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# Influence of metazooplankton on interactions of bacteria and phytoplankton in an oligotrophic lake

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**Abstract.** Commensalism based on organic carbon supplied by phytoplankton and competition for mineral nutrients are important interactions between bacteria and phytoplankton in oligotrophic clear-water systems. Both interactions are influenced by zooplankton activity. To examine the relationship between algae and bacteria in Lake La Caldera, we studied: the correlations among phytoplankton, bacteria and phosphorus (P) dynamics; the ratio of organic carbon supplied by algae to organic carbon demand by bacteria; and the importance of P remineralized by metazooplankton for both communities. Phytoplankton and bacteria had a similar seasonal dynamics, and there was a significant and positive relationship between bacterial abundance and algal biomass ($P < 0.01$). However, the release of organic carbon from phytoplankton was usually higher than the bacterioplankton carbon requirement. P available via zooplankton remineralization satisfied between 74 and 316% of the minimum P demands of algae and bacteria. To elucidate whether zooplankton operate similarly on algae and bacterial growth or indirectly influence bacterial growth through phytoplankton metabolism, we performed zooplankton manipulation experiments. High zooplankton biomass in these experiments stimulated both primary and bacterial production, but release of organic carbon from phytoplankton declined. These results suggest a direct stimulus of bacterial growth, so algae and bacteria can balance grazing losses by compensatory growth. Further, the algal decrease of the organic carbon supply for bacteria could, over time, lead to a change in the algae–bacteria interaction from competition to commensalism. This reduction in organic carbon excretion could affect the balance of the competitive interaction.

## Introduction

Early studies considered heterotrophic bacteria to be carbon limited and regenerators of inorganic nutrients which constitute a large proportion of the nutrient supply to producers (Axler *et al.*, 1981). This approach implied that bacteria were commensal organisms depending on organic matter from the food web. Exudation of organic compounds by phytoplankton has been considered as a major energy source for bacteria (Cole *et al.*, 1982; Vadstein *et al.*, 1989).

Recent studies, however, suggest that bacteria, in several lakes, are phosphorus (P) or nitrogen (N) limited instead of carbon (C) limited (Currie, 1990; Toolan *et al.*, 1991; Coveney and Wetzel, 1992; Pace, 1993). Bacterial composition and demand for high amounts of mineral nutrients (Jansson, 1988; Vadstein *et al.*, 1988; Vadstein and Olsen, 1989) suggest a different role in food webs (Hessen and Andersen, 1990; Vadstein *et al.*, 1993). Direct competition between phytoplankton and bacteria for mineral resources is an important interaction in nutrient-limited systems (Currie, 1990; Thingstad *et al.*, 1993). Usually, bacteria dominate P uptake at low concentrations and algae at higher concentrations (Currie and Kalff, 1984; Cotner and Wetzel, 1992).

These generalizations lead to an apparent paradox: phytoplankton, stressed by the lack of mineral nutrients, stimulate bacteria, their possible competitors, via organic C exudation (Bratbak and Thingstad, 1985). This apparent conflict has been explained by including microzooplankton grazing as a controlling factor of bacterial biomass which balances the competition between phytoplankton and bacteria (Stone, 1990; Rothhaupt, 1992; Thingstad and Rassoulzadegan, 1995).

The role of metazooplankton in these interactions, however, has been largely ignored in spite of their recognized influence on both communities (Güde, 1988; Sterner, 1990). Metazooplankton not only alter algal and bacterial communities by reducing their densities through consumption (Elser and Goldman, 1991; Pace and Funke, 1991), but also by increasing the per capita availability of inorganic and/or organic nutrients via excretion or sloppy feeding (Riemann *et al.*, 1986; Sterner, 1990; Peduzzi and Herndl, 1992; Sterner *et al.*, 1995). Although several studies have amply demonstrated that mineral nutrients released by zooplankton are readily available and can satisfy a substantial proportion of algal nutrient requirements (LaRow and MacNaught, 1978; den Oude and Gulati, 1988; Reche, 1995), the role of consumer recycling in controlling bacterial growth has only recently been studied (Chrzanowski *et al.*, 1995; Sterner *et al.*, 1995). Nutrient release by zooplankton, moreover, is variable in space and time. This heterogeneity may influence the partitioning of P uptake between algae and bacteria (Rothhaupt and Güde, 1992).

