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Dissolved organic carbon released by zooplankton grazing activity – a high-quality substrate pool for bacteria

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Abstract. Experiments were designed to investigate whether processes related to zooplankton feeding have a positive effect on bacterial growth. Bacterial abundance and [^{3}H]thymidine incorporation rates were followed in grazer-free batch cultures originally containing either *Scenedesmus quadricauda* or *Rhodomonas lacustris* as food sources, and *Daphnia cucullata* or *Eudiaptomus graciloides* as grazers. Compared with controls lacking either animals or algae, a significantly higher bacterial abundance and productivity occurred in cultures which contained both phyto- and zooplankton. The same experimental methodology was tested during the decline of a diatom spring bloom in a eutrophic, temperate lake. A significant increase in bacterial biomass was observed due to the grazing activity of *in situ* mesozooplankters during the clear-water phase. Our results demonstrated that the dissolved carbon pathway from mesozooplankton to bacteria averaged 57% (26–78%) of the algal carbon filtered from suspension.

Introduction

In the photic zone of aquatic pelagic systems, bacterial production is positively related to primary production (Cole *et al.*, 1988). Nevertheless, there is a disagreement concerning which of two internal sources of bacterial substrate is quantitatively most important: exudates from healthy algae versus release from grazing processes. In general, bacterial carbon requirements seem not to be met by the release of phytoplankton extracellular organic carbon (EOC) (Baines and Pace, 1991), which leaves room for other quantitatively important processes (Riemann and Søndergaard, 1986).

A significant amount of dissolved organic matter (DOM) can be released through processes of sloppy feeding, excretion and defaecation, which suggests that herbivory might be an important source of bacterial substrate (Lampert, 1978; Güde, 1988). The importance of zooplankton-mediated release of DOM over very short time scales, based on digestion theory, was proposed by Jumars *et al.* (1989). They argued that optimal digestion theory predicts that in periods of high food concentration, zooplankters would decrease their assimilation efficiency to optimize the nutritional gain from ingested food. Therefore, faecal pellets would contain more unabsorbed digestive products which would leak rapidly into the surrounding water.

Previous studies have demonstrated a significant loss of DOM in association with meso- and macrozooplankton feeding on phytoplankton (e.g. Johannes and Webb, 1965; Webb and Johannes, 1967; Lampert, 1978; Copping and Lorenzen, 1980). The loss is explained by excretion, by leakage of DOM from faecal pellets (Lampert, 1978) and by leakage of DOM from broken cells during 'sloppy feeding' (e.g. Conover, 1966). Lampert (1978) and Copping and Lorenzen (1980) quantified zooplankton-mediated DOM loss based on ^{14}C-labelled phytoplankton,

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and found that *Daphnia pulex* and *Calanus hyperboreus* released between 10–17% and 8–20% of ingested algal carbon, respectively. Olsen *et al.* (1986) found that *Daphnia* species released 18–100% of ingested algal carbon as dissolved and particulate carbon, and that the dissolved fraction averaged 21% of the total carbon released.

Other studies have specifically related zooplankton feeding to an additional flux of dissolved free amino acids (DFAAs). Studies performed in the Celtic Sea indicated that DFAAs observed in the water column could be related to the activity of zooplankton (Williams and Poulet, 1986; Poulet *et al.*, 1991). Eppley *et al.* (1981) and Riemann *et al.* (1986) also found increased DFAA release due to zooplankton grazing activity; however, released DFAAs did not always result in enhanced bacterial growth rates.

More direct evidence for micro- and mesozooplankton grazing activity providing dissolved organic substrates for bacteria stems from experiments that attempt to include all substrate sources in a quantitative evaluation (Güde, 1988; Arndt *et al.*, 1992; Peduzzi and Herndl, 1992). Enhanced bacterial growth and abundance caused by herbivores were found in these studies.

The purpose of the study was to assess the quantitative importance of zooplankton-mediated DOM production as a substrate for pelagic bacteria. The contribution of zooplankton-mediated DOM was quantified as an increase in bacterial biomass or [^{3}H]thymidine incorporation rates. The experiments were conducted under controlled laboratory conditions with either phytoplankton, zooplankton, or both, and *in situ* during the decline of a diatom spring bloom.

Method

Study site

The study was carried out in Frederiksborg Slotssø, Denmark, during spring 1991. Frederiksborg Slotssø is a small (0.21 km^2) dimictic, eutrophic lake with a mean depth of 3.1 m and a maximum depth of 8 m (Andersen and Jacobsen, 1979). The *in situ* experiments were performed on March 25, April 15, May 7 and May 28 during the decline of the spring bloom (Søndergaard *et al.*, 1995).

