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Qualitative Importance of the Microbial Loop and Plankton Community Structure in a Eutrophic Lake during a Bloom of Cyanobacteria*

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Abstract. Plankton community structure and major pools and fluxes of carbon were observed before and after culmination of a bloom of cyanobacteria in eutrophic Frederiksborg Slotssø, Denmark. Biomass changes of heterotrophic nanoflagellates, ciliates, microzooplankton (50 to 140 μm), and macrozooplankton (larger than 140 μm) were compared to phytoplankton and bacterial production as well as micro- and macrozooplankton ingestion rates of phytoplankton and bacteria. The carbon budget was used as a means to examine causal relationships in the plankton community. Phytoplankton biomass decreased and algae smaller than 20 μm replaced *Aphanizomenon* after the culmination of cyanobacteria. Bacterial net production peaked shortly after the culmination of the bloom (510 μg C liter^{-1} d^{-1}) and decreased thereafter to a level of approximately 124 μg C liter^{-1} d^{-1}. Phytoplankton extracellular release of organic carbon accounted for only 4–9% of bacterial carbon demand. Cyclopoid copepods and small-sized cladocerans started to grow after the culmination, but food limitation probably controlled the biomass after the collapse of the bloom. Grazing of micro- and macrozooplankton were estimated from in situ experiments using labeled bacteria and algae. Macrozooplankton grazed 22% of bacterial net production during the bloom and 86% after the bloom, while microzooplankton (nauplii, rotifers and ciliates larger than 50 μm) ingested low amounts of bacteria and removed 10–16% of bacterial carbon. Both macro- and microzooplankton grazed algae smaller than 20 μm, although they did not control algal biomass. From calculated clearance rates it was found that heterotrophic nanoflagellates (40–440 ml^{-1}) grazed 3–4% of the bacterial production, while ciliates smaller than 50 μm removed 19–39% of bacterial production, supporting the idea that ciliates are an important link between bacteria and higher trophic levels. During and after the bloom of *Aphanizomenon,* major fluxes of carbon between bacteria, ciliates and crus-

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taceans were observed, and heterotrophic nanoflagellates played a minor role in the pelagic food web.

Introduction

The Plankton Ecology Group (PEG) introduced a model which predicted seasonal changes in planktonic communities and interactions between phytoplankton and zooplankton [64]. The model was based on observations from twenty-four European lakes, covering a two-order range of total phosphorous and phytoplankton biomass.

The PEG model did not, however, include bacteria, nanoflagellates, and ciliates in the analysis of interactions between zooplankton and filamentous cyanobacteria [18, 26, 51]. The general concept is that larger crustaceans species tend to decrease in numbers during severe blooms of cyanobacteria and are replaced by smaller species, which are able to feed on pico- and nanoplankton. Co-existence of *Daphnia* and cyanobacteria is, however, also reported [13, 36, 37, 59]. A major reason for the exclusion of large crustaceans is the difficulty they have in avoiding the ingestion of inedible algae along with the more edible food items [10, 43]. Direct toxic effects of cyanobacteria have also been demonstrated [18, 34].

In eutrophic lakes, zooplankton communities are exposed to strong predatory pressures caused by large populations of planktivorous fish and fish larvae [31, 46]. As fish predation and blooms of cyanobacteria are in progress at the same time, it is difficult to analyze causal relationships leading to changes in the zooplankton communities.

Recently, bacteria, flagellates, and small ciliates were considered as significant components of freshwater food webs [49]. These microheterotrophic organisms feed on bacterioplankton and form a "microbial loop" to the traditional grazer food chain [4, 61, 66]. Although large numbers of heterotrophic bacteria, nanoflagellates, and ciliates frequently have been reported in the literature [42, 57, 60, 65], few studies have included these microheterotrophs in a quantitative context.

In eutrophic Frederiksborg Slotssø, a summer depression of macrozooplankton biomass often covaries with high predation from young-of-the-year fish-stocks and dominance of netphytoplankton [56]. Further, it has been reported that heterotrophic nanoflagellates can exploit food sources when *Daphnia* are absent [52]. Dominance of filamentous cyanobacteria in combination with high fish predation might create favorable conditions for the development of microheterotrophic organisms [41]. If so, the lack of *Daphnia* and the inability of microheterotrophs to feed on most phytoplankton will determine the fate of algal production. However, quantitative studies on microbial activity relative to the grazer food chain in lakes are sparse [61, 66]. Recently, Weisse et al. [71] demonstrated that more than 50% of the primary production was channelled through the microbial loop to higher trophic levels during a spring bloom in a mesotrophic pre-alpine lake. Similar quantitative information does not exist from eutrophic lakes having dense cyanobacteria blooms.

It was the objective of this study to quantify plankton community structure

and carbon cycling during a bloom of cyanobacteria and to quantify consumption of bacterio- and phytoplankton by heterotrophs before and after the culmination of a cyanobacterial bloom.

Materials and Methods

Water samples were collected one to two times per week during August and September 1988 from Frederiksborg Slotssø, Denmark. The lake is shallow (mean depth 3.1 m, maximum depth 8 m) and highly eutrophic, with an annual primary production of about 400 g C m^{-2} [3, 30].

The temperature and oxygen content were recorded at each meter by a Yellow Spring Instrument (oxygen-temperature probe) before withdrawal of water samples. Water was collected between 9 and 11 a.m. from 1, 3, 5, and 7 m depths with a 5 liter plexiglass water-sampler. All analyses were carried out in duplicate except enumeration of zooplankton larger than 50 μm.

Chemical analyses of PO_4-P, NO_3-N and NH_4-N were carried out according to Murphy and Riley [39], Crosby [12] and Solórzano [63], respectively. Irradiance was recorded with a 2 phi quantum sensor connected to a LI-COR integrator.

Primary production was measured by the ^{14}C method. Samples (125 ml) from 0, 0.25, 1, and 2 Secchi-depths were incubated with 2 μCi ^{14}C bicarbonate (The International Agency for ^{14}C Determination) from noon to sunset. The phytoplankton was filtered onto 1.0 μm Nuclepore polycarbonate filters, which were then placed in vials to which 100 μl of 1 N HCl were added. Finally, 10 ml of scintillation cocktail (Aqualyte, Baker) were added and the samples were radioassayed by liquid scintillation counting (Tracor, Analytical). Release of extracellular organic carbon (EOC) was measured by filtering 10 ml of the 1.0 μm filtrates through a 0.2 μm filter (Nuclepore). The 0.2 μm filtrate was then added 200 μl of 1 N HCl and bubbled for 30 min [67]. Finally, 10 ml scintillation cocktail (Dynagel, Baker) was added. Bacterial EOC net uptake (B_n) was measured from ^{14}C uptake in the size fraction of 0.2 μm to 1 μm. Gross EOC release was calculated from (B_n/0.5) + EOC, assuming a bacterial growth yield of 50% [68].