Metazooplankton can influence the interactions between algae and bacteria in two ways (Figure 1). If bacteria and phytoplankton are primarily P limited, and therefore in an intense competition, metazooplankton can balance this interaction through selective predation and nutrient recycling. Alternatively, if bacterial growth is basically controlled by organic C availability, metazooplankton, by

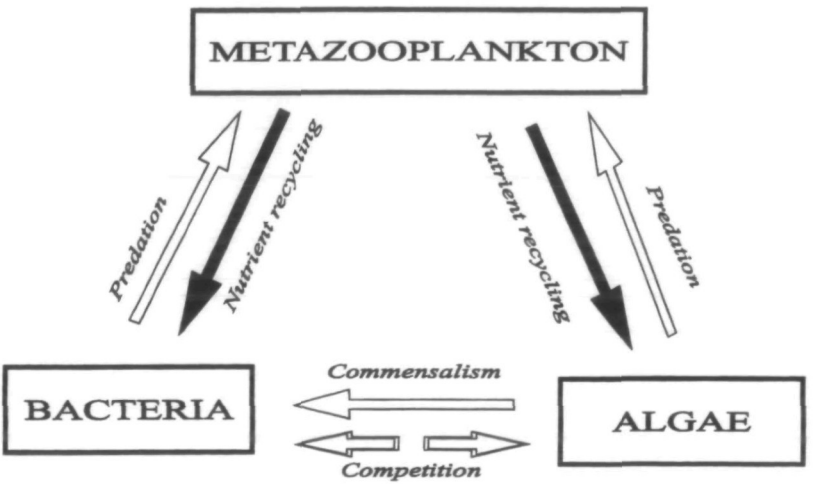


**Fig. 1.** Scheme of possible interactions among bacteria, algae and metazooplankton. Selective predation and/or nutrient recycling by zooplankton could balance the competitive or commensal interaction between phytoplankton and bacteria.

promoting a stimulus of primary production and then an increase in organic C release, can stimulate bacteria due to their commensal relationship with phytoplankton.

From this perspective, we studied how metazooplankton maintain the balance of contrasting interactions between phytoplankton and bacteria. We studied a clear-water oligotrophic system (Lake La Caldera) where metazooplankton are an important supplier of mineral resources and the main predator of both communities (Echevarría *et al.*, 1990; Carrillo *et al.*, 1995; Reche, 1995). First, we analyzed the relationships among the dynamics of phytoplankton, bacteria and P [total P (TP), P from zooplankton remineralization] and we have assessed the importance of organic C exudation from algae relative to bacterial demands. Then, we performed experiments designed to elucidate whether nutrient recycling by metazooplankton stimulates both algal and bacterial growth or whether bacteria are indirectly stimulated through phytoplankton activity.

## Site description

La Caldera is a small lake located in the Sierra Nevada of southern Spain on siliceous bedrock in a glacial cirque at an elevation of 3050 m a.s.l. It has a surface area of ~2 ha and a maximum depth of 6 m. There are no visible inlets or outlets, and the catchment area is small (~1.8 ha). Macrophytes and littoral vegetation are absent. The lake is highly transparent (e.g. Secchi depth equals water column depth).

Inputs of allochthonous nutrients to this lake are largely restricted to the ice-melt period and pH ranges from 7.8 to 9. The balance between new and regenerated production shifts towards dominance by regeneration as the season progresses. The pelagic community is very simple. Fish are absent. Contrasting with nearby lake Las Yeguas (Cruz-Pizarro *et al.*, 1994), ciliates were rarely found. This fact was a common characteristic with previous studies (Cruz-Pizarro, 1983; Carrillo *et al.*, 1990; Echevarría *et al.*, 1990). Heterotrophic nanoflagellates were below detection with traditional epifluorescence methods. Therefore, copepods, rotifers and cladocerans are the main predators both on algae and on bacteria (Reche, 1995; I.Reche *et al.*, in preparation).

## Method

### *Sampling and abiotic measurements*

Routine sampling spanned the ice-free period from July to September of 1992 and 1993. Measurements include: temperature, pH, photosynthetically active radiation (PAR), and abundance and biomass of bacteria, phytoplankton and zooplankton. In addition, primary and bacterial production were measured at four clearly differentiated phases of the plankton succession (Carrillo *et al.*, 1995).

Temperature and pH were recorded, with a YSI probe, and PAR was measured with a spherical quantum sensor LICOR (LI 193SA), from the surface to the bottom at 20 cm intervals.

The samples were taken with a Van Dorn bottle (8 l) at four depths (surface, and 25%, 50% and 75% of extinction of PAR) at a central station ($Z_{max}$ = 6 m). Subsamples from each depth were stored at 4°C for laboratory determination of alkalinity, TP, ammonium, nitrite and nitrate (dissolved inorganic nitrogen, DIN) [American Public Health Association (APHA), 1976]. Subsamples for algal, bacterial and zooplankton abundance and biomass determinations were fixed with Lugol's iodine solution, 3% and 4% formaldehyde, respectively, for later analysis.

