Elemental composition (C:N:P) and growth rates of bacteria and *Rhodomonas* grazed by *Daphnia*

Tobias Vrede

*Institute of Limnology, Uppsala University, Sweden*

*Present address: Department of Microbiology, University of Bergen, Jahnebakken 5, NO-5020 Bergen, Norway*

**Abstract.** The elemental composition and growth rate of *Rhodomonas* and heterotrophic bacteria were studied in batch cultures in the presence and absence of *Daphnia* and at two different levels of phosphorus limitation. The elemental content of single cells was measured with X-ray microanalysis. Simultaneously, dilution experiments were performed in order to estimate grazing losses, growth rates and dominant nutrient sources for bacteria and *Rhodomonas*. The phosphorus:carbon (P:C) ratios of the bacteria were generally higher in the experiment with the stronger P limitation of the system. High P:C ratios were taken as an indication that bacteria were carbon limited. The presence of *Daphnia* resulted in a further increase in bacterial P:C ratios and increased specific growth rates. Thus, grazing increased the availability both of inorganic nutrients and organic substrates, stimulating the growth of the bacteria. P:C ratios of *Rhodomonas* decreased with increasing P limitation of the system. Only at strong P limitation did the presence of *Daphnia* result in increased P:C ratios of *Rhodomonas* compared with the control without daphnids. This study shows that the elemental content and growth rate of heterotrophic bacteria and *Rhodomonas* are influenced by grazing and nutrient regeneration by daphnids. The response is dynamic and depends on the level of nutrient limitation of the system.

**Introduction**

Herbivorous crustacean zooplankton are important in the cycling of inorganic nutrients and organic matter within the pelagic food web. Besides the negative effects on phytoplankton and bacterioplankton due to zooplankton grazing, there is also a trade-off with stimulation of phytoplankton and bacterioplankton growth mediated through nutrient release by the grazers (e.g. Sterner, 1986). Both phytoplankton and bacteria may benefit from the activity of zooplankton. Regeneration of phosphorus (P) by herbivorous zooplankton can support a large part of the primary production (Lehman, 1980). Likewise, release of organic matter from zooplankton is of importance for bacterial production (Jumars et al., 1989; Peduzzi and Herndl, 1992). During recent years, it has become evident that bacterioplankton growth can also be limited by inorganic nutrients, especially P (Toolan et al., 1991; Morris and Lewis, 1992). This results in competition for inorganic nutrients between phytoplankton and bacteria. The outcome seems to be in favor of bacterioplankton because they have a higher affinity for and thus a more efficient P uptake than phytoplankton (Currie and Kalff, 1984a,b). Bacterioplankton and phytoplankton also have a commensalistic relationship because primary production is an important source of organic substrates for the bacteria. The balance between these competitive and commensalistic interactions is, therefore, of importance for the flows of nutrients and energy in planktonic food webs. Only a few studies have considered both phytoplankton and bacterioplankton growth in connection with grazing and nutrient cycling by herbivorous zooplankton (e.g. Vareschi, 1994; Sterner et al., 1995).
T.Vrede

The nutrient flow within the food web and the competition for nutrients are also influenced by stoichiometric constraints. Zooplankton have a more or less fixed intraspecific stoichiometry, while both bacterial and phytoplankton stoichiometries are highly variable (Reynolds, 1984; Andersen and Hessen, 1991; Fagerbakke et al., 1996). The available food resources might have both higher or lower carbon (C), nitrogen (N) or P content than that required by zooplankton. This implies that zooplankton have to excrete excess amounts of some elements in order to assimilate C, N and P in the right proportions to match their bodily demands. Therefore, the stoichiometry of the food particles affects the nutrient release rates (Olsen et al., 1986; Hessen and Andersen, 1992; Sterner et al., 1992). The stoichiometry of the food organisms is expected to vary across a gradient of nutrient availability. At high P concentrations, both phytoplankton and bacterioplankton can store surplus P as polyphosphate. According to stoichiometric models, this will result in high rates of P regeneration from zooplankton (Hessen and Andersen, 1992). On the other hand, when the system is strongly P limited, the organisms become P starved and approach their minimum cell quota for P. This, in turn, is predicted to result in low P regeneration rates at the same time as phytoplankton and bacterioplankton to a higher degree are dependent on regenerated P. Therefore, it is important to include the stoichiometry of food organisms and herbivores when studying the flow of nutrients within the planktonic food web.

