Picocyanobacterial photosynthetic efficiency under *Daphnia* grazing pressure

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*Daphnia hyalina* was used to assess the impact of zooplankton on the photosynthetic activity of picocyanobacteria. A phosphorus-limited laboratory system was designed, composed of 3-μm filtered lake water with natural assemblages to which non-axenic *Synechococcus* sp. was added. Different treatments with and without *Daphnia* were arranged; aliquots from these were sampled during 3-day incubations to measure changes in picocyanobacterial net primary production (NPP), excreted organic carbon (EOC), dissolved organic carbon (DOC) and photosynthetic parameters (photosynthesis–irradiance response curves). Bacterial number, biovolume and production were measured in both the treatment and control to evaluate possible bacterial interferences. A significant increase of picocyanobacterial photosynthesis and efficiency (2- and 3-fold increase of P$_{\max}$ and α respectively) was observed in bottles supplemented with *Daphnia*, whereas cell-specific bacterial production did not. At the same time, the EOC/NPP ratio was 2.3 times lower with the grazer. This result clearly shows the impact of P recycling mediated by a grazer on picocyanobacterial growth and production.

INTRODUCTION

Investigations during the past decade have shown that the presence of *Daphnia* can substantially alter the structure and function of microbial food webs in freshwater ecosystems (Jürgens, 1994; Jürgens et al., 1994, 1997). Suspension-feeding cladocerans may have a direct effect on microorganisms by grazing and an indirect effect by acting as nutrient regenerators and/or as dissolved organic carbon (DOC) ‘producers’ (by sloppy feeding on algae and by excretion and dissolution of the faeces). Studies in this area have been devoted mainly to clarifying the effects of zooplankton grazing on bacterial growth (Peduzzi and Herndl, 1992; Chrzansowsky et al., 1995) or on total phytoplankton production (Reche et al., 1997) and in modifying algal–bacterial interactions (Sterner et al., 1995; Reche et al., 1997). Reche et al. (Reche et al., 1997) argue that zooplankton affect both primary and bacterial production by converting algal–bacterial interactions from competition to commensalism through nutrient excretion. Phytoplankton and bacterioplankton production was stimulated in a resource-limited environment, and both autotrophic and heterotrophic microorganisms reacted to zooplankton grazing with a compensatory growth (Reche et al., 1997). Similarly, enclosure experiments carried out in a eutrophic reservoir showed an increase in bacterial production within 48 h of treatment with *Daphnia* because of the release of DOC through *Daphnia* sloppy feeding (Kamjunke and Zehrer, 1999).

To date, there are no data demonstrating the impact of *Daphnia* on picocyanobacterial production and photosynthetic efficiency. Picoplankton-sized algae are the smallest efficiently collected algae (De Mott, 1985; Sterner, 1989), but picocyanobacteria have been observed whole in the gut of *Daphnia* (Stockner and Antia, 1986). As most picocyanobacteria are not broken during the ingestion process by *Daphnia* (Stockner et al., 2000), there should not be any release of organic carbon because of sloppy feeding. Therefore, when only picocyanobacteria are present, the release of organic carbon by *Daphnia* can only come from a fraction of the faecal material which dissolves immediately. Furthermore, it is known that the dominance of *Daphnia* in low-phosphorus ecosystems (hence, *Daphnia* is having a low C:P ratio) results in a low P release (Carrillo et al., 1996). All these points are against a stimulation of picocyanobacterial production under *Daphnia* grazing. Hence, the hypothesis of a possible replenishing of nutrients of undamaged
picocyanobacteria during passage through the gut should be taken into account (Stockner, 1991).

Under this scenario, we explored whether the indirect effect of Daphnia feeding on picocyanobacteria could be an increase in the photosynthetic efficiency of picocyanobacteria. This would result in a more efficient biomass production. We compared the cell-specific photosynthetic rate of Synechococcus sp. with and without Daphnia present, in a 72 h laboratory incubation also measuring excreted organic carbon (EOC) and DOC. As we used non-axenic Synechococcus cultures, we also measured bacterial activity and number and biomass variations to evaluate possible bacterial interferences. To gather specific information on the photosynthetic parameters ($\alpha$, $P_{\text{max}}$ and $E_{\text{d}}$) of picocyanobacteria with and without Daphnia, we performed a second study measuring photosynthesis–irradiance response (P/E) curves.