During the period of investigation, chlorophyll decreased from ~50 to 30 μg l^{-1} and temperature increased from 5 to 14°C (Table I). The zooplankton community changed during the period from a community dominated by rotifers, copepod nauplii and few adult *Eudiaptomus graciloides* and *Bosmina* sp. to a community dominated by adult cladocerans and copepods. Among the cladocerans, *Bosmina*

Table I. *In situ* conditions during the four field studies and estimated community grazing rates

Period	Temperature (°C)	Chlorophyll (μg l^{-1})	Algal biomass (mg C l^{-1})	Community grazing rate (mg C l^{-1} day^{-1})
25 March	5.5	46	3.3	76
15 April	10.5	53	3.8	130
7 May	14	30	2.1	256
28 May	14	35	2.5	793

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sp. dominated until early May and was succeeded by *Daphnia cucullata*. The phytoplankton community was dominated by *Stephanodiscus* sp., *Cryptomonas* sp., *Rhodomonas* sp. and *Oocystis* sp. More than 80% of the chlorophyll was in the nanoplankton size fraction (<20 μm). Further details on carbon cycling, community structure and seasonal succession can be found elsewhere (Markager *et al.*, 1994; Søndergaard *et al.*, 1995).

Laboratory experiments

The experimental approach in the present study is presented in Figure 1. Lake water stored at 5°C was used in the experiments. Before an experiment, the lake water was filtered (GF/F) and aerated. The experiments were performed in duplicate. All glassware used was acid washed and Blue Cap bottles were pre-combusted (550°C). All filtrations were performed with low vacuum (<0.2 bar). The experiments were initiated by adding zooplankton to filtered lake water added cultures of algae that were diluted to a known cell concentration. The feeding experiments were stopped after 3 h by draining the samples through screens. The growth of bacteria was then measured by incubating the filtrate in bottles stored in darkness at a constant temperature (19°C) in a shaking water bath. At regular time intervals, subsamples were taken for measurements of total counts of bacteria and bacterial production (only in experiment D). Variations between experiments were due to different algal and zooplankton species and screening treatment of the water samples. Initial concentrations of algae and animals are given in Table II. Experiment A (Table II) was based on the protocol of Güde (1988). Since algae were not removed during the growth of bacteria, an artefact arises from leaching of dissolved organic carbon (DOC) from intact or broken algae. Another problem with experiment A could be loss of bacteria due to bacterivores during the incubation period. Therefore, in experiments B and C, water was filtered through 0.2 μm pore size filters after removal of algae and animals, and a predator-free bacterial inoculum (<0.6 μm) was added. This procedure allowed incubation of substrate from the different treatments with natural bacteria for several days, and we assumed that the substrate available to bacteria was metabolized during the incubations.

In situ experiments

Plankton samples collected in the middle of the lake with a 3 l heart valve water sampler at 0, 1, 2 and 3 m depths were pooled for *in situ* feeding experiments. The following size classes were represented in the feeding experiments: <20 μm (nanoplankton), <140 μm (microplankton), unscreened water and concentrated mesozooplankton (>140 μm). The concentrated mesozooplankton samples contained animals collected on a 140 μm mesh net by draining the contents of an unscreened sample three times. The animals were added to an unscreened sample, thus representing a 4-fold higher concentration of mesozooplankton than the lake. During sieving, the net was kept below the water surface to protect the retained animals against air exposure. During feeding, the samples (6 l) were protected from light and stored in the lake. Owing to a relatively low temperature

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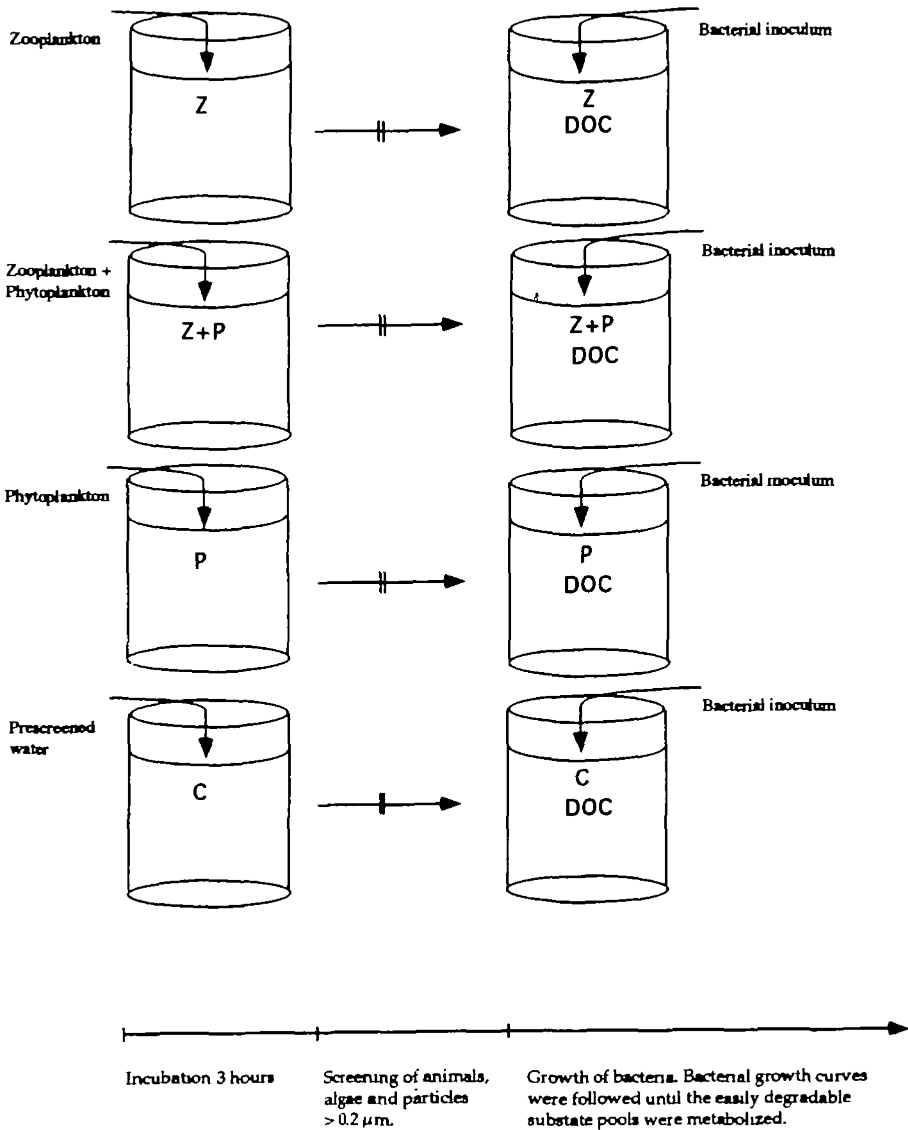