It can be concluded that both competitive and commensalistic interactions between bacterioplankton and phytoplankton, as well as the stoichiometry of the organisms in the system, must be taken into consideration when studying the role of zooplankton in nutrient cycling in planktonic systems. This paper focuses on the effects of grazing by Daphnia on the elemental composition and growth rate of Rhodomonas and heterotrophic bacteria grown at differing levels of P limitation. To the author's knowledge, this is the first attempt to use X-ray microanalysis including analysis of light elements simultaneously on both prokaryotes and eukaryotes.

Method

Experiments were conducted with non-axenic batch cultures of Rhodomonas lacustris Pascher and Ruttner containing unknown heterotrophic bacteria. The aim was to study the effects of Daphnia grazing on the elemental composition (C:N:P) and growth rates of Rhodomonas and bacteria. The cultures were incubated in the absence or presence of Daphnia. The elemental content of single Rhodomonas and bacterial cells was measured with X-ray microanalysis (XRMA). This technique gives a high resolution of the intra- and interspecific distribution of elements in the organisms in the food web, which cannot be obtained by chemical analysis of bulk samples. In addition to the presence/absence treatments described above, a dilution series of cultures with Rhodomonas, bacteria and Daphnia was incubated simultaneously. The purpose was to estimate grazing losses, maximum and realized specific growth rates, as well as dominant nutrient sources supporting the growth of the microorganisms. The design of the
dilution series part of the experiment largely followed that of Andersen et al. (1991).

The experiment was repeated twice, differing in the level of P limitation of Rhodomonas growth in the stock batch culture. The first experiment (experiment 1) was started when Rhodomonas was in late exponential/early stationary growth phase and the soluble reactive phosphorus (SRP) concentration had fallen below 18 µg P l⁻¹ (Figure 1). After subsampling ~2.5 l of the culture for use in the first experiment, the stock batch culture was diluted to 4.5 l with P-free medium. The second experiment (experiment 2) began 5 days later. By then, Rhodomonas had been in P-limited stationary growth phase for several days and the SRP concentration was steadily low at 3.8 ± 1.3 µg P l⁻¹ (Figure 1). Before each experiment was started, the subsample from the stock batch culture was diluted with P-free medium to an approximate density of 10⁴ Rhodomonas cells ml⁻¹, which was estimated from in vivo fluorescence measurements. This density will be referred to as ‘non-diluted culture’ below.

Culturing and growth conditions

Before the experiments, the non-axenic culture of Rhodomonas containing heterotrophic bacteria was grown in a 4.5 l batch in modified 3xL16 medium (Lindström, 1991). The medium was modified with B vitamins and soil extract, and the P content was reduced to 10% of the original. This ensured that P would eventually be the limiting nutrient for Rhodomonas growth. The temperature was 16°C and the light intensity was ~35 µE m⁻² s⁻¹ at the surface of the culture during the light period of the 14:10 h light:dark cycle. Rhodomonas density in the batch culture was estimated as in vivo fluorescence with a Turner 112 fluorometer (excitation filter 5-60, emission filter 2-64). A clone of Daphnia galeata Sars was maintained in 3xL16 medium and fed exponentially growing Rhodomonas.

![Graph](https://example.com/graph.png)

**Fig. 1.** Rhodomonas density (in vivo fluorescence) and soluble reactive phosphorus (SRP) concentration in the stock batch culture. The arrows indicate the start of experiment 1 and 2, respectively. Immediately after subsampling from the stock culture for use in experiment 1, P-free medium was added.
Experimental set-up

