Influence of cladoceran grazing activity on dissolved organic matter, enzymatic hydrolysis and bacterial growth

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To assess the influence of grazing by cladocerans on dissolved organic matter (DOM), glycolytic and proteolytic activities and bacterial growth were measured by in situ incubation of lake water from the epilimnion of an oligotrophic reservoir in three different treatments: in absence of zooplankton, and in presence of zooplankton (natural abundance and concentrated four-fold). These experiments were conducted at two periods in the succession of plankton populations (May and June 1998), that differed in the quality of the prey ingested (Eudorina sp. compared to Cryptomonas sp. and Rhodomonas sp.) and their grazing intensity (31.8 ± 2.2 µg C l–1 day–1 compared to 10.2 ± 0.5 µg C l–1 day–1). A systematic increase in bacterial biomass was measured in the treatments containing the highest zooplankton concentrations. The DOM concentrations produced in situ showed few significant differences between the three treatments, but the assimilation of DOM was higher in the presence of zooplankton than in their absence. These results show that the influence of cladocerans on the DOM was more of a qualitative than a quantitative nature. The protein compounds derived from the grazing activities of metazoans seem to be a major nutrient source for growth for bacteria (r = 0.81, P < 0.05). In this study, the highest hydrolytic activities were recorded in the presence of high concentrations of metazoan zooplankton. However, the processes that regulated these activities differed between the two experimental dates (repression compared to enzyme stimulation). Grazing activities could lead to an increase in phytoplanktonic excretion during the growth phase, and therefore the production of low molecular weight compounds that are easily assimilated by the bacterial plankton.

INTRODUCTION

Studies on the process of producing dissolved organic matter (DOM) in the euphotic zone of aquatic ecosystems have demonstrated the importance of the phytoplanktonic compartment (Mague et al., 1980). DOM derived from photosynthetic production, either from algal excretion (Baines and Pace, 1991; Maurin et al., 1997) or cell lysis (Fuhrman, 1992), is a key factor in the regulation of bacterial production. The existence of correlation between bacterial production and primary production in the euphotic zone of aquatic ecosystems has been demonstrated (Cole et al., 1988). However, the flows of DOM produced by zooplankton activity (faecal material, excretion by metazoan zooplankton and release of organic substrates of phytoplanktonic origin during ‘sloppy feeding’) could be a considerable source of carbon for the growth of bacterial populations. Lampert (Lampert, 1978) has shown that the dissolved organic carbon released during ‘sloppy feeding’ by Daphnia pulex accounts for between 4 and 17% of the algal carbon ingested, and Olsen et al. (Olsen et al., 1986) demonstrated that almost all of this carbon occurs in the faeces of the metazoan zooplankton. Grazing activity, by increasing the availability of organic substrates, could therefore assist in the growth of heterotrophic bacteria (Jumars et al., 1989; Peduzzi and Herndl, 1992; Vrede, 1998).

Olsen et al. (Olsen et al., 1986) showed that the pool of DOM, composed of amino acids, sugars, fatty acids and other more complex compounds produced by various planktonic processes (phytoplankton and/or zooplankton) was more or less efficiently utilised by bacteria. Seasonal and experimental studies have shown that increases in the concentration of dissolved proteins or carbohydrates in
the water column can be related to the grazing activities of the zooplankton (Arndt et al., 1992). Hygum et al. (Hygum et al., 1997) suggested that this DOM could represent a pool of high quality substrates for bacteria. The composition of the DOM released by phytoplankton excretion, mainly consisting of low molecular weight compounds, differs from that produced by ‘sloppy feeding’ which consists mainly of highly polymerised molecules (Christ and Rai, 1995). The degree of polymerisation has a direct influence on the intensity of enzymatic hydrolysis, which is thought to be a limiting stage in bacterial assimilation and therefore in the growth of these populations (Chróst, 1989, 1991).