METHOD

Experimental design

Epilimnetic lake water (5 m depth) from Lago Maggiore, a large, deep oligotrophic lake in northern Italy (Manca et al, 1992), was filtered through 3 $\mu$m Millipore nitrocellulose filters. Natural bacteria and picocyanobacteria were passed through the filters, while the other plankton components were retained. We inspected the filtrate by epifluorescence microscopy, after DAPI (4',6-diamino-2-phenylindole) staining, to check for the presence of small nanoflagellates, finding them to be absent. In August 2002, when the experiment was carried out, phycoerythrin-rich picocyanobacteria were not present and phycoerythrin-rich picocyanobacteria were scarce in the euphotic zone of Lago Maggiore. To increase the number of picocyanobacteria, non-axenic Synechococcus sp. sourced from a phycoerythrin-rich culture [isolated from Lago Maggiore, size 1.78 $\pm$ 0.041 $\times$ 0.94 $\pm$ 0.017 $\mu$m, mean biovolume 0.74 $\pm$ 0.21 $\mu$m$^3$ cell$^{-1}$; LM 94, AF 330248 (Ernst et al., 2003)] was added to the filtered lake water to give $\sim$300 $\times$ 10$^3$ cells mL$^{-1}$, which is the highest abundance found in the euphotic zone of Lago Maggiore (Callieri and Piscia, 2002). The addition of Synechococcus was made to a 10 L bottle from which three replicate samples were taken for counting at time 0. Then, incubation bottles of 1 L were filled with this water. Three bottles (replicates) were left as controls and four as treatment as determined in the first experiment. After that time, 20 mL glass vials were filled with water sampled from the 1 L bottles, with care being taken not to pick up any daphnids. We added $^{14}$C to the vials and incubated them for 4 h in an incubator for P/E curve measurement.

Laboratory methods

Samples for counting were immediately preserved using 0.2 $\mu$m filtered cacodylate-buffered formaldehyde (final concentration 2% v/v). Picocyanobacteria were concentrated on 0.2 $\mu$m pore size polycarbonate filters (Nucleopore) and counted by autofluorescence of phycoerythrin [Zeiss Axiosplan microscope equipped with an HBO 100 W lamp, a Neofluar x100 objective, x1.25 additional magnification, and filter sets for blue and green light excitation, Zeiss filter set 09 (BP450-490, FT510 and LP520) and Zeiss filter set 14 (LP510-KP560, FT580 and LP590)]. Cells were measured by semiautomatic image...
analysis, and volumes were calculated as ellipsoids from cell length and width. Cell volumes were transformed to carbon using a conversion factor of 200 fg C µm⁻³ (Weisse, 1993). The growth rate was calculated as

$$k = \ln \left( \frac{N_t}{N_0} \right) / t$$

where $N_t$ is the cell number at time $t$, $N_0$ is the cell number at initial time and $t$ is time in days.

Total bacteria (natural assemblages plus bacteria associated with non-axenic *Synechococcus* culture) were counted after DAPI (4′,6-diamino-2-phenylindole) staining (final concentration 0.2% wt/vol) using 0.2 µm pore size black polycarbonate filters (Nuclepore) following Porter and Feig (Porter and Feig, 1980). At least 400 bacterial cells were counted on at least 10 fields of individual filters. Between 100 and 200 cells were sized using a semiautomatic image analysis system, and the volumes were calculated (Image Pro Plus). Bacterial carbon biomass was calculated according to the allometric relationship between cell volume ($V$) and carbon content ($C$) reported by Norland (Norland, 1993), as $C = 120V^{0.72}$, in fg cell⁻¹.

DOC analyses were performed with a Shimadzu analyser (5000A) after the samples had been filtered through MilliQ-washed 0.2 µm Millipore filters. SRP, total phosphorus (TP) and total inorganic nitrogen were determined according to Valderrama (Valderrama, 1981).