Algal cultures both with and without daphnids were incubated for 2 days in 300 ml stoppered glass bottles (Figure 2, set-up A). All treatments were performed in duplicate, except that only one bottle without Daphnia was incubated in experiment 1. Temperature and light conditions were as above. The bottles were attached to a horizontal axis rotating at 2 r.p.m. Preliminary tests showed that growth rates of Rhodomonas and bacteria did not differ either between 300 and 120 ml flasks or between 1, 2 or 3 days incubation length. Therefore, the experiments were performed in 300 ml bottles in order to minimize the bottle effects and incubated for 2 days in order to increase the chance of obtaining measurable responses. To the Daphnia treatments, 10 animals of similar age and size were added. Daphnids were first rinsed in sterile-filtered culture and transferred with a wide-bore pipette to beakers with non-diluted culture and then to the incubation flasks. Samples for analysis of dissolved P concentration, XRMA, and
abundance of bacteria and *Rhodomonas* were taken from the algal culture at the start of the experiments as well as from the incubation flasks at the end of the experiments. At the end of an experiment, daphnids were collected by filtering the whole flask volume through a 200 μm nylon net before subsampling for the analyses mentioned above.

Two dilution series with a gradient of *Rhodomonas*, bacterial and *Daphnia* biomass were also incubated in each experiment (Figure 2, set-up B). To one of the dilution series, 12 μg P l⁻¹ and 0.6 mg C l⁻¹ were added in the form of K₂HPO₄ and glucose. The P and C were added in order to relieve P limitation of algal and bacterial growth and possible C limitation of bacterial growth. The other dilution series received no addition of P or C. All dilutions were duplicated both with and without P and C additions. The flasks with non-diluted culture without nutrient additions are identical to the *Daphnia* treatment described above.

The dilutions were made by mixing non-diluted culture with sterile-filtered culture. The filtration was accomplished in two steps. First, non-diluted culture was filtered through a GF/C glass fiber filter (Whatman, 47 mm diameter, washed with deionized water), without underpressure and without letting the filters become dry. This filtration step collected all *Rhodomonas* with a minimum of cell breakage. The *in vivo* fluorescence of the filtrate was <1% of the fluorescence in non-filtered culture and SRP concentrations did not increase in the filtrate. In the second filtration step, the filtrate was filtered through a 0.2 μm pore size polycarbonate filter (MSI, 47 mm diameter) with a GF/C supporting filter at <3.5 atm underpressure.

Daphnids were added to the flasks in the dilution series as above, except that they were transferred to beakers with appropriately diluted culture before being transferred to the flasks. *Daphnia* density was proportional to the fraction of undiluted culture, with 10 individuals per flask in non-diluted cultures and one individual per flask in the flasks with 10% undiluted culture. Samples for analysis of dissolved P concentrations and bacterial and *Rhodomonas* abundance were taken as described above.

*Chemical and biological analyses*

The SRP concentration in filtered (Munktell MGC glass fiber filter, pore size ~1.2 μm, washed in deionized water) culture medium was determined by the molybdate blue reaction (Murphy and Riley, 1962). The total dissolved phosphorus (TDP) concentration was determined as SRP after persulfate oxidation of filtered culture (Menzel and Corwin, 1965). Dissolved organic phosphorus (DOP) was calculated as the difference between TDP and SRP.

C, N and P contents of single bacterial and *Rhodomonas* cells were determined with XRMA (Heldal, 1993; Norland et al., 1995). Cells were collected by centrifugation on 100 mesh copper grids coated with formvar film. Bacteria were harvested from a 50 mm water column at 3100 g for 30 min, and *Rhodomonas* were harvested from an 80 mm water column at 500 g for 20 min.

Duplicate samples for analysis of bacterial and *Rhodomonas* abundance were preserved with formaldehyde (4% final concentration).
Rhodomonas cells were stained with acridine orange, collected on black 0.2 μm pore size polycarbonate filters (MSI) and counted in an epifluorescence microscope (Hobbie et al., 1977). Bacterial and Rhodomonas biomass was calculated as the product of abundance and average cellular C content obtained by XRMA.

