JOURNAL OF PLANKTON RESEARCH | VOLUME 26 | NUMBER 12 | PAGES 1471–1477 | 2004

Picocyanobacterial photosynthetic efficiency under *Daphnia* grazing pressure

CRISTIANA CALLIERI*, ESTEBAN BALSEIRO[1], ROBERTO BERTONI AND BEATRIZ MODENUTTI[1]

CNR – INSTITUTE OF ECOSYSTEM STUDY, SECTION OF HYDROBIOLOGY AND ECOLOGY OF INLAND WATERS, VERBANIA PALLANZA, ITALY AND [1]CONICET – UNIVERSIDAD NACIONAL DEL COMAHUE, LABORATORIO DE LIMNOLOGÍA, BARILOCHE, ARGENTINA

*CORRESPONDING AUTHOR: c.callieri@ise.cnr.it

Received March 19, 2004; accepted in principle 14 July, 2004; accepted for publication August 4, 2004; published online August 20, 2004

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; Chrzanowsky *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

doi:10.1093/plankt/fbh134, available online at www.plankt.oupjournals.org

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 (α, P_{max} and E_k) 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 μm 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 (α′,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, phycocyanin-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 ± 0.041 × 0.94 ± 0.017 μm, mean biovolume 0.74 ± 0.21 μm^3 $cell^{-1}$; LM 94, AF 330248 (Ernst *et al.*, 2003)] was added to the filtered lake water to give ~300 × 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 treatments. To each treatment replicate, 40 *Daphnia hyalina* from Lago Maggiore were added. In this lake, a *D. hyalina* density of 12 ind. L^{-1} has been recorded (de Bernardi and Canali, 1975). Daphnids of similar size (1450 ± 46.3 μm body length without caudal spine) were selected under the microscope, rinsed with 0.2 μm filtered lake water and added to the bottles. The incubation was run at 20°C and with light supplied at 60 μmol photons m^{-2} s^{-1} (LI-COR 1400 data logger, LI-193SA Spherical Quantum Sensor) in a 14:10 h light:dark cycle. The first experiment lasted 72 h, with a 75 mL sample taken every 24 h for measurements of photosynthetic production, picocyanobacterial excretion, microorganism numbers and DOC. In order to evaluate the possible DOC uptake by bacteria, we counted cell number and estimated biomass and production of the heterotrophic bacterial assemblages (natural assemblages plus bacteria associated with *Synechococcus* culture), as explained in Laboratory methods. Soluble reactive phosphorus (SRP) was only measured at the beginning of the experiment, since classical chemical analysis may not detect small variations (Moutin *et al.*, 2002). In P-limited systems, picocyanobacteria or bacteria may immediately consume the P released by *Daphnia* (Vadstein and Olsen, 1989). Thus, we derived PO_4 uptake from carbon of net primary production (NPP) and carbon of bacterial production (see Laboratory methods), assuming a C:P ratio of 106 and 50 respectively (Moutin *et al.*, 2002).

All measurements were carried out in duplicate. An additional sample for DOC analyses, bacterial production and cell numbers was taken 12 h into the incubation. At the end of the experiment the volume was 740 mL, a cumulative decrease of 26% relative to the initial volume. At the end of the experiment, the bottles were inspected and the (good) condition of the daphnids was confirmed through the observation of their swimming behaviour under a stereomicroscope.

The second experiment employed the same conditions as the first one. In addition, we measured the photosynthetic parameters with P/E curves at 48 h, time long enough to observe differences between control and 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 μm filtered cacodylate-buffered formaldehyde (final concentration 2% v/v). Picocyanobacteria were concentrated on 0.2 μm pore size polycarbonate filters (Nuclepore) and counted by autofluorescence of phycoerythrin [Zeiss Axioplan microscope equipped with an HBO 100 W lamp, a Neofluar ×100 objective, ×1.25 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

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

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 (α',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 ^{14}C technique (Steeman Nielsen, 1951, 1952). Dark bottle measurements were substituted by the time 0 organic ^{14}C 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 $NaH^{14}CO_3$. After incubation, samples were filtered using plastic disposable syringes with a plastic 25 mm 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 $DI^{14}C$ (Gächter and Mares, 1979). Radioactivity was measured in a Beckman LS 6000 TA scintillation counter. The total inorganic carbon (TIC) was calculated from pH and alkalinity measurements.