Fig. 1. Schematic diagram of the experimental protocol.

(Table I), the feeding experiments lasted 5 h and were stopped by draining the contents through screens. The principal protocol of the bacterial growth response in these experiments was the same as for the laboratory experiments.

Problems with bacterivores occurred in the first two *in situ* experiments, and the incubations were stopped before the bacteria reached the stationary phase. To prevent bacterivory, the filtration technique was changed in the remaining experiments. After the water was drained through screens, it was further filtered

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Table II. Experimental conditions for the laboratory experiments

Experiment	Zooplankton (ind. l^{-1})	Algal biomass [μg C l^{-1} (cells l^{-1})]	Grazing (h)	Temperature (°C)
A	200 *D.cucullata*[a]	308 (7.2 × 10^6) *S.quadricauda*	3	19
B	480 *D.cucullata*[b]	210 (4.9 × 10^6) *S.quadricauda*	3	19
C	400 *D.cucullata*[b]	208 (6.1 × 10^6) *R.lacustris*	3	15
D	250/500 *E.graciloides*[c]	406 (9.5 × 10^6) *S.quadricauda*	3	19

[a]Size 1.5–2.0 mm; [b]size 0.8–1.5 mm; [c]size 0.8–1.4 mm.

by tangential flow filtration (Millipore Minitan, 0.2 μm) and a bacterial inoculum was prepared from a 0.6 μm filtrate. *In situ* conditions and estimated community grazing rates (Markager *et al.*, 1994) are shown in Table I. The experiments were performed in duplicate, except for the May 7 experiments.

Laboratory cultures

Non-axenic strains of the cryptophyte *Rhodomonas lacustris* Pasch. et Ruttn. and the green alga *Scenedesmus quadricauda* Turp. were obtained from the Norwegian Institute for Water Research (NIVA) culture collection. The algae were grown on a modified Z8 medium (Staub 1961, modified NIVA 1972, 1976). Medium for *R.lacustris* was diluted with distilled water to a concentration of 20% and vitamins were added. *Rhodomonas lacustris* and *S.quadricauda* were grown in a 12/12 h light/dark cycle at a temperature of 15 and 19°C, respectively. To obtain exponential growth, fresh medium was added to *S.quadricauda* 3–4 days before an experiment. The slow-growing *R.lacustris* received fresh medium 6–10 days before an experiment.

For all experiments, individuals from the same laboratory cultures of the cladoceran *D.cucullata* (0.8–2.0 mm) and the copepod *E.graciloides* (0.8–1.4 mm) were used. The herbivores, originally isolated from the lake, were grown in 40 l vessels at 19°C in filtered lake water with slow aeration. When *R.lacustris* was used in the experiments, the animals were allowed to acclimate for 24 h to the food concentration and temperature. Because ingestion rates increase significantly with size (e.g. Peters and Downing, 1984), we varied the number of animals used in each experiment depending on the average size of the animals available.

Analysis

Chlorophyll (chl) *a* was measured from the pooled lake water samples according to Jespersen and Christoffersen (1987). Phytoplankton carbon biomass was calculated from a C:Chl ratio of 72 ± 14 (Søndergaard *et al.*, 1995).

The algal biomass in the laboratory experiments was estimated from cell dimensions of 50 cells of each species. Average cell length and width were 12.4 and 3.9 μm for *R.lacustris*, and 11.5 and 5.9 μm for *S.quadricauda*. Cell volumes averaged 197.5 and 260.6 μm^3, and were determined by fitting the surface area to geometrical formulae and converting to carbon units according to Strathmann (1967). The calculated carbon content was 34 pg C $cell^{-1}$ for *R.lacustris* and 42.8 pg C $cell^{-1}$ for *S.quadricauda*.

