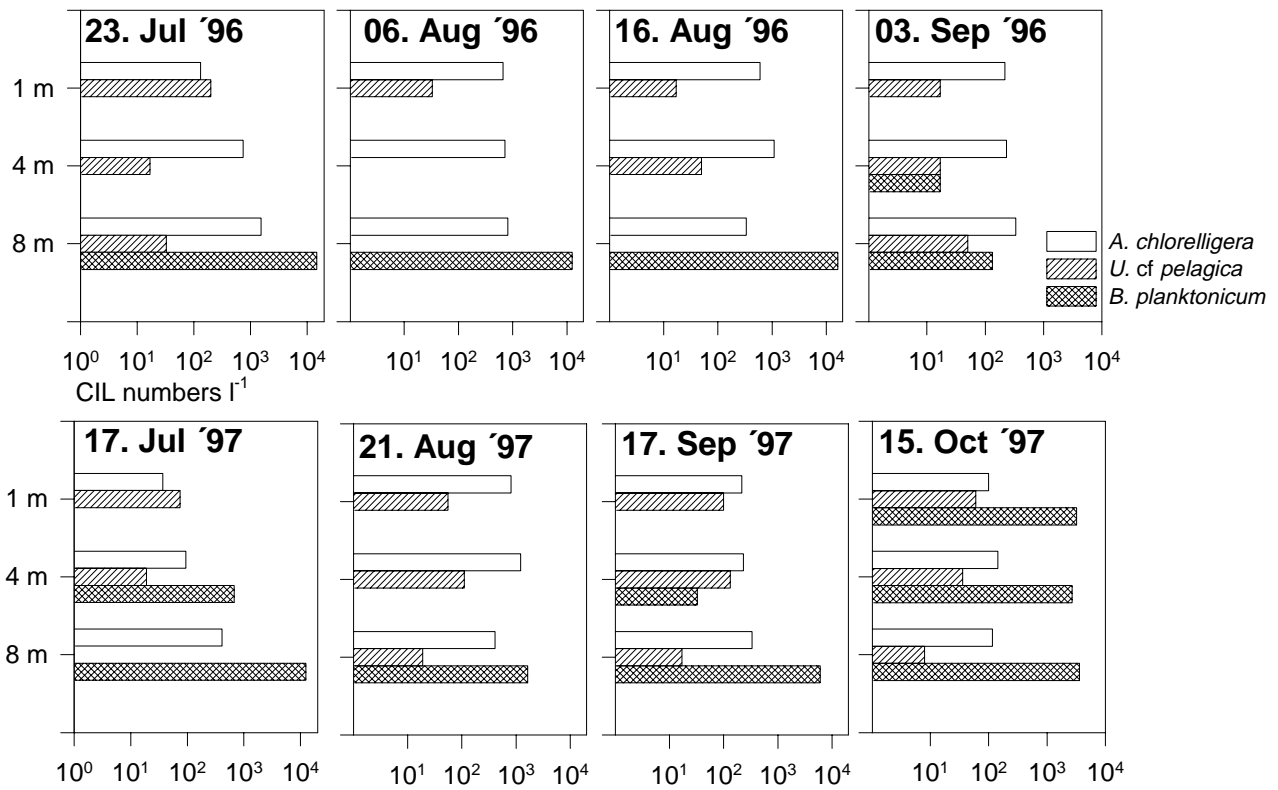


Fig. 3. Temporal and spatial distribution of abundant ciliate species during the ice-free period 1996 and 1997.