NPP of picocyanobacteria was measured by the ¹⁴C technique (Steeman Nielsen, 1951, 1952). Dark bottle measurements were substituted by the time 0 organic ¹⁴C measurement by adding the isotope to the dark bottle and immediately filtering and analysing (Fahnenstiel et al., 1994). The samples were incubated for 4 h in a thermostatic chamber (20°C; 60 µmol photons m⁻² s⁻¹), in 20 mL glass vials after addition of 24.8 kBq of NaH¹⁴CO₃. After incubation, samples were filtered using plastic disposable syringes with a plastic 25 µm filter holder fitted with 0.22 µm pore size nitrocellulose membranes (Millipore), and the filters were acidified with 100 µL of 1 M HCl. EOC was estimated in the filtrate, after acid bubbling in a hood for 1 h in order to remove DI¹⁴C (Gächter and Mares, 1979). Radioactivity was measured in a Beckman LS 6000 TA scintillation counter. The latter was immediately fixed by adding 90 µL of 100% trichloroacetic acid (TCA) (5% final concentration). After 1 h of incubation in the dark at 20°C, 90 µL of 100% TCA was added to the samples to stop experimental incubations. The samples were then maintained at 4°C for 30 min to extract macromolecules, after which they were filtered using a plastic syringe filled with 13 mm filter holders (Millipore, SX0001500) with 0.22 µm pore size nitrocellulose with 13 mm diameter filters (Millipore, GSWP01300). In sequence, 2 mL of 5% TCA and 2 mL of ethanol 80% were then added to each syringe and subsequently removed by filtration. The filters were removed from the syringe filter holder and placed in 6 mL scintillation vials with 2 mL scintillation cocktail (Packard, Filter Count). Radioassaying was carried out with a Beckman LS 6000 TA scintillation counter. Bacterial

$$P = P_{\text{max}} \times \tanh \left( \frac{\alpha E}{P_{\text{max}}} \right),$$

i.e.

$$P = P_{\text{max}} \left( e^{\alpha E/E_{\text{max}} - e^{-\alpha E/E_{\text{max}}}} \right)$$

where $P$ is the chlorophyll-specific production, $P_{\text{max}}$ is the chlorophyll-specific photosynthesis at light saturation, $E$ is the radiation and $\alpha$ is the initial slope. Data were fitted using Sigma Plot 2001 (SPSS Inc.) to perform the nonlinear least-squares regression.

The activity of bacteria assemblages was evaluated as a whole from ³H-thymidine and ¹⁴C-leucine uptake rates (Kirchman et al., 1985; Simon and Azam, 1989). Small volumes of samples and tracers were used following Smith and Azam’s (Smith and Azam, 1992) method, modified as follows. Filtration was used instead of centrifugation to separate incorporated labelled tracers. Samples of 1.7 mL (three replicates and one control) were incubated in 2.5 mL disposable plastic syringes closed at the bottom end with a sealed syringe needle. At the beginning of the incubation, 20 µL of ³H-thymidine (specific activity: thymidine 3.11 TBq mmol⁻¹, Amersham, UK) and ¹⁴C-leucine (specific activity: leucine 11.5 GBq mmol⁻¹, Amersham, UK), both at a 20 nM final concentration, was added to samples and control. The latter was immediately fixed by adding 90 µL of 100% trichloroacetic acid (TCA) (5% final concentration). After 1 h of incubation in the dark at 20°C, 90 µL of 100% TCA was added to the samples to stop experimental incubations. The samples were then maintained at 4°C for 30 min to extract macromolecules, after which they were filtered using a plastic syringe filled with a 3 µm filter holder (Millipore, SX0001300) with 0.22 µm pore size nitrocellulose with 13 mm diameter filters (Millipore, GSWP01300). In sequence, 2 mL of 5% TCA and 2 mL of ethanol 80% were then added to each syringe and subsequently removed by filtration. The filters were removed from the syringe filter holder and placed in 6 mL scintillation vials with 2 mL scintillation cocktail (Packard, Filter Count). Radioassaying was carried out with a Beckman LS 6000 TA scintillation counter. Bacterial
production was computed according to Smits and Riemann (Smits and Riemann, 1988) and Lee and Fuhrman (Lee and Fuhrman, 1987) for thymidine and according to Simon and Azam (Simon and Azam, 1989) for leucine. Statistical analysis was performed applying t test and two way ANOVA using Sigma Stat 2.03 (SPSS Inc.).