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Bacteria were preserved with formalin (final concentration 2% v/v) and counted within a week. Samples (0.5–2 ml) were stained with acridine orange (AO) following Hobbie *et al.* (1977). For each prepared slide, counts were made of the number of bacterial cells within an ocular grid in 10 randomly selected fields (magnification 1250×). Microscopy was performed with an Olympus BH2 epifluoresence microscope equipped with a 100 W mercury burner and Olympus blue filter set B (BP 490 excitation filter, DM 500, 0-515 barrier filter built in). Bacterial production was measured by [^{3}H]thymidine incorporation, using a conversion factor of 2×10^{18} cells mol^{-1} of thymidine incorporated as described by Smits and Riemann (1988). Bacterial abundance and production were converted to units of carbon by a factor of 20 fg C $cell^{-1}$ according to Lee and Fuhrman (1987). The biovolume of bacteria was measured for the May 28 experiment by enlarged epifluoresence photographs (AO). Pictures were taken randomly and ~50 cells were measured per subsample.

Zooplankton clearance experiments

Average clearance rates by *D.cucullata* and *E.graciloides* were measured using ^{14}C-labelled cultures of *Scenedesmus* and *Rhodomonas*. Uniform ^{14}C labelling of the algae was accomplished during growth for 3–9 days in a closed system with added $NaH^{14}CO_3$ (0.1 µCi ml^{-1}). Twenty to 30 *E.graciloides* and *D.cucullata* were placed in 100 ml of labelled algae suspension and were allowed to graze for 3 h. The zooplankton were then gently screened on 118 µm mesh net, the algae on 2 µm filters (Nuclepore) and bacteria on 0.2 µm filters (Sartorius). The filters and nets were transferred to scintillation vials. Tissue solubilizer (Beckman) was added 2 h before the addition of scintillation cocktail to vials containing animals. The dissolved ^{14}C activity (0.2 µm filtrate) was also measured. Clearance rates (ml $animal^{-1}$ h^{-1}) were calculated from the decrease in radioactivity in the 2 µm fraction compared with the control samples containing only the ^{14}C-labelled algae. Ingestion rates (µg C $animal^{-1}$ h^{-1}) were estimated by multiplying the clearance rates by the concentration of algae added (Table III).

Results

Laboratory experiments

Grazing activity by *Daphnia* or *Eudiaptomus* on cultures of *Scenedesmus* or *Rhodomonas* stimulated bacterial growth (Figures 2 and 3). In experiments A, B

Table III. Clearance and ingestion rates in feeding experiments with *D.cucullata* and *E.graciloides* as grazers, and *S.quadricauda* and *R.lacustris* as food sources

Species	Clearance rates (ml $animal^{-1}$ h^{-1})	Ingestion rates (µg C $animal^{-1}$ h^{-1})	Algal concentration [mg C l^{-1} (cells ml^{-1})]
D.cucullata[a]/*S.quadricauda*	0.55 ± 0.062	0.33	0.6 (14100)
D.cucullata[b]/*R.lacustris*	0.12 ± 0.068	0.156	1.3 (38600)
E.graciloides[c]/*S.quadricauda*	0.056 ± 0.038	0.038	0.6 (14100)
E.graciloides[c]/*R.lacustris*	0.044 ± 0.0028	0.057	1.3 (38600)

[a]Size 1.5–2.0 mm; [b]size 0.8–1.5 mm; [c]size 0.8–1.4 mm.

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and C, a significantly higher bacterial biomass was observed in the samples containing both plankton organisms (ZP) compared with all other samples. The results of the ANOVA test comparing the ZP samples with those containing only algae, zooplankton or pre-filtered water are shown in Table IV. Owing to the protocol in experiments B and C, where the bacteria were added as a small inoculum, the statistical tests were carried out for the periods 40 and 208 h, and 72 and 163 h, respectively.

In experiment D, bacterial production increased in all treatments during the first 3 h (Figure 3). In the time interval 6–21 h, only the treatments with feeding *E.graciloides* showed increased or constant bacterial production. There were no significant differences between samples containing 250 or 500 individuals l^{-1}. Bacterial production was significantly higher in both ZP treatments compared with only algae or pre-filtered water samples. The significance levels are given in Table IV.

Clearance and ingestion rates were measured in grazing experiments using ^{14}C-labelled cultures of *S.quadricauda* and *R.lacustris* as food, and *E.graciloides* and *D.cucullata* as grazers (Table III). The calculated ingestion rates were consistent with rates for *Daphnia* sp. and *Eudiaptomus* grazing on *Scenedesmus acutus* measured by Muck and Lampert (1984). This confirms that the animals were feeding normally during the experiments.

In situ measurements

In situ grazing experiments were carried out four times in the period from March to May in Frederiksborg Slotssø. A comparison of the estimated bacterial carbon biomass in the various manipulations of the lake water samples showed elevated growth in samples which originally contained the mesozooplankton community. In experiments from March 25 and April 15, no significant difference in bacterial biomass was found among the different treatments. In the experiment from May 7 (Figure 4), unscreened and concentrated mesozooplankton samples were pooled in the statistical test due to single samples in each treatment. There was a significant difference in bacterial biomass between the pooled mesozooplankton samples and both the nanoplankton (<20 μm) samples ($P < 0.01$) and the microplankton (<140 μm) samples ($P < 0.05$) for the time interval 42–212 h. In the May 28 (Figure 4) experiment, no significant difference was found between the unscreened samples and the micro- and nanoplankton samples, or between the concentrated and the unscreened samples. When concentrated mesozooplankton and unscreened samples were pooled, there was a significantly higher bacterial biomass in the pooled samples compared with the nanoplankton ($P < 0.05$) and microplankton ($P < 0.01$) samples for the time interval 46–233 h. There were no significant differences between the microplankton and the nanoplankton samples.