The P/E curves were obtained using a 20 mL subsample (after 48 h of incubation, experiment 2) of all the replicates with *Daphnia* (paying attention not to pick the daphnids) and the controls. After the addition of 24.8 kBq of $NaH^{14}CO_3$, the 20 mL glass vials were incubated for 4 h at five different photon flux densities (900, 516, 252, 44.3 and 4.8 μmol photons m^{-2} s^{-1}) in an incubator filled with circulating water at a strictly controlled temperature (20 ± 0.2°C) and with rotation (4 r.p.m.) of the incubation vials. After incubation, the samples were treated in the same way as for the photosynthetic production measurements. Chlorophyll *a* was measured at the beginning of the 4 h incubation with an LS2 fluorometer after 1-h methanol extraction of the pigment from cells retained on the 0.2 μm filter (Nuclepore). The P/E data were normalized to chlorophyll *a* and then fitted to the model described by Jassby and Platt (Jassby and Platt, 1976):

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

i.e.

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

where P is the chlorophyll-specific production, $P_{\max}$ is the chlorophyll-specific photosynthesis at light saturation, E is the radiation and α 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 ^{3}H-thymidine and ^{14}C-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 ^{3}H-thymidine (specific activity: thymidine 3.11 TBq $mmol^{-1}$, Amersham, UK) and ^{14}C-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 fitted with 13 mm filter holders (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 μm^3 $cell^{-1}$) 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). ^{14}C-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 PO_4 uptake

Table I: Number and biomass of picocyanobacteria and bacteria, during the first experiment

Hour	Picocyanobacterial number (10^4 cells mL^{-1})		Bacterial number (10^6 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			Bacterial production (μg C L^{-1} h^{-1}) ($n = 9$)					
	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.

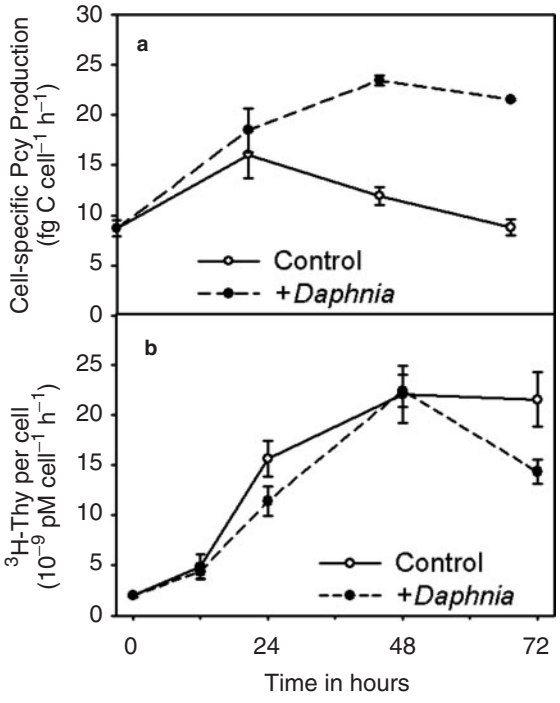

Fig. 1. Picocyanobacterial (Pcy) (**a**) and bacterial (**b**) cell-specific production, with and without *D. hyalina*. Vertical bars show standard deviation. Thy, thymidine.

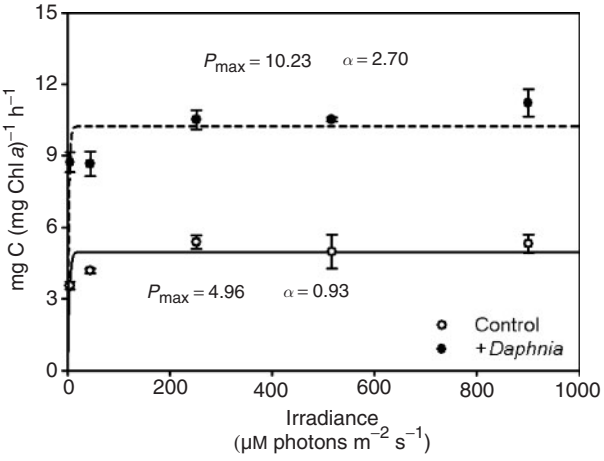

Fig. 2. Photosynthesis–irradiance (P/E) response curves of experiment 2, comparing results with and without *D. hyalina*. Vertical bars show standard deviation. P_{max}, maximum photosynthetic rate per chlorophyll *a* unit; α, maximum light utilization coefficient (efficiency).