*Phytoplankton, bacterial and zooplankton standing stocks*

Phytoplankton samples were sedimented and the cells were counted in 100 randomly selected fields at 1000× magnification. For every sample, 20 cells of each species were measured by image analysis (LEICA, Quantimet 500) to estimate cell volume according to an appropriate geometric shape. Cell volumes were converted to C using a conversion factor of 150 fg C $\mu m^{-3}$ (Vadstein *et al.*, 1988).

Bacteria were stained with DAPI, concentrated on a 0.2 μm Nuclepore filter, and counted by epifluorescence microscopy (Porter and Feig, 1980). At least 400 cells were counted for each sample. Preparations from the samples were photographed and bacterial diameter was measured by image analysis (LEICA, Quantimet 500) on digitized negatives. Bacterial biomass (C $cell^{-1}$) was obtained using a conversion factor dependent on cell volume (Simon and Azam, 1989).

To determine the zooplankton abundance and biomass, the samples were identified and counted using an inverted microscope and measured by image analysis. Twenty individuals of each species were measured from every sample. The biomass of crustaceans and rotifers was estimated using standard length–mass relationships (Botrell *et al.*, 1976) or relationships developed specifically for the most important species of Lake La Caldera (Cruz-Pizarro, 1983; Morales-Baquero *et al.*, 1988).

*Primary production, organic carbon exudation and bacterial production*

Primary production was measured by the $^{14}C$ technique (Steemann-Nielsen, 1952) by adding 10 μCi of $NaH^{14}CO_3$ (sp. act. 8.4 Ci $mmol^{-1}$, NEN Dupont) to 130 ml samples. Clear and dark Pyrex bottles were incubated at each depth (surface, and 25%, 50%, 75% of extinction of the PAR) for 4 h.

Apparent net primary production (total organic carbon, TOC) was measured by acidifying a 4 ml subsample in a 20 ml scintillation vial (100 μl of 1 N HCl, pH <2) and allowing the vial to stand open in a hood for 24 h (no bubbling) as recommended by Lignell (1990). Particulate primary production was determined by filtering an aliquot of 30 ml onto 1.0 μm (particulate organic carbon, POC, 1 μm) and onto 0.2 μm (POC 0.2–1 μm) 25 mm Nuclepore filters (serial filtrations). To minimize cell breakage, we applied low pressure (<100 mm of Hg). The filters were placed in scintillation vials, the inorganic $^{14}C$ was removed by adding 100 μl of 1 N HCl. The filtrate <0.2 μm (dissolved organic carbon, DOC) was also collected and treated in the same way as the TOC. After 12 h, the filters were counted in a scintillation counter provided with autocalibration (Beckman LS 6000TA).

Total $CO_2$ in the lake water was calculated from measurements of alkalinity and pH (APHA, 1985).

The $^{14}C$ retained on the 0.2 μm filters corresponds to the exudates incorporated by bacteria, given that autofluorescent picoplankton <1 μm are absent throughout the ice-free period. These values (POC 0.2–1 μm) were corrected for retention of bacteria on the 1 μm filter (as the percentage [$^3H$]thymidine retained on 1 μm).

Because of respiration, the amount of exudation assimilated by bacteria is underestimated by this procedure, and so the gross assimilation of exudates (GEA) was calculated by assuming 40% respiration (Bell and Sakshaug, 1980).

The gross exudation rate (GE) is represented as:

$$GE = GEA + DOC$$

Gross primary production (GPP) was calculated in accordance with Lignell (1990) by correcting the apparent net primary production for the losses due to bacterial respiration.

Net bacterial production (NBP) was measured at two depths (surface and bottom). The water profile is homogeneous for numerous parameters (i.e. temperature, chlorophyll concentration, primary production, previous measurements of bacterial production, etc.).

Bacterial production was measured based on [$^3H$]thymidine incorporation measurements. Ten samples were incubated for 30 min with 10 nM (methyl-$^3H$)thymidine (sp. act. 80–84 Ci $mmol^{-1}$, Amersham), a saturating concentration for this system (Reche, 1995). The incubations were stopped by adding NaOH, causing base hydrolysis, which breaks down RNA. After incubation, a treatment with cold trichloroacetic acid (TCA) was carried out for 20 min, leading to the precipitation of the DNA and of the proteins. The samples were filtered through 0.2 μm Nuclepore filters and rinsed twice with cold 5% TCA. Subsequently, half of the samples (3 + 2 blanks) were submitted to an enzymatic digestion procedure with DNase I (Boehringer), so that only proteins remained in the TCA precipitate (Robarts *et al.*, 1986). The difference between the two treatments was the [$^3H$]thymidine incorporated into DNA. [$^3H$]Thymidine incorporated was converted to number of cells produced by multiplying by $2 \times 10^{18}$ cells $mol^{-1}$ thymidine incorporated (Bell, 1990). The amount of C produced was estimated from the volume conversion factors obtained in the water column.