In the May 28 experiments, the size of the bacterial cells increased sixfold during the incubation period (Figure 5). There were no significant differences between bacterial size and treatment. The average cell volume of the inoculum was 0.035 ± 0.013 μm^3 (mean + SE), whereas in early stationary phase the mean cell size was 0.216 ± 0.032 μm^3 and in the late stationary phase 0.162 ± 0.023 μm^3.

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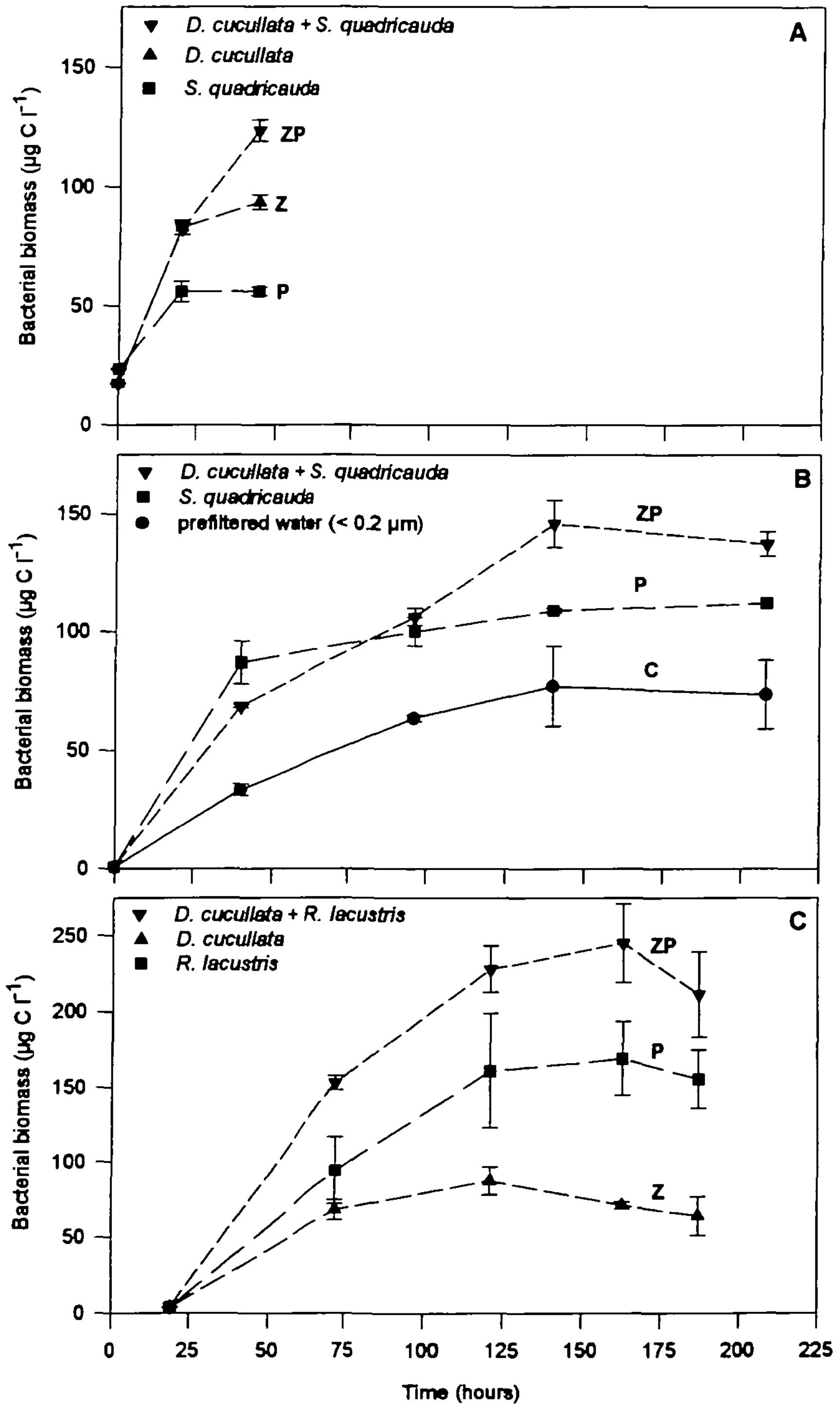