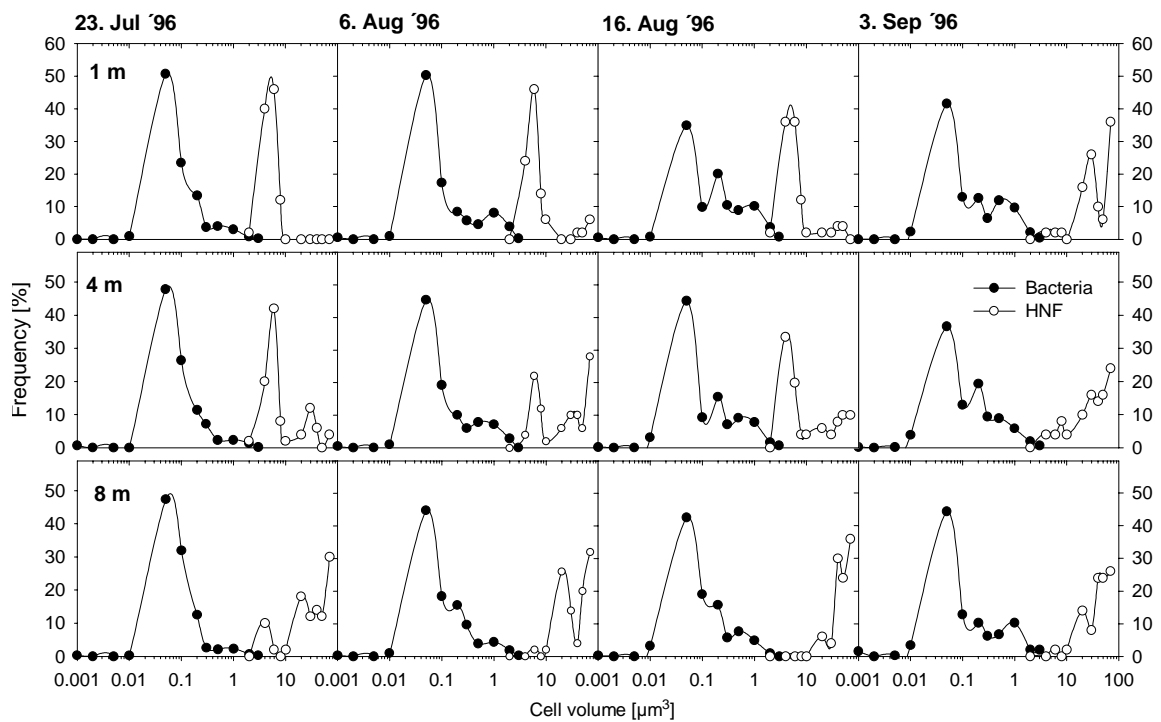


Fig. 4. Cell volume distribution of bacteria and heterotrophic nanoflagellates during summer 1996.

Bacterial biomass ranged between 5.4 and 15.3 $\mu\text{gC l}^{-1}$ and was significantly influenced by the length of the cells ($r_s = 0.68$, $p < 0.001$; Tab. 4). Filaments longer than 6.4 μm formed 16 to 67% of the bacterial standing stock (Fig. 7). This means that a considerable amount of bacterial biomass cannot be ingested by flagellates and smaller ciliates. The mean carbon content of a single bacterial cell was between 16.4–37.1 fg with lowest values occurring at 8 m depth.

The biomass of HNF ranged between 0.5 and 13.6 $\mu\text{gC l}^{-1}$ from 1–6 m depth, highest values were observed in both years in August at 8 m depth with 26.1 and 25.3 $\mu\text{gC l}^{-1}$, respectively. At this time also ANF, with the dominant genus *Rhodomonas* sp., showed a biomass peak of 67 $\mu\text{gC l}^{-1}$ at 8 m, whereas in other depths the biomass was equivalent to the bacterial standing stock (Tab. 3).

The biomass of *Askenasia chlorelligera* ($L_{\text{mean}} =$

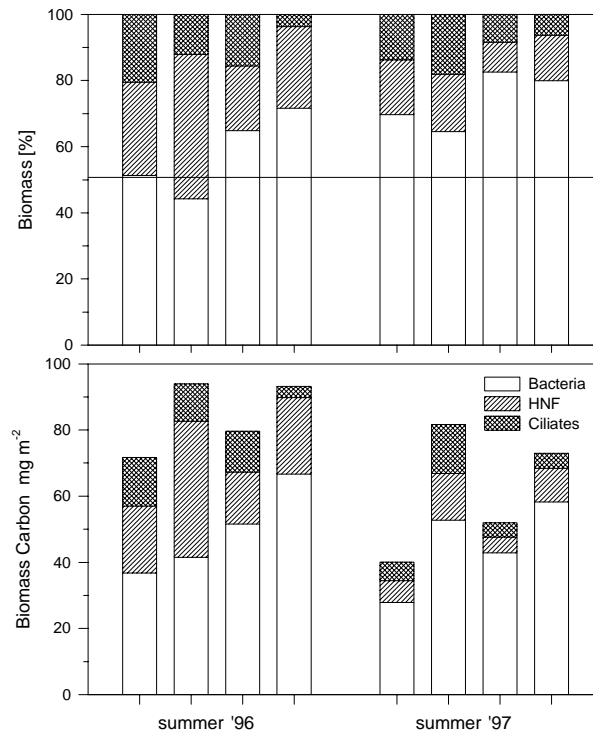


Fig. 5. Total microbial biomass of bacteria, heterotrophic nanoflagellates and ciliates in the water column during summer 1996 and 1997 (weighted means).