Calculations and statistical analyses
The apparent specific growth rate of bacteria was calculated assuming exponential growth:

\[ \mu = \frac{\ln N_f - \ln N_i}{t} \]

where \( \mu \) is the specific growth rate, \( N_0 \) and \( N_f \) are initial and final abundance, and \( t \) is the incubation time. In the dilution series with P and C additions, it was assumed that algae and bacteria grew with the maximum specific growth rate. Hence, the decrease in apparent growth rate was interpreted as an effect of increasing grazing pressure. From this series, the maximum specific growth rate (\( \mu_{\text{max}} \)), grazing coefficient (\( g \)) and incipient limiting concentration of saturated feeding (ILC) were estimated by fitting the data to a Holling type 1 functional response curve by piecewise linear regression (Sterner et al., 1995). The mean growth rate in the dilution series without P or C addition was calculated as the apparent growth rate plus estimated grazing losses. General trends in mean growth rate across the dilution gradient were tested with linear regression analysis. Elemental ratios in bacteria and Rhodomonas are shown as pooled data for each treatment. Differences between treatments with and without Daphnia were tested with unpaired t-tests. Coefficients of variation in elemental ratios were calculated on non-transformed data both within (\( CV_w \)) and between (\( CV_A \)) treatments. The calculations were made according to Fagerbakke et al. (1996) with the modification that their ‘\( CV_A \)’ is divided by the grand average.

Results
In undiluted cultures, the Rhodomonas biomass was always larger than the bacterial biomass in experiment 1 (Figure 3A). During the experiment, the Rhodomonas biomass increased, especially in the absence of daphnids. The bacterial biomass decreased both with and without grazers, but more when daphnids were present. In experiment 2, the bacterial biomass was always larger than the Rhodomonas biomass (Figure 3B). With Daphnia absent, the biomass of Rhodomonas remained stable, while the bacterial biomass almost doubled. In the presence of daphnids, both bacterial and Rhodomonas biomasses decreased.

In the presence of Daphnia in experiment 1, the bacterial P:C ratio increased slightly but insignificantly (Figure 4A, Table I). In contrast, the bacterial P:C ratio remained stable when daphnids were absent. During experiment 2, the bacterial P:C ratio increased both in the presence and absence of daphnids (Figure 4B,
Table 1). The P:C ratio was higher in the Daphnia treatment than without animals. Polyphosphate granules were common in the bacteria, especially when daphnids were present. Bacterial P:C ratios were generally higher in experiment 2 than in experiment 1 (Figure 4A and B). The total range of bacterial P:C ratios was 0.008–0.093 for individual cells and 0.041–0.067 for treatment averages (Table II, Figure 4A and B).

Bacterial N:C ratios increased from the start both in the absence and presence of daphnids in both experiments, and were generally higher in experiment 2 than in experiment 1 (Figure 4C and D). The total range of bacterial N:C ratios was 0.14–0.48 for individual cells and 0.22–0.28 for treatment averages (Table II, Figure 4C and D).

The Rhodomonas P:C ratio decreased similarly both in the presence and absence of Daphnia in experiment 1 (Figure 4A). In experiment 2, the Rhodomonas P:C ratio remained low in the absence of grazers and increased in the presence of animals (Figure 4B, Table I). Rhodomonas P:C ratios were lower in experiment 2 compared with experiment 1, except in the presence of Daphnia (Figure 4A and B). The range of Rhodomonas P:C ratios was 0.005–0.044 for individual cells and 0.013–0.023 for treatment averages (Table II, Figure 4A and B).

Treatment averages of Rhodomonas N:C ratios varied between 0.18 and 0.23 (Figure 4C and D). The total range was 0.08–0.38 for individual cells (Table II).

Plots of bacterial apparent growth rate versus percentage undiluted culture were non-linear in both experiments, indicating saturated feeding of Daphnia above concentrations of ~60% undiluted culture (Figure 5A, Table III). Both the maximum apparent growth rate ($\mu_{\text{max}}$) and the grazing coefficient (g) were higher in the first experiment compared with the second experiment (Table III). The grazing-corrected mean specific growth rates of bacteria were generally higher in
the first experiment than in the second (Figure 5B). Bacterial growth rates increased at higher fractions of undiluted culture in both experiments (Figure 5B, Table IV). The bacterial growth rates in undiluted cultures without P and C addition were close to the estimated $V_L$ when Daphnia were present (Figure 5, Table III). In both experiments, the absence of daphnids resulted in much lower specific growth rates of bacteria than in the presence of grazers (Figure 5B). Growth rates of *Rhodomonas* were very scattered and did not permit calculations either of maximum apparent growth rates ($\mu_{max}$) or grazing coefficients ($g$).