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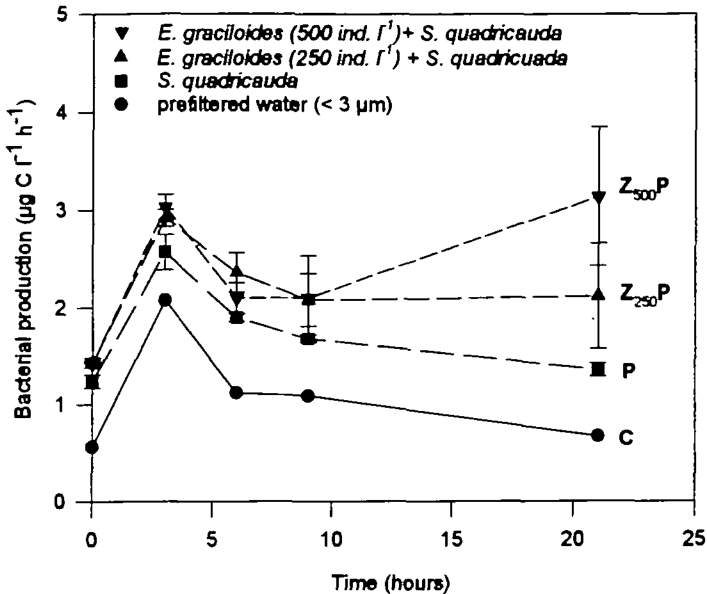

Fig. 3. Development of bacterial production in growth cultures after a serial filtration of the plankton organisms. Subsamples were taken immediately after removal of the copepods (time 0) and then at regular time intervals. Pre-filtered water represents water filtered through a 3 µm membrane filter ($n = 1$). Vertical bars represent the SEM from measurements from duplicate (▼, ■) and triplicate (▲) experiments.

Discussion

The hypothesis that herbivores can enhance bacterial growth and thus the activity of the microbial food web was supported by our results. Results from four laboratory studies showed unequivocally that bacterial production and biomass were enhanced after zooplankton grazing activity. These observations are consistent with results from other studies on increased bacterial biomass and production due to zooplankton-mediated DOC (Güde, 1988; Arndt *et al.*, 1992; Peduzzi and Herndl, 1992).

Zooplankton-mediated substrate loss rates in the laboratory experiments can be estimated by comparing ingestion rates (µg C animal^{-1} h^{-1}) with enhanced bacterial biomass due to produced substrate from feeding animals (ZP – P), expressed as zooplankton-mediated bacterial biomass (µg C animal^{-1} h^{-1}). The calculations showed that the average substrate loss rate was 28% (13–39%) of ingested carbon per animal per hour (Table V). Assuming a 50% bacterial growth efficiency

Fig. 2. Development of bacterial biomass in growth cultures after serial filtration of the plankton organisms. (**A**) Subsamples were taken immediately after removal of the daphnids (time 0) and then at regular time intervals. (**B**) and (**C**) Both animals and algae were removed and the water was filtered (0.2 µm) before a predator-free bacterial inoculum was added. Vertical bars represent the SEM from duplicate experiments.

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Table IV. Results of the ANOVA test comparing the increase in bacterial biomass in zooplankton + phytoplankton treatments with those containing only phytoplankton, zooplankton or pre-filtered water in the laboratory experiments

Experiment	Parameter tested	Zooplankton	Phytoplankton	Pre-filtered water < 2 μm
A	Zooplankton + phytoplankton	***	****	
B	Zooplankton + phytoplankton		*	****
C	Zooplankton + phytoplankton	****	*	
		Zooplankton_{500} + phytoplankton	Phytoplankton	Pre-filtered water < 3 μm
D	Zooplankton_{500} + phytoplankton		**	**
	Zooplankton_{250} + phytoplankton	–	***	****

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.005$; ****$P \leq 0.001$; –, not significant.

(Azam *et al.*, 1983), the average loss rate should be doubled to 57% (26–78%). Our calculations may be overestimated because the observed bacterial response in the ZP bottles may include carbon released by sloppy feeding; however, the food algae in our experiments usually seem to be swallowed whole (Lampert, 1978). Lampert found that sloppy feeding accounted for no more than 4% of the ingested carbon from algae species, comparable with the algae used. Furthermore, Jumars *et al.* (1989) argued that in periods of high food concentrations, which is the case in our experiments, zooplankters would decrease their assimilation efficiency to optimize the nutritional gain from ingested food. Faecal pellets and faeces would, therefore, contain more unabsorbed digestive products which would leak rapidly into the surrounding water. In this regard, leakage from faecal pellets and faeces appears more significant than sloppy feeding and excretion for mesozooplankton-mediated DOM production.

Our calculations may be biased due to uncertainties in the conversion factors used for bacterial biomass and production, but the observed enhanced responses in bacterial densities and growth related to herbivorous feeding activity demonstrated that by-products of animal feeding may be in the range of 50% of the ingested carbon. Although the results obtained from the laboratory experiments cannot simply be transferred to the field situation, our field results showed that bacterial activity can be enhanced by the presence of mesozooplankton. The estimated community grazing rates during the 5 h *in situ* experiments were in the range of 15–165 μg C l^{-1} (Table I). With the assumption that 50% of the ingested carbon is available as substrate for bacteria and a 50% growth efficiency for bacteria, we may expect a bacterial biomass response between 4 and 41 μg C l^{-1} in the experiments. This estimate seems to be within an acceptable range compared with the measured bacterial response in the four *in situ* experiments.