The aim of this study was to determine the influence on the bacterial growth of various organic compounds (proteins and carbohydrates) produced by metazoan zooplankton processes releasing DOM. To this effect, variables such as the bacterial biomass, the free and bound amino acids and carbohydrate concentrations, and bacterial proteolytic and glycolytic activities were measured in the presence and absence of zooplankton and in relation to differing grazing intensities on the phytoplanktonic populations.

**METHOD**

**Study site**

The experiments were performed in May and June 1998, when the zooplankton community in the euphotic zone (1 m depth) was dominated by cladocerans, in the small oligotrophic Sep Reservoir (Massif Central—France) (area 33 ha, maximum depth 35 m, mean depth 14 m) (Thouvenot, 1999). Lake water was sampled using an 8 l Van Dorn-type bottle. The water temperature and dissolved oxygen were determined with a multiparameter probe (YSI GRANT 3800).

**In situ experiments**

**In situ analysis**

The chlorophyll content was determined by spectrophotometry, after extraction with acetone (Strickland and Parsons, 1972). Phytoplankton primary production was determined as described by Tadonléké-Dzatchou (Tadonléké-Dzatchou, 1999). Phytoplankton primary production was performed in duplicate water samples. Light and dark 125 ml bottles were incubated in situ (1 m depth) with the addition of 2.5 µCi of [14C]sodium bicarbonate (specific activity 50-62 mCi mmol⁻¹; Amersham). Subsamples (20 ml) were filtered through 0.45 µm pore-size membrane filters after an incubation time of 3 h. Filters were then rinsed twice with 0.1 N HCl and distilled water, and stored in a scintillation liquid (Ready protein) until counting of radioactivity with an LKB liquid scintillation counter.

The phytoplankton community was counted after Utermöhl’s method (Utermöhl, 1958) using a Letz-type inverted microscope (Wild M40). For calculation of specific biomass, the conversion factor used was 1 mg fresh weight per 10⁶ µm³; biomasses were calculated in carbon according to Blomqvist et al. (Blomqvist et al., 1995). The metazoan zooplankton was counted under a binocular microscope (Wild M-EZ) in a Dolfuss chamber. To prevent the plankton from moving about or drying out, a few drops of 10% alcohol glycerine solution were added. Animals were made more visible by staining with a few drops of rose Bengal, which stains organic matter. When the number of animals in a sample was too high it was subsampled using a Motoda box (Motoda, 1959). Rotifers were counted in a Seldewick–Rafter cell, after subsampling. The metazoans collected were identified using the keys described by Thouvenot et al. (Thouvenot et al., 2000). All the zooplankton organisms counted (at least 300) were measured. Some triplicate counts were conducted during the study of this ecosystem, the resulting coefficients of variation being 11% for rotifers, 7% for calanoids, 24% for cyclopoids and 13% for cladocerans (Thouvenot et al., 2000). The dry weight (DW) of each taxon was calculated using the formulae of Bottrell et al. (Bottrell et al., 1976).

**Grazing experiments**

Plankton samples were collected using an 8 l Van Dorn-type bottle. The water needed for an experiment was mixed to uniformity in a container. Three types of treatments were used: ‘without zooplankton’ using water samples filtered through a 100 µm mesh size net, ‘with 1*zooplankton’ using undisturbed water samples, and ‘with 4*zooplankton’ using water samples with four times the normal metazooplankton concentration (Figure 1A). In this treatment, the zooplankton was concentrated three times by straining the water volumes slowly through a 100 µm mesh net, and the animals were added to unfiltered samples, thus representing a four-fold higher concentration of metazooplankton than in the lake. The experiments were performed in triplicate clear 5 l glass bottles. Preliminary results showed that the various treatments did not lead to any significant changes (α = 5%) in the concentrations of the various organic compounds measured. The abundances of the cladoceran Daphnia longispina (the dominant species) varied from about 85 to 340 individuals l⁻¹ respectively in the treatments ‘without zooplankton’ and ‘with 4*zooplankton’. These values are similar to those encountered in a similar study (Hygum et al., 1997). The experiments lasted 6 h (Hygum et al., 1997). The bottles were suspended at 1 m depth in
the Sep reservoir, and were emptied by draining the contents through screens (50 µm). The samples were then taken immediately to the laboratory, for the rest of the experiments. At the same time as these experiments, the grazing intensity of the metazoan zooplankton was measured using the method described by Thouvenot et al. (Thouvenot et al., 2000).