was 9.4 and 18.4 × 10^{-9} nmol cell^{-1} h^{-1} (picocyanobacteria) and 1.4 and 1.5 × 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

	Experiment 1	Experiment 2
EOC		
Control t_{48}	1.66 ± 0.34	0.58 ± 0.19
+*Daphnia* t_{48}	0.44 ± 0.05	0.29 ± 0.07
EOC:NPP		
Control t_{48}	0.31 ± 0.074	0.17 ± 0.047
+*Daphnia* t_{48}	0.11 ± 0.010	0.10 ± 0.026

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 (α and P_{max}, Fig. 2). Under *Daphnia* grazing, picocyanobacteria doubled their maximum photosynthetic rate (P_{max}) and almost tripled their photosynthetic efficiency ($\alpha_{control}$ = 0.93; $\alpha_{Daphnia}$ = 2.70). E_k (light saturation parameter = P_{max}/α) 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*

(Brendelberger, 1985). 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_{max} expresses the photosynthetic capacity as it is the chlorophyll-specific photosynthesis at light saturation, while α 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_{max} and α were two and three times higher than those in the control. E_k was lower in the *Daphnia* treatment, implying that picocyanobacteria not only reach higher P_{max} values but also reach P_{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.

ACKNOWLEDGEMENTS

We are grateful to Dr P. L. Starkweather for the critical review of an earlier version of the manuscript. This study was carried out in the framework of the International Cooperation Program between CNR (Italy) and CONICET (Argentina). We also thank A. Calderoni for providing chemical data.

REFERENCES

Balseiro, E. G., Modenutti, B. E. and Queimaliños, C. P. (1997) Nutrient recycling and shifts in N:P ratio by different zooplankton structures in a South Andes lake. *J. Plankton Res.*, **19**, 805–817.

de Bernardi, R. and Canali, S. (1975) Population dynamics of pelagic cladocerans in Lago Maggiore. *Mem. Ist. Ital. Idrobiol.*, **32**, 365–392.

Brendelberger, H. (1985) Filter mesh-size and retention efficiency for small particles: comparative studies with Cladocera. *Arch. Hydrobiol. Beih.*, **21**, 135–146.

Callieri, C. and Piscia, R. (2002) Photosynthetic efficiency and seasonality of autotrophic picoplankton in Lago Maggiore after its recovery. *Freshwater Biol.*, **47**, 941–956.

Carrillo, P., Reche, I. and Cruz-Pizarro, L. (1996) Quantification of the phosphorus released by zooplankton in an oligotrophic lake (La Caldera, Spain) – regulating factors and adjustment to theoretical-models. *J. Plankton Res.*, **18**, 1567–1586.

Chrzanowsky, T. H., Sterner, R. W. and Elser, J. J. (1995) Nutrient enrichment and nutrient regeneration stimulate bacterioplankton growth. *Microb. Ecol.*, **29**, 221–230.

De Mott, W.R. (1985) Relations between filter mesh-size, feeding mode, and capture efficiency for cladocerans feeding on ultrafine particles. *Arch. Hydrobiol. Beih.*, **21**, 125–134.

Ernst, A., Becker, S., Wollenzien, V. I. A. *et al.* (2003) Ecosystem dependent adaptive radiations of picocyanobacteria inferred from 16S rRNA and ITS-1 sequence analysis. *Microbiology*, **149**, 217–228.

Fahnenstiel, G. L., Redalje, D. G. and Lohrenz, S. E. (1994) Has the importance of photoautotrophic picoplankton been overestimated? *Limnol. Oceanogr.*, **39**, 432–438.

Gächter, R. and Mares, A. (1979) Comments on the acidification and bubbling methods for determining phytoplankton production. *Oikos*, **33**, 69–73.

Jassby, A. D. and Platt, T. (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.*, **21**, 540–547.

Jürgens, K. (1994) Impact of *Daphnia* on planktonic microbial food web. A review. *Mar. Microb. Food Webs*, **8**, 295–324.

Jürgens, K., Gasol, J. M., Massana, R. *et al.* (1994) Control of heterotrophic bacteria and protozoans by *Daphnia pulex* in the epilimnion of Lake Cisó. *Arch. Hydrobiol.*, **131**, 55–78.

Jürgens, K., Arndt, H. and Zimmermann, H. (1997) Impact of metazoan and protozoan grazers on bacterial biomass distribution in microcosm experiments. *Aquat. Microb. Ecol.*, **12**, 131–138.