Tab. 3. Mean biomass (mgC m^{-3}) of the microbial components (minimum and maximum values are in brackets). Mean values are weighted over the water column; $n = 5$, except for ciliates ($n = 3$). n.d. not determined.

Date	ANF	HNF	BAC	CIL
23. Jul 1996	n.d.	3.39 (1.3 - 18.0)	8.58 (6.5 - 10.9)	3.22 (1.2 - 10.8)
06. Aug 1996	n.d.	6.23 (1.9 - 26.1)	8.90 (7.6 - 9.6)	2.48 (2.0 - 5.1)
16. Aug 1996	n.d.	2.81 (1.2 - 11.4)	11.29 (6.1 - 12.5)	2.72 (1.8 - 5.1)
03. Sep 1996	n.d.	4.62 (4.0 - 9.1)	14.58 (11.6 - 15.3)	0.75 (0.7 - 1.1)
17. Jul 1997	8.11 (6.4 - 14.0)	1.43 (0.6 - 3.4)	6.11 (5.4 - 7.6)	1.21 (0.5 - 5.6)
21. Aug 1997	6.31 (2.5 - 67.0)	3.10 (1.3 - 25.3)	11.52 (7.1 - 12.8)	3.24 (1.7 - 4.8)
17. Sep 1997	7.21 (2.3 - 23.9)	1.02 (0.5 - 4.4)	9.39 (7.4 - 12.1)	0.96 (0.9 - 1.2)
15. Oct 1997	11.85 (7.1 - 20.9)	2.21 (1.5 - 3.2)	12.75 (11.1 - 14.5)	0.99 (0.9 - 1.0)

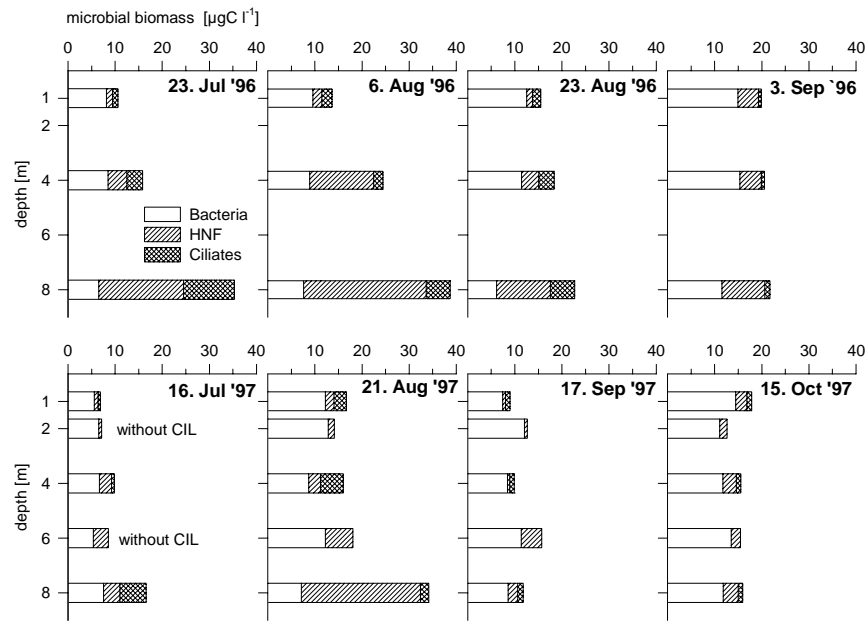


Fig. 6. Spatial distribution of heterotrophic microbial biomass. Note that 2 and 6 m in 1997 are without ciliate biomass. HNF – heterotrophic nanoflagellates; CIL - ciliates

Tab. 4. Spearman Rank correlation matrix for microbial parameters for the ice-free periods of 1996 and 1997; n= 32, except for ciliates (n= 24); level of significance: * p<0.05; ** p<0.01; *** p<0.001. CIL – ciliate abundance; BAC – bacterial abundance; HNF – heterotrophic nanoflagellates; BL – mean bacterial cell length; BB – mean bacterial biomass; chl a – chlorophyll a.