A mortality rate was calculated for each species of phytoplankton, as follows:

$$\mu_w = \ln B_{w, t} - \ln B_{w, t0} / t$$  
$$\mu_z = \ln B_{z, t} - \ln B_{z, t0} / t$$

where \( \mu \) is the exponential growth rate with (\( \mu_w \)) and without (\( \mu_z \)) zooplankton; \( B_t \) and \( B_{t0} \) are the biomass (\( B_{w,t} \), \( B_{z,t} \)) in the enclosure with zooplankton and \( B_{w,t0} \), \( B_{z,t0} \) without at the beginning and the end of the experiment.

The differences between the treatment with and without zooplankton reduces to:

$$d_t = \mu_w - \mu_z$$

The carbon flow grazed, \( F \) (µgC/l/day) was computed as follows:

$$F = B_{w0} \times d_t$$

The experiments were run in triplicate; the reported mortalities are the means of the three replicates.

**Laboratory experiments**

The experimental approach in this study is shown in Figure 1B. All the bottles (1 litre) were acid washed and pre-combusted. Water samples from the different treatments were filtered through 0.2 µm sterile polycarbonate Millipore filters, under low vacuum (<0.2 bar) and a 1 ml predator-free bacterial inoculum (<1.2 µm) was added. The absence of predator (heterotrophic flagellates) was verified by epifluorescence microscopy after primulin coloration (Bloem and Bar-Gilissen, 1986). This inoculum came from a water sample collected in the reservoir at a depth of 1 m on the day of the experiments; it was the same in all three treatments. Subsamples for measuring the growth of bacteria (abundance and biomass), DOM content and proteolytic and glycolytic enzyme activities were collected immediately. Then the bottles were stored in darkness at a constant temperature (19°C) in a shaking incubator (about 30 r.p.h.), and during the phase of bacterial growth (about 40 h) further subsamples were taken at regular intervals for the assays described above.

**Laboratory analysis**

**Abundance and bacterial biomass**

Heterotrophic bacteria were fixed in 2% (final concentration) formaldehyde. One to five millilitre samples were filtered on 0.2 µm black polycarbonate filters (Millipore), stained with 1 µg l⁻¹ final concentration 4,6-diamidino-2-phenylindole (DAPI), and counted by means of epifluorescence microscopy (Peters and Fieg, 1980). Between 400 and 1000 bacterial cells were counted in 50–80 microscopic fields (the coefficient of variation was inferior to 7%). We calculated bacterial biomass by measuring every counted cell, length (L), and width (W), with an analysis image system. Bacterial biovolumes were calculated according to the formula \( \pi/6 \times W_{\text{mic}} \times L \times W/3 \) (Jaasby, 1975). The densities were converted to biomass using a conversion factor of 220 fg C µm⁻³ (Beatley and Dunbulla, 1984).

**Dissolved organic matter**

Water samples prefiltred through prewashed (with sterilized water) 0.2 µm pore size polycarbonate filters were used to determine dissolved organic matter. Dissolved combined amino acids (DCAA) were determined by the 'micro BCA Protein Assay Reagent' Kit (Pierce). Bovine serum albumin was used as a standard. The coefficients of variation for the DCAA analysis varied between 0.6% and 6.0%. Dissolved free monosaccharides (DFCHO) were determined according to Barney and Sieburth (Barney and Sieburth, 1977) and Johnson and Sieburth (Johnson and Sieburth, 1977), using glucose as a standard. Total dissolved carbohydrate (TDCCHO) concentrations were determined after hydrolysis with HCl (1 N; 100°C, 15 h). TDCCHO minus DFCHO gave dissolved combined carbohydrates (DCCHO).