Phytoplankton chlorophyll *a* was extracted overnight in 96% ethanol and measured spectrophotometrically without correction for degradation products [29].

A 100 ml water sample from each depth was fixed with acid Lugol's solution (0.4% final concentration) and later used for taxonomic identifications of phytoplankton using an inverted microscope.

Enumeration of bacteria was made by epifluorescence microscopy [25]. Cell numbers were converted to carbon biomass by multiplying with an average cell volume of 0.160 μm^3 ([44; B. Riemann unpublished data] and a carbon content of 0.35 pg C μm^{-3} [7]. Bacterial heterotrophic production was determined from ^3H-thymidine incorporation into bacterial DNA [17]. ^3H-thymidine was added to water samples from each depth to a final concentration of 15 nM and incubated in situ for 20 min. Incubations were terminated by adding formaldehyde to a final concentration of 1%, followed by filtration of the samples through 0.45 μm filters (Sartorius). Radioactivity of the filters was assayed by liquid scintillation counting, after addition of 1 ml ethyl acetate and 10 ml scintillation cocktail (Aqualyte, Beckman). A conversion factor of 2.15×10^{18} cells mol^{-1} of thymidine incorporated into cold trichloroacetic acid precipitate was applied [62]. A growth yield of 50% [54, 58] were used in further calculations.

Heterotrophic nanoflagellates were enumerated by epifluorescence microscopy on proflavine hemisulphate stained preparations mounted in paraffin oil and stored at 5°C [22]. Biovolumes were calculated from measurements of diameters of 30 individuals per filter, assuming spherical forms. The carbon biomass was obtained from multiplication by 0.12 pg C μm^{-3} [15]. Flagellate community ingestion rates of bacteria were calculated from clearance rates of 10^5 body-volumes $hour^{-1}$ [16] multiplied by the carbon biomass of the bacteria.

Ciliates in 10 ml of unfiltered water from each depth were counted. Acid Lugol's solution (0.4% final solution) was used as preservative. The number of individuals was counted in an inverted microscope in two size classes (<50 μm, >50 μm). Volumes were calculated from measurements of linear dimensions and simple geometric formulae, and finally converted to carbon biomass using

a conversion factor of 0.05 pg C μm^{-3} [5]. Clearance rates were calculated using 10^5 body-volumes [16] and transformed to community ingestion rates by multiplying by population density and bacteria biomass.

Micro- and macrozooplankton abundance were estimated from selective filtrations (50–140 μm and >140 μm) of 2 liter samples from each depth that had been fixed with 70% ethanol. Generally, two to three subsamples were counted in an inverted microscope, but in cases where fewer than 100 individuals were counted, the entire sample was assessed. Animals were identified to species and development stage except the cyclopoid copepods, which were pooled in groups of adults and copepodits. The dry weight of the dominating species of macrozooplankton was determined by weighing 100 fresh individuals on an electrobalance after drying at 60°C for 24 hours. Carbon was assumed to constitute 50% of dry weight. Biovolumes of microzooplankton were estimated from measurements of length and width and converted to biomasses assuming a specific gravity of 1, a dry to wet weight ratio of 0.1 and, finally, a dry weight to carbon ratio of 0.5 [38].

Determination of zooplankton ingestion rates of bacteria followed Bjørnsen et al. [7], using two size fractions of the zooplankton (>140 μm, 50–140 μm) and ^{3}H-thymidine labeled bacteria. Ingestion rates were obtained by multiplying filtering rates by the actual bacterial biomass from each depth. Ingestion rates of radiolabeled phytoplankton were also carried out in the two size classes of zooplankton. One or two days before the grazing experiment, 20–100 μCi ^{14}C-bicarbonate was added to 1 liter of 20 μm filtered natural phytoplankton and incubated at 0.5 Secchi depth. Just before the experiment, the labeled algae were filtered through a 20 μm mesh net and mixed with unlabeled natural lake water from each depth in the ratio of 1:6. Incubations lasted 15–20 min, and the water was immediately filtered through 140 μm and 50 μm mesh nets, respectively. The filters were washed 3 times with particle-free lake water and immediately placed in vials. In the laboratory tissue solubilizer (150 μl Sololyte, Baker) was added, and after a minimum of 20 hours at 20°C, 10 ml scintillation cocktail (Aqualyte, Baker) were added. Radioactivity of the feeding suspension was measured from 10 ml samples obtained at time zero and after the feeding period. Samples were filtered onto 0.45 μm Sartorious filters, washed with distilled water, and placed in vials with 1 ml of ethyl acetate. After 1 hour, 10 ml of scintillation cocktail were added. Further details and calculations are described in Bosselmann and Riemann [9].

Estimates of biological activities from 1, 3, 5, and 7 m depths were averaged to μg C $liter^{-1}$. Units of chlorophyll *a* were transformed to carbon using a factor of 50 estimated during an *Aphanizomenon* bloom in Frederiskborg Slotssø [55]. Measurements of carbon pools and fluxes were integrated with time for the construction of a carbon budget under a set of assumptions: (i) flagellate and ciliate (<50 μm) ingestion rates were based upon body volume clearance rates as suggested by Fenchel [16]; (ii) it was suggested that small ciliates and flagellates were bacterivorous (see review by Beaver and Crisman [6]); (iii) the growth yield of all heterotrophic organisms, except bacteria, was set to 30%, as has been reported for nanoflagellates [8], ciliates [16], and micro- and macrozooplankton [9]; (iv) tentative values of excretion rates (sloppy feeding, feces) of 35% of ingested carbon were used to calculate flagellate, ciliate, and zooplankton contributions to bacterial substrates [70].