RESULTS

In the two experiments at initial time, SRP concentration in the experimental flasks was 0.26 ± 0.01 μM, TP was 0.49 ± 0.01 μM and total inorganic nitrogen was 60.7 ± 0.01 μM. The N:P ratio was >23, typical of P-limited systems (Wetzel, 2001). In the first experiment, after 24 h, the cells in the control (in the absence of grazers) had a growth rate (k in 48 h) of 0.21 day⁻¹. Comparing the treatments and the controls, we found a significant difference (two way ANOVA) for all the parameters except for DOC. In the treatment with Daphnia, picocyanobacterial number and biomass decreased significantly (P < 0.001) during the 72 h of the experiment, whereas we observed a more complex trend in control (Table I). In the treatment, NPP dropped, as well as the cell number (Tables I and II). However, picocyanobacterial cell-specific production was enhanced in the presence of Daphnia (P < 0.001; Fig. 1a). In this treatment, cell-specific picocyanobacterial production increased from 8.7 to 21.5 fg C cell⁻¹ h⁻¹ in 72 h, a result 2 and 2.4 times higher than in the control at 48 and 72 h respectively (Fig. 1a). Bacteria assemblage (initial time: mean cell size 0.66 ± 0.015 × 0.41 ± 0.012 μm, mean biovolume 0.51 ± 0.025 μm³ cell⁻¹) numbers and biomass increased until the 48 h, with values significantly higher in the controls than in the treatments (P < 0.001; Table I). Conversely, bacterial production expressed as thymidine and leucine incorporation increased both in the control and in the Daphnia treatment (Table II), but cell-specific bacterial production was unaffected by the grazer presence (two way ANOVA, P > 0.05) (Fig. 1b). ¹⁴C-Leucine data of cell-specific bacterial production are not shown in Fig. 1 as they mirrored thymidine estimates, though with higher values. After 48 h, the calculated PO₄ uptake

| Table I: Number and biomass of picocyanobacteria and bacteria, during the first experiment |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Hour   | Picocyanobacterial number (10⁶ cells mL⁻¹) | Bacterial number (10⁶ cells mL⁻¹) | Picocyanobacterial biomass (μg C L⁻¹) | Bacterial biomass (μg C L⁻¹) |
|        | Control + Daphnia | Control + Daphnia | Control + Daphnia | Control + Daphnia |
| 0      | 44.45 ± 8.3 44.45 ± 8.3 | 5.72 ± 0.24 5.72 ± 0.24 | 92 ± 11.7 92 ± 11.7 | 421 ± 21 421 ± 21 |
| 12     | 35.28 ± 4.5 30.00 ± 3.3 | 4.42 ± 1.53 4.42 ± 0.70 | 82 ± 14.2 71 ± 4.1 | 282 ± 96 347 ± 117 |
| 24     | 32.85 ± 7.1 21.26 ± 4.7 | 5.81 ± 0.76 4.97 ± 0.77 | 74 ± 14.0 56 ± 8.5 | 705 ± 128 446 ± 145 |
| 48     | 45.46 ± 4.7 17.25 ± 0.7 | 9.81 ± 2.07 6.47 ± 1.16 | 96 ± 12.1 46 ± 3.7 | 1228 ± 241 792 ± 180 |
| 72     | 49.91 ± 4.6 12.05 ± 0.5 | 5.37 ± 0.63 4.17 ± 0.48 | 104 ± 17.0 30 ± 3.7 | 535 ± 26 409 ± 32 |

Values are expressed as mean ± standard deviation (control, n = 3; treatment, n = 4).