**Extracellular enzyme activity**

Extracellular enzyme activities were determined using artificial substrates (Christ, 1991; Richardot et al., 1999). To investigate the hydrolytic degradation of carbohydrates (α- and β-linked to glucose) and proteins, 4-methylumbelliferyl (MUF)-α-glucoside, MUF-β-glucoside, and L-leucine-β-naphthylamide (Sigma) were added, respectively. Various amounts of L-leucine-β-naphthylamide [five concentrations between 25 and 1000 µM] and MUF-substrates [five concentrations between 0.25 and 15 µM] for α-GlA and between 2 and 100 µM for β-GlA were added to samples in order to establish substrate saturation curves to enable the calculation of apparent Vₘₐₓ. In 1998, substrates were added at saturation concentration, 15 µM for α-glucosidase activity (αGlA), 100 µM for β-glucosidase activity (βGlA), and 1000 µM for leucine aminopeptidase activity (LAP). Samples were incubated with substrates at 20°C in the dark for 2–6 h. Fluorescence was
read with a spectrofluorometer (Kontron SFM-25) at 365 nm excitation and 460 nm emission (α and β-GlcA) and at 340 nm excitation and 410 nm emission (LAP). Calibration was performed in water samples, with standard solutions of MUF and β-naphthylamine.

**Statistical analysis**
The data obtained (three replicates) were subject to an analysis of variance followed by a Scheffé’s test. We compared each experiment for each time of incubation...
(abundance and bacterial biomass, enzyme activities). We compared each experiment for initial and final time for DOM and we compared initial and final time for each experiment (specific enzyme activities).

**RESULTS**

**In situ measurements**

The dissolved oxygen concentration and temperature varied little between the two experiment periods (Table I). The chlorophyll a contents were 3.1 µg l⁻¹ in May and 10.5 µg l⁻¹ in June, while the primary production was 9.3 ± 0.9 µg C l⁻¹ h⁻¹ in May, but only 3.3 ± 0.07 µg C l⁻¹ h⁻¹ in June (Table I). The phytoplankton community was represented mainly by Euchlorophyceae, with *Eudorina* sp. accounting for 85% of the phytoplankton biomass in May and 95% in June (Table I). The zooplankton community was dominated by adult cladocerans (essentially *Daphnia longispina*) which accounted for 84% of the total metazoan biomass in May and 91% in June (Table I).

The carbon flow passing through the metazoan zooplankton was 31.8 ± 2.2 µg C l⁻¹ day⁻¹ in May and 10.2 ± 0.5 µg C l⁻¹ day⁻¹ in June (Table I). In May, more than 90% carbon biomass came from *Eudorina* sp., whereas in June, the carbon from the Cryptophyceae *Rhodomonas* sp. and *Cryptomonas* sp. accounted for 44 and 37% of the total carbon flow, respectively.

**Laboratory experiments**

**Bacterial abundance and biomass**

In the absence of predators, changes in bacterial abundance and/or biomass can be used to estimate bacterial growth. Bacterial growth (abundance and biomass) was always highest in the ‘with 4*zooplankton’ treatments (Figure 2). In May, the bacterial abundances and the biomasses in the treatments only containing phytoplankton (1.9 × 10⁶ ± 0.1 × 10⁶ cells ml⁻¹ and 12.8 ± 1.4 µC l⁻¹ at the last sampling time) were significantly lower than those in the treatments containing metazoan zooplankton (2.4 × 10⁶ ± 0.3 × 10⁶ cells ml⁻¹ and 25.1 ± 0.1 µC l⁻¹ with 1*zooplankton and 2.6 × 10⁶ ± 0.3 × 10⁶ cells ml⁻¹ and 26.2 ± 1.9 µC l⁻¹ with 4*zooplankton at the last sampling time). In June, the bacterial abundances and biomasses did not differ significantly between the ‘without zooplankton’ and ‘with 1*zooplankton’ treatments except at the last sampling time (80 hours), where the bacterial abundance and the biomass of prokaryotes were significantly higher in the ‘without zooplankton’ treatment than in the ‘with 1*zooplankton’ treatment.