Results

Physical-Chemical Parameters

The lake was stratified with a thermocline around 5 m depth until the middle of September. Oxygen saturation was less than 5% below 5–6 m depth, but increased gradually at the end of the period. Complete mixing of the water column occurred on 20 September (Fig. 1). Inorganic nutrients revealed distinct vertical gradients until mixing occurred. Phosphate increased gradually with time in the epilimnion (Fig. 2) and was never below 100 μg P $liter^{-1}$. Nitrate was less than 10 μg N $liter^{-1}$ in the epilimnion on 15 August (Fig. 2), after

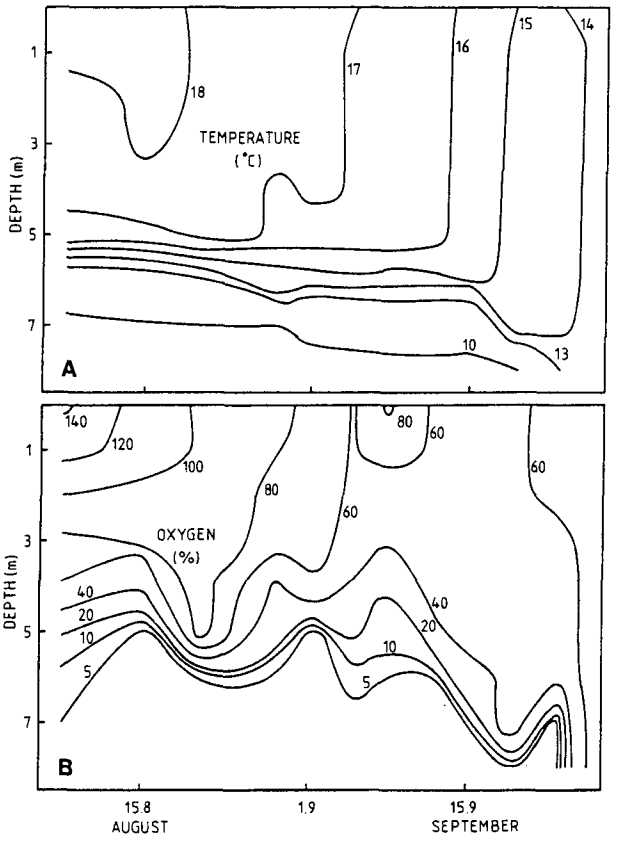

Fig. 1. Temperature (**A**) and oxygen saturation (**B**) isopleth diagrams.

which the concentration followed the pattern for phosphate. High values of nitrate were observed in the boundary layer between epi- and hypolimnion. A sharp separation was also found for ammonium (Fig. 2). Low values of ammonium covaried with low values of nitrate.

Phytoplankton

Chlorophyll *a* concentrations peaked in late August with 187 μg chlorophyll *a* liter^{-1} at the surface (Fig. 3). A subsequent rapid decrease at all depths was followed by a steady increase throughout September. Primary production was high during the build-up of new phytoplankton biomass and matched peaks of irradiance (Fig. 4). Size distributions of phytoplankton biomass and primary production showed increasing importance of cells <10 μm with time (Fig. 5), although algal cells of >50 μm contributed more than 50% to the total biomass and primary production throughout the experimental period. The transparency was low during the bloom (29–55 cm), but increased as the cyanobacterial bloom leveled off and stabilized at approximately 1 m (Fig. 4).

Gross EOC (data not shown) covaried ($r^2 = 0.301$) with phytoplankton pro-

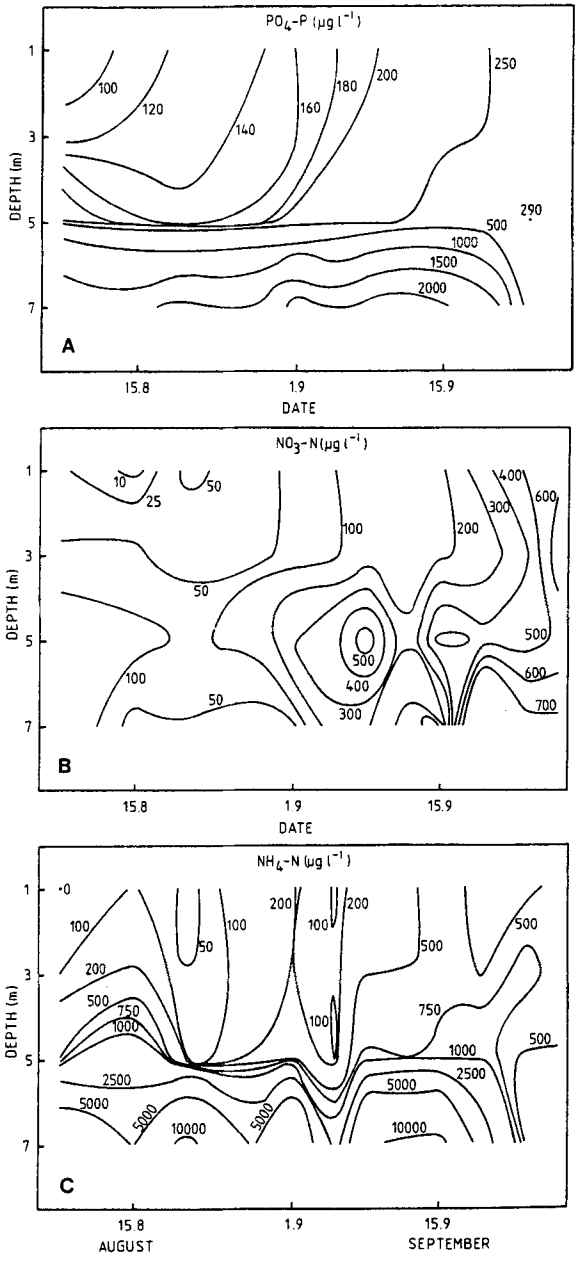

Fig. 2. Phosphate (**A**) nitrate (**B**) and ammonia (**C**) isopleth diagrams.

duction and constituted 16.3% (2.1–30.8%) of primary production. Integrated over two periods (the bloom period and the post-bloom period), gross EOC decreased from late August onwards, but increased as a percentage of bacterial gross production (Table 1).