| Table II: Production of picocyanobacteria and bacteria and changes in dissolved organic carbon (DOC) concentration during the first experiment |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Hours   | NPP (μg C L⁻¹ h⁻¹) (n = 8) | Thymidine | Leucine | DOC (μg C L⁻¹) (n = 15) |
|        | Control + Daphnia | Control + Daphnia | Control + Daphnia | Control + Daphnia |
| 0      | 3.85 ± 0.09 3.85 ± 0.09 | 0.46 ± 0.08 0.46 ± 0.08 | 0.09 ± 0.02 0.09 ± 0.02 | 1027 ± 17 1027 ± 17 |
| 12     | — — | 0.77 ± 0.12 0.74 ± 0.10 | 0.60 ± 0.07 0.48 ± 0.03 | 946 ± 13 1033 ± 48 |
| 24     | 5.08 ± 0.33 3.78 ± 0.13 | 3.56 ± 0.25 2.23 ± 0.48 | 18.2 ± 1.69 10.6 ± 0.28 | 1081 ± 30 1131 ± 31 |
| 48     | 5.38 ± 0.22 4.04 ± 0.10 | 8.39 ± 0.59 5.69 ± 0.45 | 24.2 ± 0.73 17.1 ± 0.90 | 1118 ± 125 1109 ± 59 |
| 72     | 4.37 ± 0.60 2.60 ± 0.10 | 4.55 ± 0.47 2.35 ± 0.15 | 14.0 ± 1.36 5.73 ± 0.29 | 984 ± 72 1003 ± 40 |

Values are expressed as mean ± standard deviation. NPP, net primary production.
was 9.4 and 18.4 $\times 10^{-9}$ nmol cell$^{-1}$ h$^{-1}$ (picocyanobacteria) and 1.4 and 1.5 $\times 10^{-9}$ nmol cell$^{-1}$ h$^{-1}$ (bacteria), for control and Daphnia treatment respectively.

DOC concentration in the controls and treatments was not significantly different ($P > 0.05$) (Table II). The EOC was higher in the control than in the Daphnia treatment by 2–3.9 times. We used the EOC:NPP ratio to evaluate the amount of organic carbon excreted per NPP unit. The EOC:NPP ratio at 48 h was higher in the control than in the Daphnia treatment ($t$ test, $P < 0.01$) (Table III).

**Table III**: Comparison of excreted organic carbon (EOC, µg C L$^{-1}$ h$^{-1}$) and of EOC:NPP (net primary production) ratios at time 48 h

<table>
<thead>
<tr>
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<th>Experiment 1</th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td>EOC</td>
<td></td>
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<tr>
<td>Control $t_{48}$</td>
<td>1.66 ± 0.34</td>
<td>0.58 ± 0.19</td>
</tr>
<tr>
<td>+Daphnia $t_{48}$</td>
<td>0.44 ± 0.05</td>
<td>0.29 ± 0.07</td>
</tr>
<tr>
<td>EOC:NPP</td>
<td></td>
<td></td>
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<tr>
<td>Control $t_{48}$</td>
<td>0.31 ± 0.074</td>
<td>0.17 ± 0.047</td>
</tr>
<tr>
<td>+Daphnia $t_{48}$</td>
<td>0.11 ± 0.010</td>
<td>0.10 ± 0.026</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation ($n = 8$).

The P/E curve was obtained from the second experiment. Chlorophyll-a-normalized data were fitted to Jassby and Platt’s (Jassby and Platt, 1976) model to estimate the photosynthetic parameters ($\alpha$ and $P_{\text{max}}$, Fig. 2). Under Daphnia grazing, picocyanobacteria doubled their maximum photosynthetic rate ($P_{\text{max}}$) and almost tripled their photosynthetic efficiency ($\alpha_{\text{control}} = 0.93; \alpha_{\text{Daphnia}} = 2.70$). $E_{\text{K}}$ (light saturation parameter = $P_{\text{max}}/\alpha$) values were 3.79 and 5.33 in Daphnia treatment and control respectively. The cells, incubated for 2 days at an irradiance of 60 µmol photons m$^{-2}$ s$^{-1}$, were not photoinhibited at 900 µmol photons m$^{-2}$ s$^{-1}$, and at this irradiance the specific production was 5.3 mg C (mg Chl$^{-1}$) h$^{-1}$ in the control and 11.2 mg C (mg Chl$^{-1}$) h$^{-1}$ in the treatment with Daphnia.