SRP concentrations were generally lower in experiment 2 compared with experiment 1, while no difference between experiments could be seen in DOP concentration (Table V). No accumulation of SRP or DOP was observed in any experiment either with or without the presence of Daphnia. Initial concentrations of SRP and DOP did not change systematically over the dilution gradient (Figure 6). Bacterial P might contribute to DOP because of the large pore size of the

![Fig. 4. *Rhodomonas* (●) and bacterial (□) P:C and N:C ratios in undiluted cultures in experiment 1 and 2. Error bars indicate standard error.](https://academic.oup.com/plankt/article-abstract/20/3/455/1548590)
filters \((1.2 \ \mu m)\). However, average linear dimensions were \(0.7 \times 1.9 \ \mu m\) for the pooled bacterial populations \(n = 151\). Thus, most bacteria were probably retained by the filters. Both SRP and DOP concentrations were much higher than the average in the sample from the 10\% undiluted culture in the first experiment. This may be because of contamination in the sample and not because of high P content in the culture. The SRP concentration in the 20 and 30\% dilutions did

Table I. P:C ratios (average ± SE) of bacteria and Rhodomonas after incubation in the presence or absence of Daphnia. The numbers of analysed cells are shown in parentheses. Differences in the P:C ratio between treatments were tested with unpaired t-tests.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Organism</th>
<th>P:C ratio (weight:weight)</th>
<th>Daphnia present</th>
<th>Daphnia absent</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacteria</td>
<td>0.048 ± 0.002 (38)</td>
<td>0.041 ± 0.002 (16)</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhodomonas</td>
<td>0.016 ± 0.001 (19)</td>
<td>0.016 ± 0.001 (14)</td>
<td>0.947</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Bacteria</td>
<td>0.067 ± 0.003 (29)</td>
<td>0.060 ± 0.002 (27)</td>
<td>0.042</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhodomonas</td>
<td>0.017 ± 0.001 (21)</td>
<td>0.013 ± 0.001 (21)</td>
<td>0.046</td>
<td></td>
</tr>
</tbody>
</table>

Table II. P:C and N:C ratios (weight:weight) of bacteria and Rhodomonas. \(n\) is the number of analysed cells. \(CV_w\) and \(CV_A\) are the coefficients of variation within and among treatments, respectively.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Grand average</th>
<th>Total range</th>
<th>(n)</th>
<th>(CV_w)</th>
<th>(CV_A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>P:C</td>
<td>0.052</td>
<td>0.008-0.093</td>
<td>148</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>N:C</td>
<td>0.26</td>
<td>0.14-0.48</td>
<td>148</td>
<td>0.22</td>
</tr>
<tr>
<td>Rhodomonas</td>
<td>P:C</td>
<td>0.017</td>
<td>0.005-0.044</td>
<td>113</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>N:C</td>
<td>0.20</td>
<td>0.08-0.38</td>
<td>113</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Fig. 5. Bacterial specific growth rates. (A) Apparent growth rates in dilution series with phosphate (P) and glucose (C) additions. (B) Grazing-corrected mean growth rates in dilution series without P or C addition. Open symbols, experiment 1; filled symbols, experiment 2. 

\(\text{○: Daphnia present, ◦ and \bigstar: Daphnia absent. Solid lines show piecewise linear regressions (A) and linear regressions (B).} \)
Table III. Estimates (± SE) of bacterial maximum specific growth rate ($\mu_{max}$), grazing coefficient ($g$) and incipient limiting concentration (ILC) in cultures with addition of phosphate and glucose. The estimates were obtained from piecewise linear regressions fitting a Holling type 1 functional response curve to the data.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$\mu_{max}$ (day$^{-1}$)</th>
<th>$g$ (day$^{-1}$)</th>
<th>ILC (percentage undiluted culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.63 ± 0.04</td>
<td>0.0165 ± 0.0010</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>2</td>
<td>1.12 ± 0.08</td>
<td>0.0149 ± 0.0023</td>
<td>64 ± 8</td>
</tr>
</tbody>
</table>