Aphanizomenon flos-aquae dominated (Fig. 5) until the beginning of Sep-

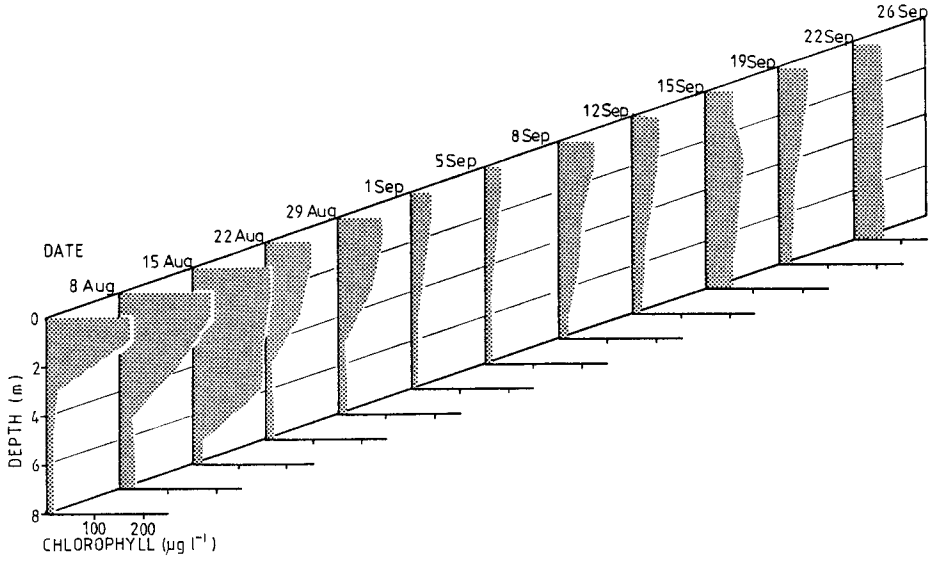

Fig. 3. Vertical changes in chlorophyll *a* during August and September. Sampling depths were 1 m, 3 m, 5 m, and 7 m.

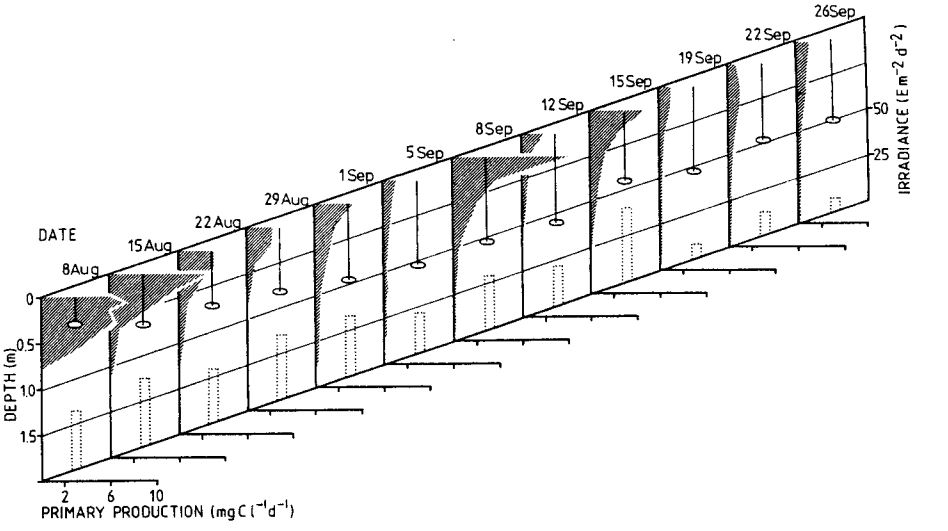

Fig. 4. Vertical changes in primary production (hatched area), transparency (illuminated by the Secchi depth), and daily surface irradiance integrated over 24 hours (dotted columns) during August and September.

tember, followed by a more diverse species composition, including *Microcystis aeruginosa, M. wesenbergii, Cryptomonas* sp., *Ceratium hirundinella, Melosira granulata, Stephanodiscus hantzschii, Pediastrum duplex, P. boryanum, Scenedesmus* spp. and *Closterium acutus.*

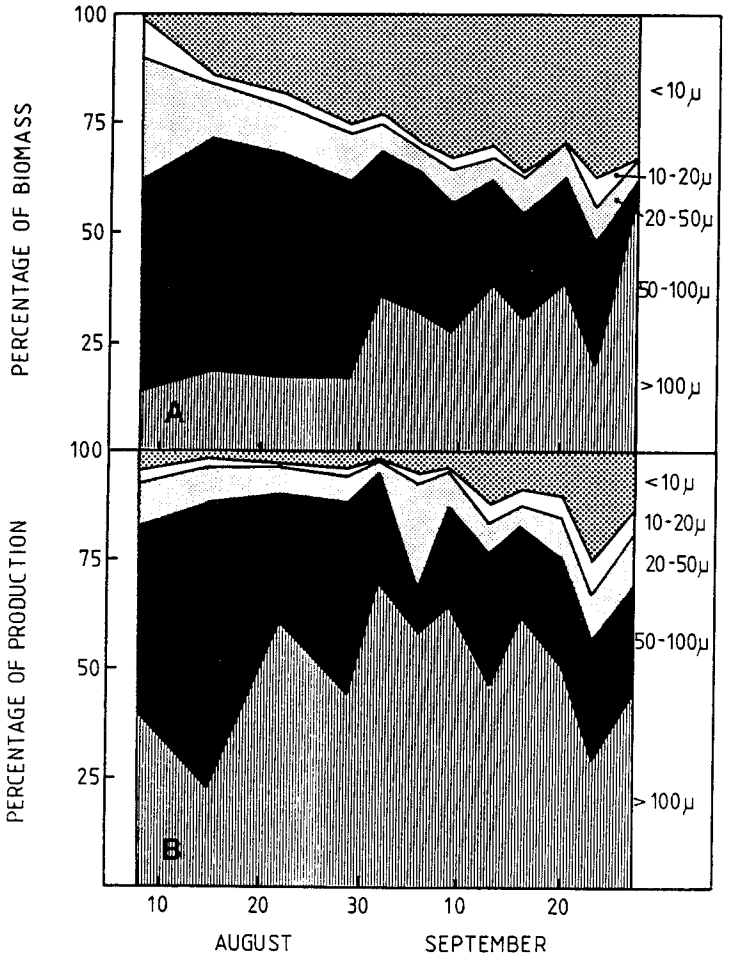

Fig. 5. Size distribution (% of total) of chlorophyll (A) and gross primary production (B).