**Dissolved organic matter**

After 6 h of incubation *in situ* and after filtration through 0.2 µm (corresponding to time *t* = 0 in the experiments conducted in the laboratory), there were no significant differences in the dissolved combined amino acids concentrations (DCAA) measured in the different treatments, in either of the experiments (Figure 3A). In June, there were no significant differences in carbohydrate concentrations (DFCHO or DCCHO) between the different treatments after 6 h incubation *in situ*. In contrast, in May, the dissolved free monosaccharide concentrations (DFCHO) were significantly higher in the ‘with 4*zooplankton’ treatment (0.9 ± 0.1 mg l⁻¹) than in the two other treatments (0.6 ± 0.1 mg l⁻¹ in the ‘without zooplankton’ and 0.6 ± 0.1 mg l⁻¹ in the ‘with 1*zooplankton’), and inversely, the dissolved combined monosaccharides concentrations (DCCHO) were lower (Figure 3B,C).

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**Table 1: Physical, chemical and biological data at 1 m depth**

<table>
<thead>
<tr>
<th></th>
<th>May</th>
<th>June</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>17.6</td>
<td>20.8</td>
</tr>
<tr>
<td>Oxygen (mg O₂ l⁻¹)</td>
<td>7.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Chl a (µg l⁻¹)</td>
<td>3.1</td>
<td>10.5</td>
</tr>
<tr>
<td>Euchlorophycean biomass (µg C l⁻¹)</td>
<td>5.3 ± 0.9</td>
<td>3.3 (± 0.07)</td>
</tr>
<tr>
<td>Dictomophycean biomass (µg C l⁻¹)</td>
<td>184.5 (± 35.7)</td>
<td>88.2 (± 20.2)</td>
</tr>
<tr>
<td>Pigmented flagellate biomass (µg C l⁻¹)</td>
<td>22 (± 11)</td>
<td>26 (± 10)</td>
</tr>
<tr>
<td>Calanoid biomass (µg DW l⁻¹)</td>
<td>171</td>
<td>134</td>
</tr>
<tr>
<td>Calanoid biomass (µg DW l⁻¹)</td>
<td>13.5</td>
<td>6</td>
</tr>
<tr>
<td>Copepod biomass (µg DW l⁻¹)</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Rotiferan biomass (µg DW l⁻¹)</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>
Table II: Grazing rate by metazooplankton, and its distribution (%) between different phytoplanktonic species