The principal source of organic carbon for bacterial production during the phytoplankton spring bloom changed from phytoplankton-dominated exudates (EOC) in the early stages of the bloom (March and April studies) to a significant mesozooplankton feeding-induced DOC production in the clear-water phase in May (Markager *et al.*, 1994). From March to mid-April, the surface temperature of

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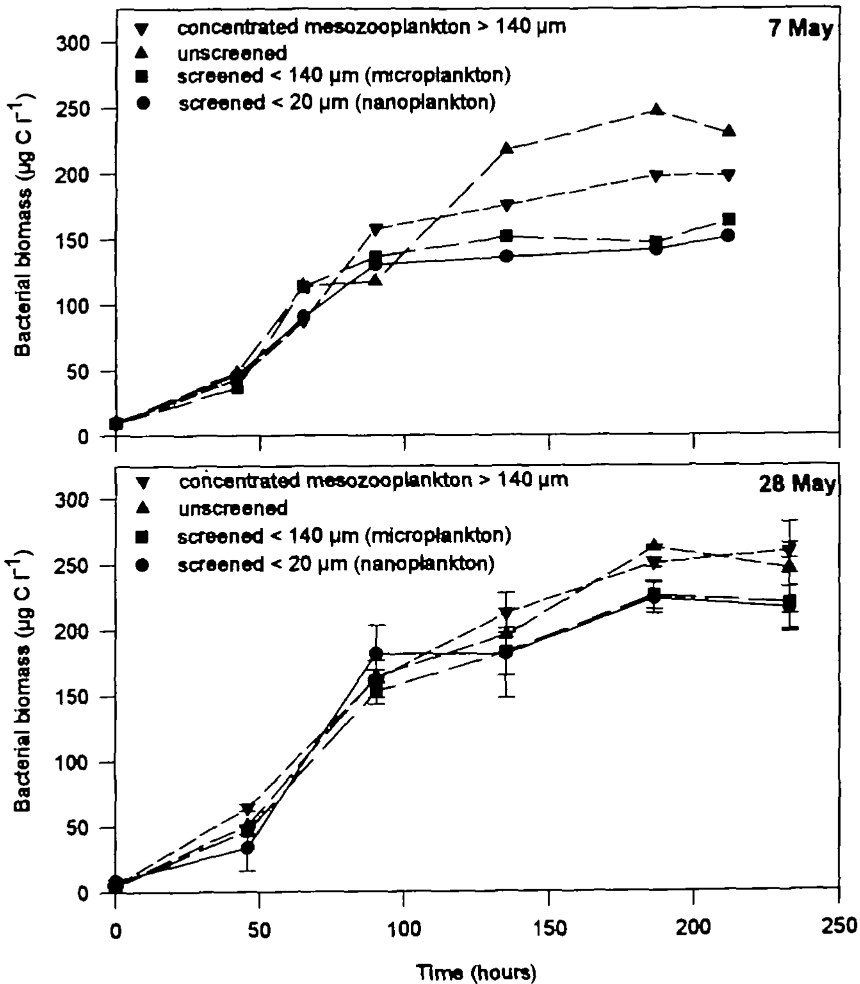

Fig. 4. Development of bacterial biomass in growth cultures after a serial filtration of the plankton community in *in situ* experiments from May 7 and 28. The cultures were started from pre-filtered (<0.2 µm) lake water with a <0.6 µm lake water filtrate added as inoculum. Vertical bars represent the SEM from measurements from duplicate experiments.

the lake increased from 5 to 10°C and the chlorophyll biomass was rather constant at ~50 µg C l^{-1} (Table I). Effects from low temperature, a relatively high phytoplankton biomass and a herbivorous biomass dominated by microzooplankters may not produce DOC that contributes substantially to bacterial production compared to EOC from primary producers. The microzooplankters are inefficient feeders on the dominant nanoplankton prey, but exert a size-selective grazing pressure on <3 µm algae (Hansen and Christoffersen, 1995). Although copepods and *Bosmina* were relatively abundant during this period, low temperature reduced both clearance and ingestion rates (Bogdan and Gilbert, 1982; Muck and Lampert,

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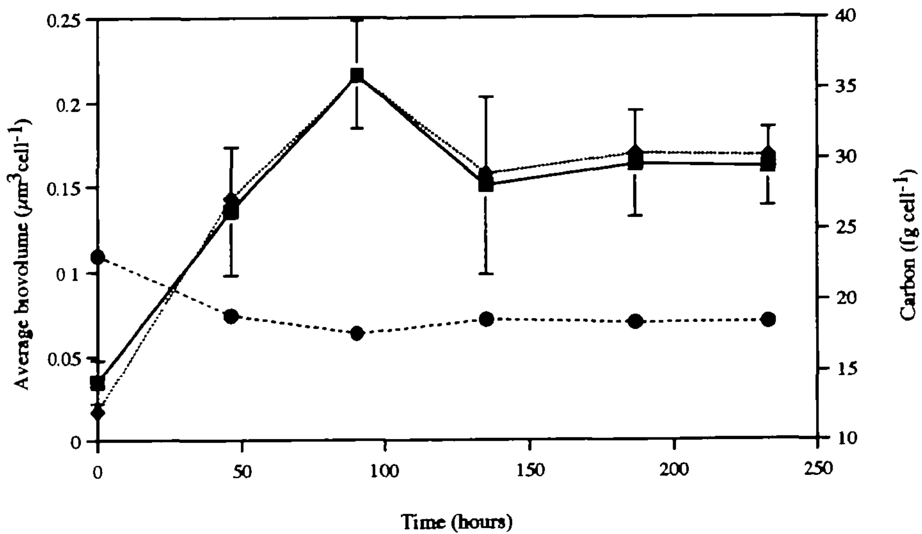

Fig. 5. Development of bacterial cell volume during the 28 May *in situ* experiment, values are the means ± SE of four treatments (■). The carbon content of the bacterial cell is calculated as a function of cell volume: (●) fairly constant carbon values of ~20 fg cell^{-1}, independent of size (Lee and Fuhrman, 1987); (◆) a cell size-dependent conversion of biovolume to carbon (Simon and Azam, 1989).