Table 1. Extracellular organic carbon as net release (EOC_n), bacterial net uptake of EOC (B_n), gross EOC release and gross EOC as percentage of bacterial gross production (BGP). The number of measurements (n) are indicated. Values are averages for the bloom period and for the post-bloom period with standard deviations in parentheses

Period	EOC_n	B_n	Gross EOC	% of BGP	n
	(μg C liter^{-1} d^{-1})			(d^{-1})	
8–29 Aug	21.2 (18.6)	4.1 (2.5)	29.3	4.3	4
1–26 Sept	12.1 (9.7)	2.7 (1.9)	21.2	9.1	7

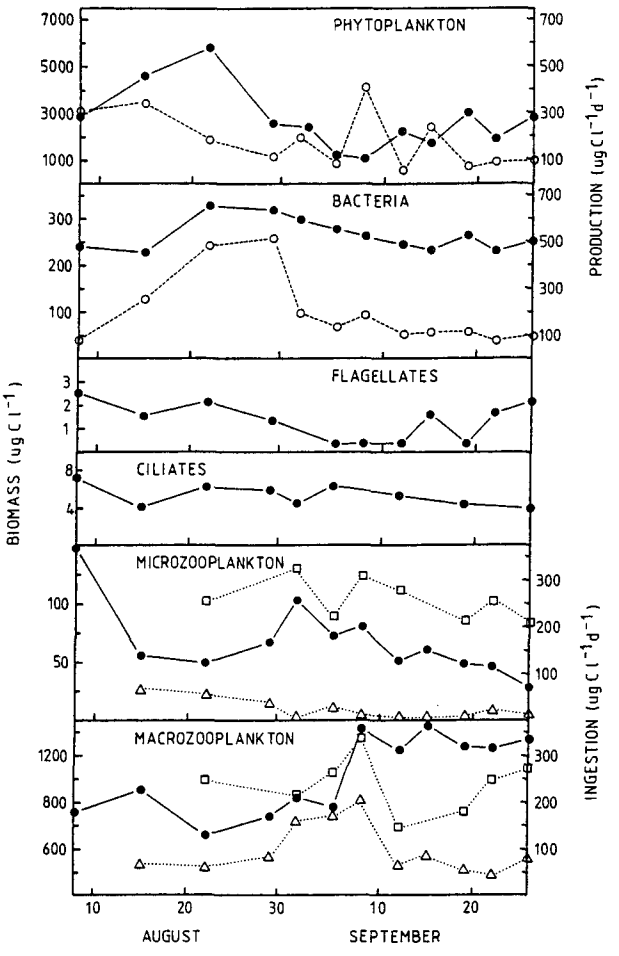

Fig. 6. Changes in phytoplankton biomass and gross primary production, bacterial biomass and gross production, flagellate biomass, ciliate biomass, microzooplankton (50–140 μm) biomass and ingestion of bacteria and phytoplankton, and macrozooplankton (> 140 μm) biomass and ingestion of bacteria and phytoplankton. Biomass, —●—; production, —○—; ingestion, . . .□. . .; (phytoplankton) or . . .△. . . (bacteria).

Heterotrophic organisms

The number of bacteria ranged between 3.2 and 6.8 × 10^6 ml^{-1}, and the biomass showed only minor changes throughout the period, with a mean value of 262 μg C $liter^{-1}$ (Fig. 6). In contrast, bacterial net production peaked at the end of August with 510 μg C $liter^{-1}$ d^{-1} and decreased thereafter to about 124 μg C $liter^{-1}$ d^{-1} (Fig. 6). The bacterial generation time was, on average, 31 hours (ranging between 14 and 22 hours, except for one date with an exceptional high generation time of 74 hours) during the bloom of *Aphanizomenon,* and increased to more than 2 days (50–72 hours) at the end of September.

The number of heterotrophic nanoflagellates was low (40–440 ml^{-1}) corresponding to a biomass ranging between 0.4 and 2.5 μg C $liter^{-1}$ (Fig. 6). Population density reached a minimum (40–60 ml^{-1}) after the bloom period of *Aphanizomenon.* Small ciliates (<50 μm) were less numerous (10–18 individ-

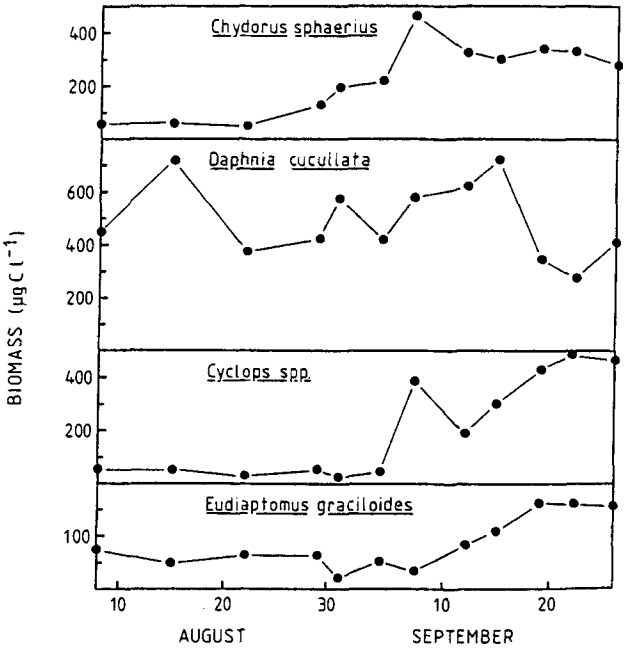

Fig. 7. Changes in biomass of the dominant macrozooplankton species with time. Note different scaling of the y-axis.

uals ml^{-1}) and showed a constant biomass of approximately 5 µg C liter^{-1} throughout the period (Fig. 6).

Microzooplankton, defined as the size fraction of 50–140 µm, included calanoid and cyclopoid copepod nauplii, ciliates larger than 50 µm and rotifers. The biomass fluctuated, with peaks in the beginning of August and again in the beginning of September, after the cyanobacteria collapsed, and averaged 50 µg C liter^{-1} (Fig. 6). The distribution of the biomass did not change much during the entire period, and nauplii and ciliates made up 59% (48–73%) and 33% (21–45%), respectively, of the microzooplankton biomass. *Vorticella* and colonies of *Epistylis* were the major component of ciliate carbon biomass during most of the period. *Keratella cochlearis* and *K. cochlearis tecta* dominated the rotifer community, which only contributed with 8% to the total microzooplankton biomass. Macrozooplankton biomass started to increase immediately after the break down of the cyanobacteria (Fig. 6). During most of the period *Daphnia cucullata* dominated the community with an average biomass of 500 µg C liter^{-1} (Fig. 7). The cyclopoid copepods and *Chydorus sphaericus* were responsible for the major part of the increase in the total biomass, whereas the increase of the calanoid copepod *Eudiaptomus graciloides* was 14% of the total increase. *Diaphanosoma brachyum* and *Ceriodaphnia quadrangula* were found to contribute negligibly (<5%) to the total biomass.