**DISCUSSION**

Previous studies emphasized the role of zooplankton as producers of dissolved carbohydrates (Peduzzi and Herndl, 1992) or nutrients (Carrillo et al., 1996; Balseiro et al., 1997) by grazing, thus supporting the growth of bacteria and algae (Sterner et al., 1995). Nevertheless, the effect of Daphnia grazing on picocyanobacteria could give different results because of the absence of sloppy feeding during ingestion and given the low C:P ratio of Daphnia. Filtered lake water with bacterial assemblages and picocyanobacteria was amended with *Synechococcus* sp. cells from a culture isolated from the same lake and with Daphnia, which has the capability of grazing on a wide cell-size spectrum (Jürgens, 1994). Daphnia can directly consume picoplankton, and in particular, *D. hyalina* was observed to feed effectively on *Synechococcus*
In the experiments, we tripled the density of daphnids with respect to the maximum encountered in Lago Maggiore (de Bernardi and Canali, 1975) to enhance the possible effects on picocyanobacteria. In our experiments, *Daphnia* grazing greatly affected picocyanobacteria by decreasing its standing stock, while increasing its cell-specific photosynthetic rate. The higher cell-specific photosynthetic rate at 48 and 72 h of incubation in the *Daphnia* treatment is likely a reaction of autotrophic prokaryotes to *Daphnia* grazing. Cell-specific picocyanobacterial phosphorus uptake consequently increased in the *Daphnia* treatment. We observed that picocyanobacterial organic carbon excretion (EOC) decreased in the *Daphnia* treatment after 48 h and was always lower with respect to the control. Although bacteria in the control had more EOC, their specific activity was not different from that in the *Daphnia* treatment. Therefore, the bacterial assemblages (natural bacteria plus those associated with the non-axenic *Synechococcus* culture) did not themselves give rise to the difference in picocyanobacterial photosynthetic efficiency found in *Daphnia* treatments and controls.

The EOC:NPP ratio in the control was approximately three times that of the *Daphnia* treatment. This means that the picocyanobacteria in the *Daphnia* treatment were much more efficient at producing biomass than those in the control. However, DOC concentration was similar in the treatment and the control, so that C release from picocyanobacteria and from *Daphnia* grazing did not contribute significantly to the background organic substrate. This result is in accordance with the expected lack of *Daphnia* sloppy feeding in the absence of eukaryotic larger algae. Nevertheless, we can infer that *Daphnia* itself released organic carbon, as the lower EOC:NPP (low dissolved carbon produced with respect to particulate) ratio in the grazer treatment indicates that the source of DOC in this treatment is mainly due to *Daphnia* and not to picocyanobacteria.

*Daphnia* presence significantly decreased the excretion of EOC from the autotrophic fraction. The measured EOC in fact represents a leftover, as part of the produced EOC can be assimilated and respired by bacteria. If bacterial production had been enhanced in the *Daphnia* treatment, it could be assumed that the decrease in EOC was due to bacterial uptake. However, in our experiments, bacterial production did not increase in the grazer treatment; hence, the decrease in EOC (and the decrease in EOC:NPP) should be attributed to a higher conversion rate of carbon fixation to biomass by picocyanobacteria.

Another evidence of the increase in picocyanobacterial autotrophic activity coupled with *Daphnia* grazing is provided by the increase in the photosynthetic parameters obtained in our second experiment. The $P_{\text{max}}$ expresses the photosynthetic capacity as it is the chlorophyll-specific photosynthesis at light saturation, while $\alpha$ is the slope of the $P/E$ curve at low light and is related to the efficiency of photosynthesis. In the presence of *Daphnia*, $P_{\text{max}}$ and $\alpha$ were two and three times higher than those in the control. $E_{\text{c}}$ was lower in the *Daphnia* treatment, implying that picocyanobacteria not only reach higher $P_{\text{max}}$ values but also reach $P_{\text{max}}$ at lower irradiance. At the same time, the EOC:NPP ratio was 2.3 times (average of the experiments) lower with the grazer. All these results support the hypothesis that picocyanobacteria generate biomass more efficiently when a grazer is present. In 48 h, the effect of *Daphnia* grazing on picocyanobacterial photosynthesis was visible and picocyanobacteria increased their photosynthetic capacity. In other words, picocyanobacteria can reach higher production at light saturation in the presence of *Daphnia*.

In conclusion, we observed an important effect of *Daphnia* grazing on picocyanobacterial functioning, with an increase of P and C cell-specific uptake and of the photosynthetic efficiency. Probably, the nutrient status of the picocyanobacterial prey is enhanced by P regeneration by the predator, especially in the light of a decreased number of prey as predation continued.

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**REFERENCES**