Table IV. Linear regressions of bacterial growth rate on percentage undiluted culture. The growth rate is the mean grazing-corrected specific growth rate in flasks without phosphate or glucose addition. Parameter values are estimates ± SE.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Slope</th>
<th>Intercept</th>
<th>$P$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0065 ± 0.0014</td>
<td>0.77 ± 0.08</td>
<td>0.0017</td>
<td>0.69</td>
</tr>
<tr>
<td>2</td>
<td>0.0081 ± 0.0014</td>
<td>0.28 ± 0.08</td>
<td>0.0004</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Discussion

Elemental composition and growth of bacteria

With increasing P limitation of the experimental system, bacteria became more dominant on a biomass basis. At the same time, bacterial P:C ratios increased, somewhat contrary to expectations. Treatment averages of bacterial P:C ratios ranged between 0.041 and 0.067. This is within the range reported by Fagerbakke et al. (1996) for natural assemblages and cultured bacteria (0.040–0.090), and in the upper half of the range reported by Goldman et al. (1987) for cultured natural assemblages of marine bacteria (0.020–0.064). In contrast, lower P:C ratios than in the present study have been reported for Pseudomonas fluorescens (average 0.020, range 0.012–0.033) grown in chemostat cultures with various N:P supply ratios (Chrzanowski and Kyle, 1996). Also, the N:C ratios in the present study (0.22–0.28) were high in comparison with those reported by Chrzanowski and Kyle (1996) (average 0.17, range 0.10–0.31) and in the upper part of the ranges reported by Fagerbakke et al. (1996) (0.17–0.29) and Goldman et al. (1987) (0.14–0.27). In the case of P. fluorescens, it was grown with an excess of organic C (Chrzanowski and Kyle, 1996). This might have resulted in storage of C, thus lowering both P:C and N:C ratios. The high P:C and N:C ratios in the present study, especially in experiment 2, therefore suggest that bacterial growth both in the presence and absence of grazers was neither P nor N limited. There was also a tendency that bacterial P:C ratios were high when N:C ratios were also high (Figure 5). This may indicate a low C content rather than a high P and N content. Considering the composition of the growth medium, it is unlikely that any inorganic ion or trace element should be limiting. Thus, it seems as if bacterial growth...
Table V. Soluble reactive phosphorus (SRP) and dissolved organic phosphorus (DOP) concentrations (average ± SE) at the start and end of experiments 1 and 2, respectively.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>SRP (µg P l⁻¹)</th>
<th>DOP (µg P l⁻¹)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Start</td>
<td>5.6 ± 0.2 a</td>
<td>5.0 ± 0.3 a</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Dilution series, no P added</td>
<td>3.3 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Non-diluted culture, Daphnia present</td>
<td>3.5 ± 0.5 b</td>
<td>4.5 ± 1.0 b</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Non-diluted culture, Daphnia absent</td>
<td>6.0</td>
<td>4.0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Start</td>
<td>2.6 ± 0.2</td>
<td>3.5 ± 0.3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Dilution series, no P added</td>
<td>1.9 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Non-diluted culture, Daphnia present</td>
<td>2.8 ± 0.8 b</td>
<td>5.5 ± 1.0 b</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Non-diluted culture, Daphnia absent</td>
<td>3.8 ± 0.5</td>
<td>4.8 ± 0.5</td>
<td>2</td>
</tr>
</tbody>
</table>

a The high value in the 10% undiluted culture is omitted (cf. Figure 6).
b Also included in the dilution series average.

Fig. 6. Initial concentrations of soluble reactive phosphorus (SRP, □) and dissolved organic phosphorus (DOP, ○) in cultures without phosphate addition in the dilution experiments. (A) Experiment 1; (B) Experiment 2.

was C limited, either by organic substrate in general or a specific biomolecule such as an essential vitamin, amino acid or fatty acid. Potential sources of organic C were exudation from Rhodomonas, sloppy feeding, defecation and excretion by zooplankton, and organic compounds originating from the soil extract which was included in the stock culture medium.