	CIL	HNF	BL	BB	BAC
Chl-a	0.55**	0.02	0.02	-0.14	0.05
BAC	0.10	-0.06	0.29	0.76***	
BB	-0.22	0	0.68***		
BL	-0.41*	-0.27			
HNF	0.14				

31.1, $w_{\text{mean}} = 31.3 \mu\text{m}$) ranged between 0.1 and $6.8 \mu\text{gC l}^{-1}$ and represented 2–49% of the ciliate biomass. Because of its abundances the small *Balanion planctonicum* ($L_{\text{mean}} = 14.6$, $w_{\text{mean}} = 11.9 \mu\text{m}$) contributed 0.2–4.3 $\mu\text{gC l}^{-1}$ (up to 51%) to the biomass of the pelagic ciliates.

4. DISCUSSION

Compared to various marine and freshwater systems, the observed bacterial abundance of $3.73 \times 10^5 \text{ cells ml}^{-1}$ is at the lower end of the range (Riemann & Christoffersen 1993), but it seems to be typical for high alpine lakes (Sattler 1998). Certainly, total bacterial numbers are useful to describe existing carbon pools in systems but in fact it is not the only variable when discussing food availability for higher trophic levels (Sherr *et al.* 1992). In this case other parameters such as the physiological activity or the shape and size of the bacterial cells are important (Gonzales *et al.* 1990; Gasol *et al.* 1995). Thus the abundance of the available

prey will be reduced by a remarkable amount when we consider that the largest flagellate in Gossenköllesee (length = 7–8 μm), may ingest only small bacteria of 2–3 μm length (see Figs 4, 7).

The occurrence of filamentous bacteria (length >10 μm) in Gossenköllesee is already known from various studies (Posch *et al.* 1997; Pernthaler *et al.* 1998). During and shortly after ice-break only small rod shaped cells occur in the water column (Pernthaler *et al.* 1997) but filamentous forms, members of the Cytophaga-Flavobacterium group (Pernthaler *et al.* 1998), develop during summer in the upper part of the water column and account for more than 50% of the total bacterial biomass.

Oligotrophic high mountain lakes generally show low abundances of heterotrophic nanoflagellates and sometimes they are under the detection limit (Sattler 1998). With the exception of the distinct peaks in August, the densities in Gossenköllesee are comparable to other alpine lakes of the Tyrol and Carinthia (0–

$1.57 \times 10^3 \text{ ml}^{-1}$) (Sattler 1998). As the abundance of the bacterioplankton is below the assumed threshold density for supporting the nutrition of heterotrophic nanoflagellates (Riemann *et al.* 1995), they might survive by mixo- or osmotrophy (Sanders 1991). Due to low particle density, low content of dissolved nutrients, and ambient light conditions even at the lake bottom, the nutrition often is based on mixotrophy (Stockner & Porter 1987; Havskum & Riemann 1996). This nutrition strategy could explain the large discrepancy between the biomasses of heterotrophic nanoflagellates and their prey bacteria, specially at 8 m depth (Fig. 6).

terial abundance and biomass because flagellates can graze effectively on newly produced bacterial cells. Riemann *et al.* (1995) showed that mixotrophic flagellates are able to switch between phago- and autotrophy within several hours by changing the chl-*a* content depending on the particle density of the prey. Specially at 8 m depth we often had problems to distinguish between auto- or heterotrophy of the dominant flagellate species because they did not show a distinct chloroplast, and the autofluorescence was very weak.

Following to the chl-*a* maxima *Balanion planctonicum* peaked at the bottom layer of the lake. This prostomatide ciliate is known to be an effective grazer on