Macrozooplankton ingested up to 202 µg C liter^{-1} d^{-1} of the bacterioplankton during the first week of September (Fig. 6). Microzooplankton also contributed to the removal of bacteria, with an average ingestion rate of 23 µg C liter^{-1} d^{-1}. Ingestion rates of nanoflagellates and ciliates averaged 10 and 63 µg C liter^{-1} d^{-1} during the cyanobacteria bloom, but decreased after the collapse

Table 2. Calculated ingestion rates of nano-flagellates and ciliates smaller than 50 μm on bacteria derived from specific body volume clearance rates and bacterial biomass (see text for explanation). Values are means for the two periods with range of estimates in brackets

	Flagellates	Ciliates <50 μm
Period	(μg C liter^{-1} d^{-1})	
8–29 Aug	10.2 (7.0–13.6)	63.2 (35.3–78.1)
1–26 Sept	4.8 (1.3–10.9)	49.6 (36.7–67.3)

Table 3. Average grazing rates (% d^{-1}) on phytoplankton and bacteria expressed as percentage of available food, which was derived from estimates of net production of algae (<100 μm) or bacteria corrected for biomass changes between first and last sampling date in each period

	Grazing (% d^{-1})			
	Bloom period		Post-bloom period	
	Algae	Bacteria	Algae	Bacteria
Flagellates	—	3.1	—	3.8
Ciliates <50 μm	—	19.3	—	39.4
Microzooplankton	156.0	15.9	439.4	9.5
Macrozooplankton	161.6	21.6	404.1	86.4
Total	317.2	59.6	843.5	139.1

(Table 2). Macrozooplankton consumed on average 250 μg C liter^{-1} d^{-1} of phytoplankton, with lower values in the middle of September, when the biomass of macrozooplankton remained high (Fig. 6). Microzooplankton grazing of phytoplankton exceeded the macrozooplankton ingestion rates (Table 3), despite a lower biomass of microzooplankton. Microzooplankton ingestion of phytoplankton decreased with grazer biomass throughout September and averaged 300 μg C liter^{-1} d^{-1}.

The results presented above were integrated over two periods, the "bloom" period (August 8–29), characterized by high phytoplankton biomass dominated by *Aphanizomenon,* and the "post-bloom" period (September 1–26), characterized by intermediate phytoplankton biomass and a more diverse phytoplankton community. Major events of the carbon budgets (Fig. 8) include a marked reduction of phytoplankton biomass from approximately 4 mg C liter^{-1} dominated by filamentous cyanobacteria, to 2 mg C^{-1}, containing a more diverse community of cyanobacteria and chlorophytes. The <20 μm fraction increased in particular after the culmination of the bloom. Bacterial biomass changed less, whereas bacterial production decreased by a factor of 2 after the culmination of cyanobacteria. Furthermore, the biomass of microzooplankton and heterotrophic flagellates decreased, and the biomass of small ciliates (<50

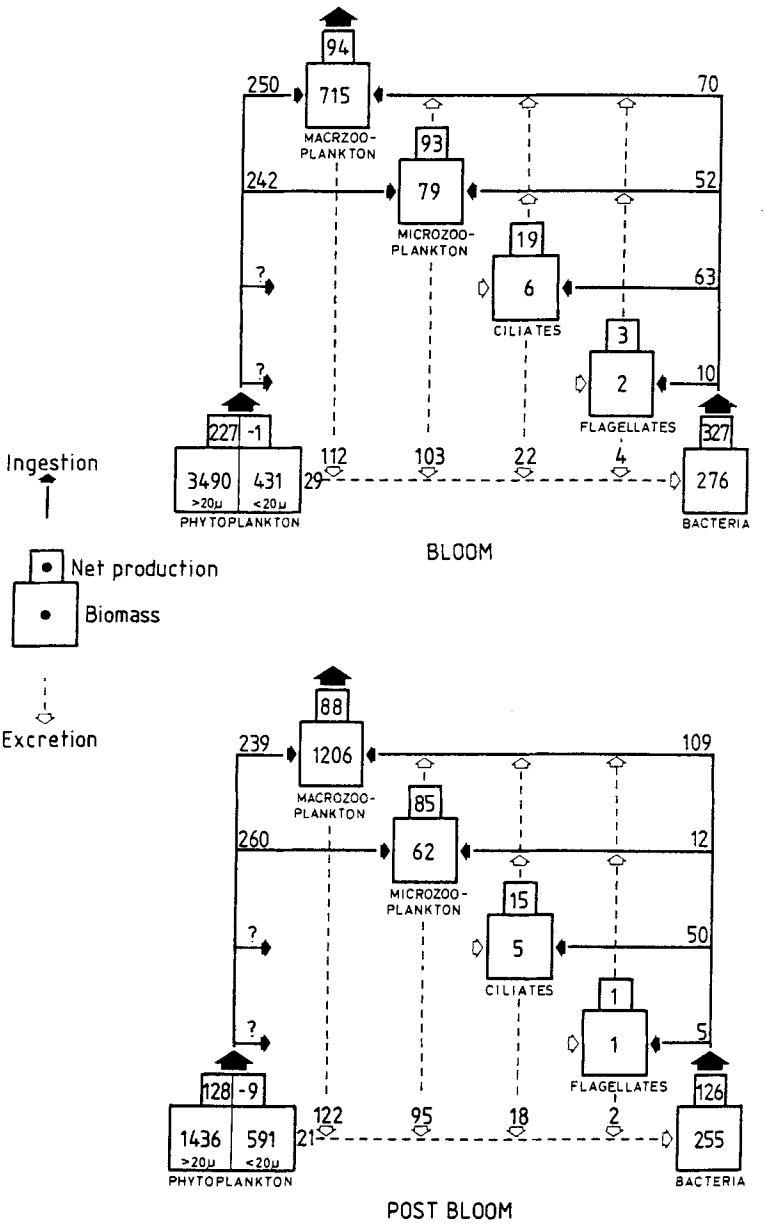

Fig. 8. Carbon flux in Frederiksborg Slotssø during August and September (1988). Boxes represent biomass (μg C liter^{-1}) or net production (μg C liter^{-1} d^{-1}). Solid lines and black arrows represent measured or calculated uptake rates (μg C liter^{-1} d^{-1}), and dashed lines and open arrows represent measured or calculated excretion rates (μg C liter^{-1} d^{-1}). Question marks indicate tentative pathways.

Table 4. Turnover times (days) of different assemblages during Period 1 (8–29 August) and Period 2 (1–26 September)

	Period 1	Period 2
Bacteria	1.3	2.2
Flagellates	0.6	0.7
Ciliates	0.3	0.3
Microzooplankton	0.5	0.7
Macrozooplankton	6	12
Phytoplankton	18	15

μm) remained constant after the bloom. In contrast, macrozooplankton biomass nearly doubled after the collapse of cyanobacteria. Population turnover rates of each trophic level (Table 4) were calculated from estimates of carbon biomass and net production. It appeared that populations of phytoplankton turned over faster after the cyanobacteria bloom, whereas all heterotrophic components revealed decreasing or constant turnover rates.