<table>
<thead>
<tr>
<th>Phytoplankton species</th>
<th>Mean length µm</th>
<th>% carbon flow between different species</th>
<th>May</th>
<th>June</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankistrodesmus gelificatum</td>
<td>16</td>
<td>0% 3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenedesmus crassus</td>
<td>35</td>
<td>0% 6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scenedesmus longus</td>
<td>20</td>
<td>0% 1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eudorina sp.</td>
<td>85</td>
<td>92% 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synedra rumpens</td>
<td>48.5</td>
<td>0% 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragilaria crotonensis</td>
<td>70</td>
<td>0% 5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melosira varians</td>
<td>&gt;50</td>
<td>0% 3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asterionella formosa</td>
<td>45.5</td>
<td>0% 0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodomonas sp.</td>
<td>9.5</td>
<td>0% 44%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptomonas sp.</td>
<td>15</td>
<td>8% 37%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Bacterial abundance and biomass immediately after adding the inoculum (time 0) and then at regular time intervals. Vertical bars represent the SD (three replicates) of each treatment. Non-statistically significant differences have same letter between two or three treatments (P<0.05).
Fig. 3. Concentration of DOM after 6 h in situ (open bars) and consumption of DOM by bacteria in laboratory experiment (final time concentrations—time 0 concentrations) (stippled bars). SDs are shown by the vertical bar on top of each bar. Non-statistically significant differences have the same letter between two or three treatments (P<0.05). Negative values indicate consumption of DOM whereas positive values signify DOM production.
At the final sampling time in the in situ experiments and in the two field experiments, the DFCHO were only assimilated by the bacterial plankton in the ‘with 4*zooplankton’ treatments, whereas the DCAA and the DCCHO were always used by the bacteria (Figure 5). In May, the consumption of DCAA in the ‘with 4*zooplankton’ treatment was significantly higher than that recorded in the ‘with 1*zooplankton’ and ‘without zooplankton’ treatments (Figure 5A). In June, there was no significant difference in the consumption of DCAA between the ‘with 4*zooplankton’ and ‘without zooplankton’ treatments, but these values, respectively –0.60 ± 0.1 mg l⁻¹ and –0.4 ± 0.1 mg l⁻¹, were significantly higher than that recorded in the ‘with 1*zooplankton’ treatment (~0.07 ± 0.03 mg l⁻¹). The consumption of DCCHO (Figure 3B) was lower in the ‘with 4*zooplankton’ treatment than in the other treatments in both field experiments. It was significantly lower than the consumption of DCHO in the ‘without zooplankton’ treatment and in the ‘with 1*zooplankton’ in May, and than that of the ‘with 1*zooplankton’ treatment in June.

Enzyme activities

The intensities of hydrolytic enzyme activities in all treatments and in both experiments were ranked as follows: LeuA > βGlcA > αGlcA (Figure 6). There were no significant differences between the ‘without zooplankton’ and ‘with 1*zooplankton’ treatments for any of the three enzyme activities studied. In contrast, the hydrolysis rates recorded in the ‘with 4*zooplankton’ treatments were always significantly higher than those measured in the two other treatments (Figure 6). An overall decrease in specific activities (enzyme activity/bacterial biomass) was recorded between 10 h (t = 1) and 40 h (t = 3) in May, whereas in June, with the exception of β-glucosidase activity in the ‘without zooplankton’ treatment, an increase in specific activities was recorded (Table III).

Discussion

In agreement with previously published results (Arndt et al., 1992; Peduzzi and Hernell, 1992), at metazoan zooplankton concentrations of the same order of magnitude, the presence of metazoan zooplankton generally led to an increase in the bacterial abundance and biomass. However, in the treatments containing the normal concentration of zooplankton, the bacterial abundances and/or the biomasses were not always significantly different from those measured in the absence of zooplankton and in June they were sometimes lower. In this respect, these results are similar to those obtained by Riemann et al. and Hygum et al., who recorded a higher bacterial growth when the bacterial plankton utilised the DOM derived solely from phytoplankton rather than in the presence of zooplankton (Riemann et al., 1986; Hygum et al., 1997). All our results indicate that the dynamics of bacterial populations are not necessarily proportional to the zooplankton concentration. Without excluding the possibility that other factors, such as inorganic nutrients or vitamins, may also limit bacterial growth, the organic matter produced in the various treatments and the processes of depolymerisation of this matter can explain the differences that were found.

Influence of the presence of zooplankton on the production and utilisation of DOM by the bacterial plankton