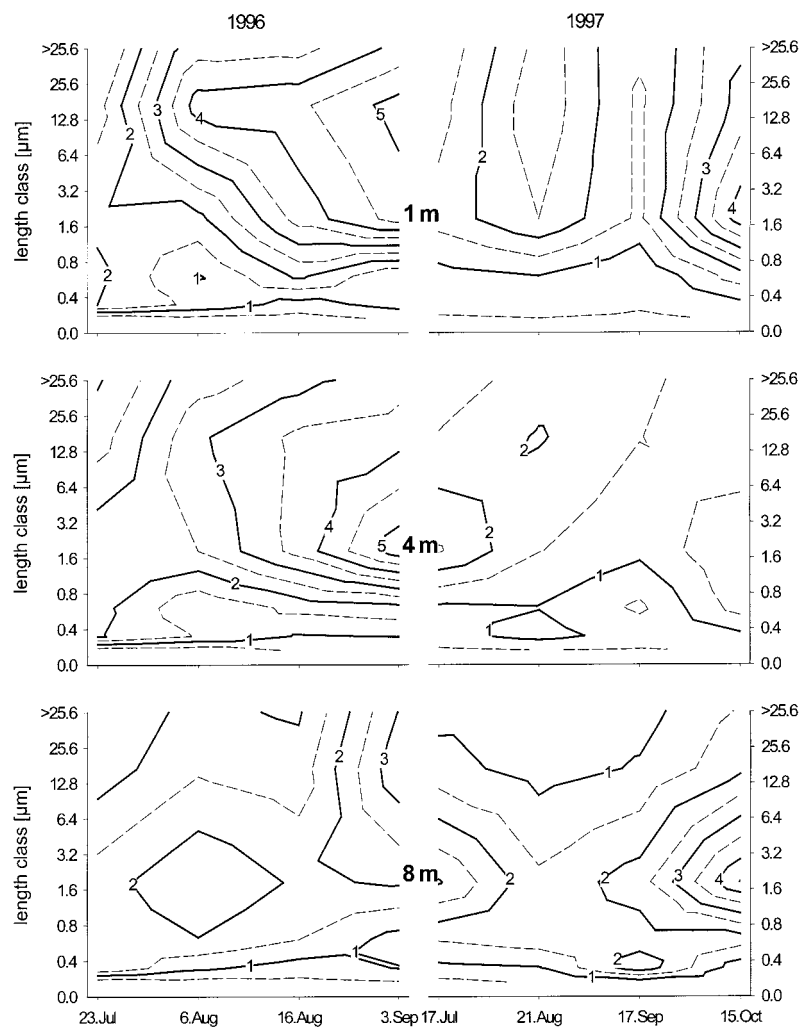


Fig. 7. Size dependant distribution of the bacterial biomass. Units are given in $\mu\text{g C l}^{-1}$.

Sanders *et al.* (1990) stated that the change from photo- to phagotrophy can occur simultaneously with increasing particle density. According to Stockner & Porter (1987), and del Giorgio *et al.* (1996) this fact could explain the small seasonal variability of the bac-

the phytoflagellate *Rhodomonas* sp. (e.g., Kentner *et al.* 1996), which was the dominant algal species in 8 m depth. Sommaruga & Psenner (1995), who studied the development of *B. planctonicum* in a subalpine lake, suggested that it may react much faster on increasing

densities of *Rhodomonas* sp. than metazooplankton, thus inducing the clear water phase in lakes which are later dominated by cladocerans. While *B. planktonicum* was restricted to layers including *Rhodomonas*, the other species, *Askenasia chlorelligera* and *Urotricha* sp., could be observed in the whole water column. The ciliate abundances of all samples were positively correlated with the chl-*a* concentrations ($r_s = 0.51$, $p < 0.01$), which suggests alivory of all ciliate groups. The negative correlation with the mean cell length of bacteria (Tab. 4) indicates that filamentous bacteria are not available as prey.

5. CONCLUSIONS

The microbial assemblages of the oligotrophic high mountain Lake Gossenköllesee are confronted with extremely low nutrient-concentrations: DOC ~ 0.4 mg l^{-1} , TDP ~ 1 μg l^{-1} , and DN ~ 0.3 mg l^{-1} . Therefore, bacterial abundances are low (3.73×10^5 cells ml^{-1}). The bacterial biomass amounted to $>50\%$ of the total heterotrophic microbial biomass. Filamentous morphotypes, which can represent $>50\%$ of the bacterial standing stock, and are thus inedible for small flagellates, seem to be typical for this lake. Although bacteria are below the reported threshold density for bacterivorous protists, the high abundance of flagellates suggests that a considerable proportion may be mixotrophic. The ciliate diversity seemed to be very poor - three abundant species which preferentially feed on algae (three species as single observations) - but can occur at high abundances (*B. planktonicum* $>16,000$ ind l^{-1}).

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