Discussion

We used the carbon budget as a tool to examine interactions between autotrophic and heterotrophic plankton populations. The bloom period of cyanobacteria and the subsequent crash of the bloom were chosen as representative of major events which are common for most eutrophic, temperate lakes [64].

The reasons for the crash of the bloom were not clear. One week before the collapse, low nitrate values (<10 μg NO_3-N liter^{-1}) and fluctuating ammonia values (0–100 μg NH_4-H liter^{-1}) were recorded in the epilimnion (Fig. 2). On the other hand, inorganic phosphate was never below 100 μg liter^{-1}, and, according to Healey and Hendzel [24], only a low intracellular content of phosphorus prevents *Aphanizomenon* from fixation of atmospheric nitrogen. Considering the high external pool of phosphate, it is not likely that phosphorus limitation prevented *Aphanizomenon* from maintaining population growth. Alternatively, it is known that low temperature affects growth of cyanobacteria. Tilman et al. [69] demonstrated on cultures of natural algal assemblages that cyanobacteria dominated the phytoplankton community at low N:P ratios at 24°C, but not at 17°C and 10°C. In this study, the temperature ranged between 17°C and 18°C (Fig. 1) during the collapse of the bloom. Hence, temperature was not optimal for growth, although the nutrient regime favored cyanobacteria. Finally, it has been demonstrated that collapse of the cyanobacteria vacuoles can occur by increased cell turgor when photosynthetic products accumulate or as a consequence of decreasing temperature [1]. The extent to which these factors contributed to the observed crash of the bloom is unclear.

Generally, phytoplankton productivity decreased during the whole period, probably due to self-shading. Phytoplankton larger than 50 μm dominated the

production throughout the period, but, after the crash, phytoplankton smaller than 50 μm increased in absolute biomass and in production relative to larger cells (Fig. 5). Shifts from large phytoplankton characterized by low specific growth rates to more rapid growing species are typical for eutrophic, temperate lakes during late summer [64]. The measured release of EOC from phytoplankton made out only 4–9% of the bacterial carbon demand. Similar values were reported by Riemann and Søndergaard [54] from the same lake, suggesting that release of EOC from cyanobacteria was of minor importance and that excretion from heterotrophs [32] or lysis products from the cyanobacteria [23] sustained the major part of the bacterial production. In fact, the calculated excretion from heterotrophs plus measured release of EOC could sustain all bacterial production during the post-bloom period and constituted 41% during the bloom period. The bloom of cyanobacteria crashed within 1 week, and a total of 25.7 g particulate C m^{-2} disappeared from the water column with a daily average loss rate of 3.7 g C m^{-2} d^{-1} (460 μg C $liter^{-1}$ d^{-1}). Hansen et al. [23] demonstrated that cyanobacteria lost up to 43% of the cellular carbon content as dissolved carbon within 24 hours after death. In subsequent experiments, Krog et al. [33] showed that almost equal amounts of different sized molecules (<700, 700–10,000, >10,000 daltons) were lost from the cyanobacteria. All molecule weight groups were utilized by the bacteria, but intermediate sized fractions were preferred. Assuming that similar relationships occurred in this study, we calculated an average daily loss of organic carbon from the decaying cyanobacteria of 198 μg C $liter^{-1}$ (406 × 0.43), corresponding to 20% of the bacterial carbon demand during the initial crash phase (22–29 August). Although these theoretical calculations are speculative, they nevertheless demonstrate a potential source of carbon that could explain the bacterial substrate sources.

The nanoflagellates contributed little to the total heterotrophic carbon biomass (Fig. 6), and the calculated grazing on bacteria corresponded to 3–4% d^{-1} of the bacterial net production (Table 3). In comparison, nanoflagellates were abundant in the lake during June 1984 [52], and the biomass of larger grazers (>140 μm) was low compared to the levels normally found in August–September [56]. It is likely that the macrozooplankton in Frederiksborg Slotssø are able to control the nanoflagellate populations, except for periods without *Daphnia*. The biomass of the microzooplankton increased while nanoflagellate biomass decreased, but we do not know if nauplii and large ciliates were able to utilize flagellates as food. Similar complex scenarios were suggested by Sherr and Sherr [61]. Ciliate biomass (<50 μm) was approximately 5 times higher than the biomass of nanoflagellates (Fig. 6) and within the range reported for other eutrophic lakes [40]. The calculated ingestion rates of bacteria averaged 29% of bacterial production (Table 3, both periods), supporting the idea that ciliates are an important link between bacterioplankton and higher trophic levels [19, 40, 42, 50, 57].

The increasing biomass of all crustaceans immediately after the crash indicated that *Aphanizomenon* had no direct toxic effect. Lampert [34] and Holm and Shapiro [27] found likewise no toxicity of *Aphanizomenon* but reported, on the other hand, low growth and reduced fecundity of *Daphnia* when fed *Aphanizomenon* as the only food. In fact, *Daphnia cucullata* decreased in this study at high cyanobacteria concentration and increased again after the bloom

had declined (Figs. 6 and 7). The increase of the cyclopoid copepod population was probably a termination of the summer diapause caused by decreasing temperature. In situ ingestion rates on phytoplankton smaller than 20 μm and bacteria revealed two distinct results. First, the microzooplankton fraction (50–140 μm; nauplii, rotifers, and ciliates caught in this fraction) had high ingestion rates of phytoplankton and low ingestion rates of bacteria relative to phytoplankton (Fig. 6, Table 3). Since rotifers, nauplii, and large ciliates fed less efficiently on small-sized particles, they turned out to be competitors to macrozooplankton in feeding on phytoplankton. The amount of bacteria removed by microzooplankton was 10–16% of bacterial production. These values are in accordance with previous findings by Bosselmann and Riemann [9, 53]. Second, the macrozooplankton community (>140 μm) ingested both small-sized (bacteria) and large food particles (phytoplankton). However, the efficiency on the small-sized fraction was 2 to 4 times lower than the efficiency on larger particles.