Grazing-corrected bacterial mean growth rates increased in high fractions of undiluted culture in both experiments (Figure 5B, Table IV). This indicates that regenerated nutrients are the dominating nutrient source supporting the bacterial growth. Basically, the argument for this interpretation is that a large number of animals consuming more food should regenerate more nutrients than a few animals feeding in a less dense culture. Therefore, the slope of the curve showing mean growth rate as a function of percentage non-diluted culture should be positive if regenerated nutrients are important (cf. Andersen et al., 1991).
Preliminary tests showed that the bacterial growth rate did not increase above 0.8 day\(^{-1}\) even at high glucose concentrations (1.5 mg C l\(^{-1}\); data not shown). This is considerably below the estimated \(\mu_{\text{max}}\) in both experiments (1.12 and 1.63 day\(^{-1}\)), and may be interpreted as if organic C in general did not limit bacterial growth. Instead, bacterial growth may have been limited by some specific biomolecule released by Daphnia. However, this is not probable considering the high growth rates in the more diluted cultures (10–30% undiluted culture) with P and C addition (Figure 5A). If bacterial growth was limited by a specific compound regenerated by Daphnia, the addition of glucose would not have resulted in such high growth rates in these flasks. Therefore, I conclude that bacterial growth was most likely limited by organic substrate in general in these experiments. Then, the low \(\mu_{\text{max}}\) in the test with high glucose concentrations remains to be explained. Possible alternative explanations are that not all bacteria are able to utilize glucose efficiently, that P became depleted, or that some density-dependent mortality factor decreased the specific growth rate in these tests (which were performed with non-diluted cultures and without Daphnia).

The increase in bacterial P content with increasing P limitation of Rhodomonas growth and in the presence of Daphnia give support to the view that bacteria are able to outcompete algae for P at low concentrations (Currie and Kalff, 1984a; Rothhaupt and Güde, 1992). The bacterial dominance of the P uptake may have been promoted in these experiments due to the rotation of the incubation bottles, since rotation decreases the patchiness of the available P. This, in turn, shifts the P uptake even more towards bacterial dominance (Rothhaupt and Güde, 1992).

**Elemental composition and growth of Rhodomonas**

In the less P-limited conditions in experiment 1, Rhodomonas P:C ratios decreased both in the presence and absence of daphnids. This can be explained as a dilution of the internal P stores due to higher C assimilation than P uptake rates. Although the cellular P:C ratio decreased, the total amount of P allocated to Rhodomonas increased almost 3-fold in the absence of daphnids, indicating a substantial P uptake. In the presence of Daphnia, Rhodomonas grew faster than they were grazed, but the amount of P allocated to Rhodomonas decreased due to the decreasing P:C ratio.

In the second experiment, P:C ratios of Rhodomonas were close to the minimal P quota (P:C = 0.01; T.Vrede unpublished data) at the start and with Daphnia absent. In the presence of grazers, the P:C ratio increased. This is probably an effect of increased P availability due to nutrient recycling rather than ingestion of P-rich bacteria. In the latter case, one would expect a similar increase in the treatment without Daphnia too. In addition, the ingestion of fluorescent polystyrene beads (diameter 0.5 \(\mu\)m) by Rhodomonas was below the detection limit in P-limited cultures and dim light (T.Vrede, unpublished data). Therefore, it seems unlikely that this Rhodomonas strain is capable of phagotrophy, at least in the conditions prevailing in the present experiments.

Average Rhodomonas N:C ratios varied between 0.18 and 0.23, which is close to the range for general optimum values for phytoplankton of 0.16–0.21 reported
by Reynolds (1984) (calculated assuming a C content of 50% of dry weight). The high N:C ratios indicate that N was not limiting.

Role of grazers

The presence of grazers promoted bacterial growth and increased the relative P content of the bacteria. Thus, nutrient regeneration by metazoan zooplankton provided an important source not only for inorganic nutrients such as N or P, but also for organic C, fueling the bacterial community. In comparison with regenerated substrates, organic compounds exuded from Rhodomonas seem to have had little importance.

In the presence of Daphnia, the bacterial growth rate in flasks without P or C addition was close to the estimated $\mu_{\text{max}}$ in both experiments, while they grew much slower when not grazed upon. Nevertheless, the increase in bacterial growth rate in the presence of grazers was still smaller than the bacterial losses due to grazing. The presence of metazoan grazers has in some cases been reported to stimulate bacterial growth (Vareschi, 1994; Chrzanowski et al., 1995). In these studies, as well as in the present one, the increase in bacterial mortality seems to equal or exceed the growth rate increase since no biomass increase in bacteria occurred.