Kamjunke, N. and Zehrer, R. F. (1999) Direct and indirect effects of strong grazing *by Daphnia galeata* on bacterial production in an enclosure experiment. *J. Plankton Res.*, **21**, 1175–1182.

Kirchman, D. L., K'nees, E. and Hodson, R. E. (1985) Leucine incorporation and its potential as a measure of a protein synthesis by bacteria in natural aquatic systems. *Appl. Environ. Microbiol.*, **49**, 599–607.

Lee, S. and Fuhrman, J. A. (1987) Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl. Environ. Microbiol.*, **56**, 1298–1303.

Manca, M., Calderoni, A. and Mosello, R. (1992) Limnological research in Lago Maggiore: studies on hydrochemistry and plankton. *Mem. Ist. Ital. Idrobiol.*, **50**, 171–200.

Moutin, T., Thingstad, T. F., Van Wambeke, F. *et al.* (2002) Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus? Limnol. Oceanogr.*, **47**, 1562–1567.

Norland, S. (1993) The relationship between biomass and volume of bacteria. In Kemp, P., Sherr, B., Sherr, E. and Cole, J. (eds), *Handbook of Methods in Aquatic Microbiology*. Lewis Publishers, Boca Raton, FL, pp. 303–308.

Peduzzi, P. and Herndl, G. J. (1992) Zooplankton activity fueling the microbial loop: differential growth response of bacteria from oligotrophic and eutrophic waters. *Limnol. Oceanogr.*, **37**, 1087–1092.

Porter, K. G. and Feig, Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943–948.

Reche, I., Carrillo, P. and Cruz-Pizarro, L. (1997) Influence of metazooplankton on interactions of bacteria and phytoplankton in an oligotrophic lake. *J. Plankton Res.*, **19**, 631–646.

Simon, M. and Azam, F. (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.*, **51**, 201–213.

Smith, D. C. and Azam, F. (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. *Mar. Microb. Food Webs*, **6**, 107–114.

Smits, J. D. and Riemann, B. (1988) Calculation of cell production from [^{3}H]thymidine incorporation with freshwater bacteria. *Appl. Environ. Microbiol.*, **54**, 2213–2219.

Steeman Nielsen, E. (1951) Measurement of the production of organic matter in the sea by means of carbon-14. *Nature*, **167**, 684–685.

Steeman Nielsen, E. (1952) The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Int. Explor. Mer.*, **18**, 117–140.

Sterner, R. W. (1989) The role of grazers in phytoplankton succession. In Sommer, U. (ed.), *Plankton Ecology: Succession in Plankton Communities*. Springer-Verlag, Berlin, pp. 107–170.

Sterner, R. W., Chrzanowski, T. H., Elser, J. J. *et al.* (1995) Sources of nitrogen and phosphorus supporting the growth of bacterioplankton and phytoplankton in an oligotrophic Canadian Shield Lake. *Limnol. Oceanogr.*, **40**, 242–249.

Stockner, J. G. (1991) Autotrophic picoplankton in freshwater ecosystems: the view from the summit. *Int. Rev. Gesamten Hydrobiol.*, **76**, 483–492.

Stockner, J. G. and Antia, N. J. (1986) Algal picoplankton from marine and freshwater: a multidisciplinary perspective. *Can. J. Fish. Aquat. Sci.*, **43**, 2472–2503.

Stockner, J., Callieri, C. and Cronberg, G. (2000) Picoplankton and other non-bloom forming cyanobacteria in lakes. In Whitton, B. and Potts, M. (eds), *Ecology of Cyanobacteria: Their Diversity in Time and Space*. Kluwer Academic Publishers, pp. 195–238.

Vadstein, O. and Olsen, Y. (1989) Chemical composition and phosphate uptake kinetics of limnetical bacterial communities cultured in chemostats under phosphorus limitation. *Limnol. Oceanogr.*, **34**, 939–946.

Valderrama, J. C. (1981) The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.*, **10**, 109–122.

Weisse, T. (1993) Dynamics of autotrophic picoplankton in marine and freshwater ecosystems. In Jones, J. G. (ed.), *Advances in Microbial Ecology*. Vol. 13. Plenum Press, pp. 327–370.

Wetzel, R. (2001) *Limnology, Lake and River Ecosystems*. 3rd ed. Academic Press, London, 1006 pp.