Ingestion rates of bacteria reached highest levels (200 μg C liter^{-1} d^{-1}) when cladocerans dominated the macrozooplankton community (Figs. 6 and 7). Previously, Pace et al. [43] and Porter et al. [47] demonstrated the ability of crustaceans to utilize bacterioplankton as food, and Riemann [52] found that a high biomass of macrozooplankton (dominated by crustaceans) controlled the bacterial biomass in enclosures without fish. The ingestion rates of phytoplankton were obtained for particles smaller than 20 μm, as we considered ingestion of larger particles to be of minor importance. However, ingestion rates exceeded the production of algae smaller than 20 μm, while, at the same time, phytoplankton biomass smaller than 20 μm increased. Furthermore, decreasing biomass of the 20–50 μm and the 50–100 μm size fractions were recorded (Fig. 5). This suggests that the size fraction used in the tracer experiments did not match the food size spectrum exploited by the zooplankton. We presumed then that the grazers utilized food particles larger than 20 μm, with an upper limit of 100 μm. It is known that *Daphnia* can ingest algae cells up to 50–80 μm [14, 35] and even larger [28]. Still, considering an enlarged food spectrum, the larger than 50 μm zooplankton community ingested on average more phytoplankton carbon than available in the "edible" size classes (Table 3). The measured ingestion rates resembled values from previous investigations in Danish lakes and other temperate, eutrophic lakes [9]. Despite the disagreement between grazing and phytoplankton growth, a considerable proportion of the phytoplankton was not edible and would undergo sedimentation or lysis.

The total carbon uptake of the macrozooplankton averaged, respectively, 60% and 32% of grazer biomass per day (daily ration) before and after the culmination of cyanobacteria. These values indicate that macrozooplankton growth was probably controlled by food concentration after the collapse of cyanobacteria. In comparison, Christoffersen and Jespersen [11] found rations of 61–68% for populations of *Eudiaptomus* on a diet of natural populations of phytoplankton during May and June in Frederiksborg Slotssø. Similar rations (53–62%) on labeled seston smaller than 33 μm were found for the mesotrophic Lake Vechten during late summer [21]. These values were minimum estimates, as the former did not include bacterial carbon, and the latter excluded algal cells >33 μm as food, suggesting that the daily ration of 32% found after the

bloom in this study was an indication of food limitation. The extent to which detritus was utilized by macrozooplankton during this study remains unknown, but preliminary laboratory experiments with natural populations of zooplankton from Fredriksborg Slotssø feeding on labeled detritus gave uptake rates comparable to our measurements of ingestion rates of live phytoplankton (J. Ingerslev, personal communication) suggesting that detritus, although of less quality, was a potential food source.

A carbon budget for the periods before and after the collapse of cyanobacteria (Fig. 8) was constructed from estimated and calculated values of pools and flow rates. The provisional carbon budget allowed us to examine gross production and ingestion and the subsequent net production in various size fractions of the plankton community. We did not make any attempt to balance the budget, and the number of assumptions applied made it far from perfect. Nevertheless, four interesting ecological scenarios became clear: 1) the carbon supply to the bacteria, 2) the control of bacterial biomass, 3) growth and food selection of the macrozooplankton, and 4) the role of heterotrophic nanoflagellates and ciliates in the lake. Concerning the first point, the suggested amount of carbon available to the bacteria matched the bacterial gross production during the post-bloom period, but could only explain 41% of the bacterial carbon requirement during the bloom period. Lysis products from the decaying cyanobacterial bloom were possibly a major substrate source for the bacteria, but still not sufficient to meet the total bacterial carbon demand. Since the bacterial production increased during the bloom period, it is likely that increased transport from the algae occurred already before the chlorophyll level dropped. Concerning the second point, the integrated grazing impact on bacteria of nanoflagellates, ciliates, and micro- and macrozooplankton accounted for 60% of the bacterial net production during the bloom period and 139% during the post-bloom period (Table 3). In our carbon budget (Fig. 8), ciliate grazing on bacteria constituted 19% in the bloom period and 39% in the post-bloom period of the total grazing on the bacteria, whereas the role of microzooplankton as bacterivores decreased from 16% to 10% in the post-bloom period. The growth of macrozooplankton during the post-bloom period also increased their role as bacterivores (from 22% to 86%), supporting the idea that natural populations of crustaceans can play a key role in controlling the biomass of bacteria [20, 52]. Findings regarding the third point, growth and food selection of the zooplankton, were in contradiction to the general conception that large grazers starve during cyanobacterial blooms [48, 51]. The bacteria made up a considerable part (40%) of the food the first week after the bloom had collapsed (bacteria constituted about 20% of the food in the remaining period), but a two-fold increase in the zooplankton biomass was not followed by an increased carbon uptake. In fact, we found that zooplankton was food limited in the period *after* the bloom, compared to spring and summer conditions in eutrophic or mesotrophic lakes [9, 11, 21]. Since all the macrozooplankton populations started to grow after the bloom, it is likely that other food sources, such as nano-sized organisms (flagellates and small ciliates) and microzooplankton were part of the macrozooplankton diet [6, 66]. Concerning the fourth point, the heterotrophic flagellates and small ciliates revealed only minor changes in population turnover rates after the cyanobacterial bloom, indicating that

Aphanizomenon did not affect any of these populations. Ciliates were supposed to be important as transformers of carbon sources to higher trophic levels, especially after the cyanobacteria bloom, as they seemed to be able to consume substantial amounts of bacterioplankton (and possibly other ultrafine particles) [6] and had high population turnover rates (Table 4). The heterotrophic nanoflagellates played a minor role in the carbon budget since their biomass remained low throughout the period. Most likely, the macrozooplankton controlled the flagellate biomass either by predation or by damaging the cells when filter-feeding. The importance of flagellates as bacterivores depends primarily on the biological structure of the lake; e.g., if cladocerans are removed by planktivorous fish, flagellates will appear in dense populations within few weeks [52].

By means of intensive sampling during the build-up and subsequent breakdown of a cyanobacterial bloom and the construction of a pelagic carbon budget, it was possible to quantify the relative importance of various consumers of bacterial and algal carbon. Control of phytoplankton growth by grazers were of minor importance relative to the high algal biomass in Frederiksborg Slotssø (2–4 g C m^{-2}). In contrast, cladocerans accounted for most of the bacterial grazing. Heterotrophic flagellates were not important as bactivores, nor was the microzooplankton, but ciliates smaller than 50 μm consumed considerable proportions of the bacterial carbon production. Earlier reports have emphasised the importance of cladocerans as algal consumers and heterotrophic flagellates as bacterial consumers [57, 70, 71]. Similar scenarios have been reported from marine studies [2, 8, 15], as most marine grazers are copepods not capable of feeding on bacteria. In freshwater, large populations of cladocerans often develop and control bacterial production [20, 45, this study]. The extent to which the macrozooplankton control the biomass of phytoplankton depends upon the composition, size structure, and quality of the food. During the bloom of cyanobacteria in Frederiksborg Slotssø, the phytoplankton was less favourable for the macrozooplankton, and small-sized heterotrophs partly subsidized their food requirement.

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