In the absence of grazers, the bacterial growth rates were higher in the second experiment than in the first (Figure 5B). This may be due to higher cell lysis or exudation rates when Rhodomonas were more severely P limited.

In the more P-limited system, the P:C ratio of Rhodomonas increased in the presence of grazers. This indicates that nutrient regeneration became more important for the nutrient status and growth of phytoplankton with increasing P limitation. In chemostats with nutrient-limited algae at steady state, an increase in the cellular content of the limiting nutrient increases the specific growth rate (Droop, 1974). Even at non-steady-state conditions, sestonic or algal C:P and C:N ratios are negatively correlated with algal growth rate, as predicted by the Droop model (Sterner, 1995). Thus, the increase in Rhodomonas P:C ratio in the Daphnia treatment in experiment 2 might result in an increased growth rate, even though the experiment was performed in batch cultures which were certainly not in steady state. Alternatively, the increased P:C ratios may reflect an increased P uptake and subsequent storage of P, which cannot immediately be used for growth and cell division because the cells need more time for recovery from severe nutrient limitation. Both these interpretations reflect some sort of stimulation of algal growth, either by increasing growth rate or by increasing the potential growth yield. The effect of nutrient regeneration on Rhodomonas growth rate could not be estimated directly, because estimates of apparent growth rate in the dilution experiments were very scattered (data not shown). Hence, the estimates of the grazing coefficients became too imprecise in both experiments to use as correction factors in the dilution series without P or C added.

Theoretical threshold P:C ratios for P limitation of Daphnia growth have been estimated at ~0.016–0.020 (Hessen, 1992). This is within the rather narrow range of P:C ratios of Rhodomonas in the present experiments and far below the P:C
ratios of the bacteria. Assuming that all P in bacteria and algae was available to the daphnids, it does not seem probable that Daphnia growth was P limited. Thus, they should have been efficient remineralizers of P. In addition, organic substrates were released in quantities large enough to stimulate bacterial growth substantially, although bacterial P uptake exceeded the net assimilation of C.

Sommer (1992) has shown that grazing results in intraspecific facilitation of Daphnia growth in chemostat cultures with P-limited algae and Daphnia. Grazing decreased the algal biomass, but at the same time the algal P:C ratios increased. The increase in algal P content was probably mainly an effect of decreased competition for P, but may also have been an effect of uptake of regenerated P. In accordance with these results, sestonic P:C ratios increased in a eutrophic pond both as an effect of particle elimination by zooplankton and regeneration of P (Urabe, 1995). It is not surprising that this occurs in chemostats or in eutrophic ponds, because P availability is high. On the other hand, it is not self-evident that it also occurs in P-limited batches where there are no external P sources.

The food quality of algae increases with increasing growth rate and increasing P:C ratio (Sterner, 1993). The reproductive rate especially seems to be very sensitive to decreases in P content and/or growth rate of the food algae (Sterner et al., 1993; Sundbom and Vrede, 1997). Thus, the results from the present experiments can be interpreted as if intraspecific facilitation also occurs in nutrient-limited systems. Although the overall quantity of food decreases, the quality increases as a result of the presence of grazers and their activity.

In conclusion, Daphnia grazing and subsequent release of organic and inorganic nutrients lead to increased cellular P content and stimulation of bacterial and Rhodomonas growth. It is also evident from the results that the elemental content of bacteria and Rhodomonas is highly dynamic. Furthermore, the overall nutrient availability in the system modifies the influence of the grazers on the elemental composition and growth rate of bacteria and Rhodomonas.

Acknowledgements

The X-ray microanalysis was done at the Department of Microbiology and the Laboratory for Electron Microscopy, University of Bergen, with invaluable help from Mikal Heldal, Kjell Magne Fagerbakke, Svein Norland and Egil Erichsen. Kjell Hellström, Jan Johansson and Raoul Figueroa are acknowledged for bacterial counting and chemical analyses. Dag Hessen, Tom Andersen, Mikal Heldal, Peter Blomqvist and Katarina Vrede made comments on the manuscript. This work was financially supported by stipends from NorFA and the Malmén foundation.

References

Elemental composition and growth of microplankton


Received on June 13, 1997; accepted on October 31, 1997.