1984; Peters and Downing, 1984). On the assumption that <10% of the algal carbon leaks from healthy phytoplankton cells (Bjørnsen, 1988), then phytoplankton EOC will contribute ~90 and 85% of total produced substrate for bacteria during March and April, respectively. This calculation is based on data concerning bacterial production (Søndergaard *et al.*, 1995) and phytoplankton biomass and zooplankton grazing (Table I). In May, the effects of herbivory by zooplankton became more important. The period is characterized by a shift towards a zooplankton community dominated by mesozooplankters (Markager *et al.*, 1994) and a temperature increase to 14°C. Using the above assumption concerning phytoplankton exuda-

Table V. Estimated algal carbon loss rates during feeding activity of *D.cucullata* in the laboratory experiments. Zooplankton-mediated bacterial biomass represents the accumulated bacterial biomass in the growth cultures which can be related to grazing activity. See the text for further explanation

Experiment	Zooplankton/ phytoplankton	Ingestion rates (µg C animal^{-1} h^{-1})	Zooplankton-mediated bacterial biomass (µg C animal^{-1} h^{-1})	Bacterial substrate production in % of ingested algal carbon (bacterial biomass/ ingestion in %)
A	*D.cucullata*/ *S.quadricauda*	0.33	0.112	34 (68)[a]
B	*D.cucullata*/ *S.quadricauda*	0.20	0.0257	13 (26)
C	*D.cucullata*/ *R.lacustris*	0.16	0.0633	39 (78)

[a]Bacterial carbon demand (net production + respiration) in parentheses.

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tion and that 50% of the food filtered from suspension by mesozooplankton is released as DOM, then ~35 and 60% of total produced bacterial substrate was due to mesozooplankton grazing activity in May.

Our results indicate that the main source of DOM in the pelagic domain in eutrophic Frederiksborg Slotssø during the clear-water phase was associated with the feeding of mesozooplankton on phytoplankton. These data are consistent with results from other studies on increased bacterial biomass and production due to zooplankton-mediated DOM in various environments (Eppley *et al.*, 1981; Roman *et al.*, 1988; Peduzzi and Herndl, 1992). However, our results are subject to at least two sources of error: (i) DOC produced during the feeding experiments can be metabolized simultaneously by bacteria; (ii) we used a constant carbon cell content of 20 fg C bacteria $cell^{-1}$ (Lee and Fuhrman, 1987). Concerning (i), our experiments did not include the metabolized substrate during zooplankton grazing (Figure 1). An estimate of this quantity can be made. The calculated average growth rate of bacteria in the laboratory experiments is 0.05 h^{-1}, we assume that bacteria have a 50% growth efficiency (Azam *et al.*, 1983) and the initial bacterial abundance was 1×10^9 cells l^{-1} (20 fg C $cell^{-1}$). The metabolized carbon is then estimated to be 6 μg C l^{-1} during the 3 h laboratory incubation. Similar estimates can be made for the *in situ* experiments. Bacterial growth rate ranged from 0.09 to 0.28 day^{-1} in the experimental period (Søndergaard *et al.*, 1995). Given an initial bacterial abundance of 10×10^9 cells l^{-1}, the metabolized carbon ranged from 7 to 23 μg C l^{-1} during the 5 h incubation. These estimates indicate that bacterial processing of substrate during the feeding experiments is expected to be a significant contribution to the total loss of DOC mediated by the grazers. Furthermore, there might be a qualitative difference in the substrate originating from phytoplankton exudates compared with zooplankton-mediated DOC, which may provide different metabolic rates. Nevertheless, if we assume that there will be a standing stock of easily degradable substrate in the lake water, these sources of error tend to offset one another in the different treatments. Secondly, regarding (ii), during bacterial growth in the experiments from May 28 we measured a continuous increase in biovolume until 90 h of incubation, followed by a decrease during substrate limitation (Figure 5). However, we found no significant biovolume differences among the treatments; thus, although a size-independent constant carbon of 20 fg $cell^{-1}$ is disputable, a size-dependent conversion of biovolume to carbon (e.g. Simon and Azam, 1989) will not change the result concerning a difference among the different treatments.

In conclusion, our laboratory results showed that ~50% of the food filtered from suspension by mesozooplankton is released as DOM and represents a significant source of utilizable substrate for heterotrophic bacteria. These findings verify the theoretical arguments for potentially high animal-caused DOM losses suggested by Jumars *et al.* (1989).

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