Differences in the DOM concentration after 6 hours’ incubation in situ were only visible in the experiment conducted in May. Søndergaard et al. (1997) showed that differences in prey quality between the two experiments could have caused the difference in DOM concentration, such as that occurring in this ecosystem (Richardot et al., 2000), could have masked variations caused by zooplankton grazing on the phytoplankton or related to phytoplankton excretion (Strom et al., 1997). However, excluding these methodological aspects, the quality of the prey and the flow of phytoplanktonic carbon transiting through the zooplankton, which was three times greater in May than in June, seem to be the main factors explaining the differences that were found. The quantity of DOM released as a result of grazing depends on the predation intensity and also the quality of the available prey, in terms of size and digestibility (Lampert, 1978). In May, 92% of the carbon flow consumed by Daphnia came from green algae of the genus Eudorina, whose large-sized colonies cannot be completely ingested or digested by the zooplankton, probably resulting in the release of large quantities of DOM either by sloppy feeding (Lampert, 1978) or in the faeces (Jumars et al., 1989). In contrast, in June, the carbon flow transiting through the metazoan zooplankton was mainly dominated by small pigmented flagellates belonging to the genera Rhizomonas and Cryptomonas. The small size of these algae enables them to be consumed whole, and they are almost certainly completely assimilated since these two genera are known to be of very high nutrient quality for zooplankton (Ahlgren et al., 1990). It therefore seems that differences in prey quality between the two experiments could have caused the difference in DOM production and its bioavailability for bacteria.

In both studies and in all the treatments, the DFCHO found in the ‘with 1*zooplankton’ treatments were always assimilated by the bacterial plankton, whereas there was a
production of these compounds in the two other treatments. The large difference in composition between the intracellular carbohydrates of prey that may be released by grazing and those exuded by the phytoplankton (Aluwihare and Repeta, 1999) could contribute to a differential assimilation of DFCHO. Furthermore, although the DCAA and DCCHO were assimilated by the bacteria, combined amino acids were always more strongly consumed in the treatments where the zooplankton had been concentrated, whereas polysaccharides were less assimilated by the bacterial plankton when the DOM was produced in the same conditions. The existence of a positive correlation positive between the consumption of DCAA and the bacterial biomass at the end of incubation in all treatments and in both experiments ($r = 0.81$, $P < 0.05$), confirms the role played by combined amino acids on bacterial growth, as has already been shown in many studies (Hoch and Kirchman, 1995). The DCAA and amino acids released by zooplankton activities could be a good quality protein source for heterotrophic bacteria, as shown by Peduzzi and Herndl, who demonstrated that amino acids originating from grazing by the cladoceran Daphnia ocultata on a bloom of dinoflagellates, were largely taken up by heterotrophic micro-organisms in contrast to those produced solely by phytoplankton or zooplankton (Peduzzi and Herndl, 1992).

These observations therefore confirm the hypotheses of Chróst and Rai (Chróst and Rai, 1993), who suggested...
<table>
<thead>
<tr>
<th>Table III: Specific enzyme activities (Enzyme activities/bacterial biomass)</th>
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<tbody>
<tr>
<td><strong>Specific enzyme activities</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>a-Glucosidase</td>
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<td></td>
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<tr>
<td>p-Glucosidase</td>
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<td></td>
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<tr>
<td>Leucine aminopeptidase</td>
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Note: Values in parentheses indicate standard error. p-values are given for statistically significant differences.
that the zooplankton, by its grazing activity, has an effect on DOC. Our experiments show that the differences in consumption of the DOM by bacteria between the different treatments, seem to depend mainly on the quality of the organic substrates rather than on the quantity of substrate.

Influence of the presence of zooplankton on enzymatic hydrolysis

The intensity of leucine aminopeptidase activity was higher than that of \( \beta \)-glucosidase, which itself was equal to or higher than the hydrolytic activity of \( \alpha \)-glucosidase, in all treatments and in both experiments. These show that supply of DOM originating from interactions between metazoan zooplankton and phytoplankton did not significantly change the proportions of the biodegradable protein and carbohydrate pool by altering the relative concentrations of proteins and carbohydrates bound by \( \alpha \) or \( \beta \) bonds.

The leucine aminopeptidase and \( \alpha \)- and \( \beta \)-glucosidase activities measured in the treatments containing only phytoplanktonic organisms were not always significantly different from those obtained in the treatments containing unfiltered water. In contrast, the enzyme activities in the water samples in which the zooplankton had been concentrated were always significantly higher than those measured in the treatments without zooplankton. In in situ experiments, positive correlations were found between changes in metazoan zooplankton biomass and abundance and changes in enzyme activities (\( \alpha \)- and \( \beta \)-glucosidase and leucine aminopeptidase) in the 0.2–100 \( \mu \)m particle fraction (Vrba et al., 1992; Richardsot et al., 1999). Various studies describing seasonal changes in hydrolytic activities in different ecosystems (Karner and Herndl, 1992) have also tended to show that the increase in these enzyme activities could result mainly from the release into the outside environment of intracellular or digestive enzymes from zooplankton, i.e. free enzymes occurring in the <0.2 \( \mu \)m fraction. Our results show that the proportion of enzyme activity accounted for by enzymes derived from cladocerans was negligible, since the hydrolytic activities studied were undetectable after filtration through 0.2 \( \mu \)m \( \tau < 0 \), even when the zooplankton was concentrated. Similarly, Chróst and Rai and Sala and Guile observed no significant increase in the activities in the <0.2 \( \mu \)m fraction, after enriching mesocosms with zooplankton (Chróst and Rai, 1993; Sala and Guile, 1996). It is therefore probable that the correlations between metazoan zooplankton densities and enzyme activities recorded in seasonal studies are not due to free enzymes released by cladocerans. It is probable that enzymes originating directly from zooplankton are less significant than free enzymes of bacterial origin and enzymes of attached bacteria (Vrba et al., 1992).

During the second experiment in June, the changes in specific enzyme activities suggested that the organic substrates derived from zooplankton grazing on phytoplankton stimulated the production of enzymes by bacteria. According to previous studies (Minster, 1985; Chróst and Rai, 1993), sloppily feeding activity releases a large quantity of high-molecular-weight metabolites, such as polysaccharides and proteins, which because of their high degree of polymerisation, have the property of de-repressing the synthesis of extracellular depolymerisation enzymes. This would allow these products to be hydrolysed into compounds that would then be assimilable by bacteria (Chróst and Overbeck, 1990). In contrast, during the first experiment, the DOM released by grazing activities seemed to repress enzyme activity. These results, which are apparently contradictory with those of the second experiment, could indicate that zooplankton grazing on phytoplankton does not always produce highly polymerised DOM. The metazoan zooplankton could also have an indirect impact on the composition of DOM by stimulating primary production resulting from the increase in the predation pressure that it exerts on the phytoplankton populations (Simon et al., 1998). It is, in fact, recognised that cellular excretion of photosynthetic products in the active growth phase gives rise to the production of low-molecular-weight organic compounds (Fogg, 1983; Chróst and Faust, 1983) which have the property of de-repressing enzyme synthesis (Chróst, 1989; Chróst and Overbeck, 1990). This latter hypothesis could be corroborated by the fact that in May, in addition to a higher mortality caused by cladoceran grazing than in June, the measurements of primary production made in the water column were also higher.

The significant differences between the specific activities therefore reflect processes of enzyme repression in May, whereas in June, with the exception of \( \beta \)-glucosidase activity in the ‘without zooplankton’ treatment, they represented a stimulation of these activities. These regulation phenomena could therefore explain the higher bacterial biomasses produced during the second experiment even though the flow of carbon transiting by the metazoan zooplankton at this time of year was lower.

The hypothesis that herbivores can enhance bacterial growth was supported by our results. These experiments show the importance of predator–prey relations on the quality of the organic substrates released and on the enzyme activities involved in the degradation of these compounds. However, the identification of the structure of the various organic compounds produced during zooplankton grazing on phytoplankton species is still to be determined.
ACKNOWLEDGEMENTS

We would like to thank ‘Agence de l’Eau, Loire-Bretagne’, ‘Ministère de l’environnement’, ‘Ministère de l’éducation nationale’, and ‘Société mixte de mise en valeur de l’Auvergne et du Limousin’ for allowing us to conduct our work on Reservoir Sep, and C. Dychlak and J. C. Romagous for their invaluable collaboration.

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