The organic carbon required by the bacterioplankton (BCR) was calculated from NBP and growth efficiency (BGE). We used values of 0.3 and 0.5 to bracket BGE (Cole and Pace, 1995).

$$BCR = NBP/BGE$$

*Phosphorus remineralized by zooplankton*

Phosphorus released by the zooplankton into the water column was determined by multiplying the phosphorus-specific release rate (SRR) by the zooplankton

biomass (mg $l^{-1}$). The SRR was calculated using the linear function obtained from several *in situ* experiments for the zooplankton of Lake La Caldera during the same studied period (see Carrillo *et al.*, 1996a). This function ($P < 0.001$) has as independent variable the individual mean size (MS) of zooplankton:

$$SRR(\mu g\ P\ mg^{-1}\ h^{-1}) = 4.91 - 6.16MS\ (mm)$$

Minimum P requirements for phytoplankton were obtained from gross primary production and the ratio 40C:1P (weight). Minimum P requirements for bacteria were estimated using the ratio 11.2C:1P (weight) proposed by Vadstein *et al.* (1988) for these organisms. We also calculated the gross P requirements of seston <40 μm using the sum of the gross C produced by algae and bacteria and the actual seston <40 μm C:P ratio in the water profile (data from Carrillo *et al.*, 1996b).

*Experimental design*

To assess the effect of metazooplankton (plankton >40 μm) on bacterial and algal growth, we conducted a set of short-term experiments *in situ* comparing bottles without zooplankton (control treatment, C) and with a zooplankton concentrate added (zooplankton treatment, Z). Three experiments were performed during summer of 1993.

To remove all zooplankters (control treatment, C) lake water was filtered through a 40 μm nitex net in a 20 l container. For treatment (Z), in another 20 l container we concentrated zooplankton 10–15 times using a 32 l Schindler–Patalas sampler equipped with a 40 μm pore size net to trap the whole zooplankton assemblage without adding algae or dissolved nutrients. Replicates were taken for each treatment. We immediately filled from the 20 l containers three clear and one dark bottle (Pyrex) with 130 ml (C treatment) or 300 ml (Z treatment) to quantify primary production and organic C exudation, and six 15 ml bottles and four blanks (final concentration 0.25 N NaOH) to quantify bacterial production.

The bottles were incubated at the depth where the zooplankton were collected, for 4 h in the case of primary production and organic C exudation bottles, and for 30 min in the case of bacterial production. The procedures to obtain these measurements were the same as those used in the water column samples, except in the serial filtration of the primary production samples in the Z treatments which included a previous filtration through 40 μm to quantify the $^{14}C$ incorporated into zooplankton.

## Results

*Environmental conditions and plankton dynamics*

During the ice-free period La Caldera has a uniform temperature profile, with surface–bottom differences of <2°C and a narrow range of temperature (8–13°C). PAR at noon reaching the surface layer ranged from 1056 to 1133 μE $cm^{-2}$ $s^{-1}$, with a vertical attenuation varying between 21 and 23% per meter in July and August, and close to 37% in September.

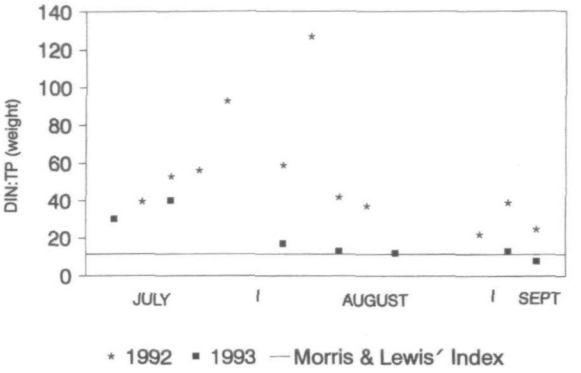


**Fig. 2.** Seasonal changes in the dissolved inorganic nitrogen (DIN):total phosphorus (TP) ratio during the ice-free period of 1992 and 1993. The horizontal line represents the P limitation threshold following the Morris and Lewis index.

TP ranged from 5.4 to 10.7 μg P $l^{-1}$ with pronounced vertical homogeneity. The DIN concentration decreased slowly over the summer from 243 to 85 μg N $l^{-1}$. The DIN:TP (by weight) ratio has been proposed by Morris and Lewis (1988) as a good indicator of nutrient limitation. A ratio higher than 12 implies a strong likelihood of P limitation. The DIN:TP ratio in La Caldera was higher than 12 during the ice-free period, except in September of 1993 (Figure 2).

Algal abundance fluctuated between 2000 and 8300 cells $ml^{-1}$ in 1992, and between 2000 and 14 000 cells $ml^{-1}$ in 1993, with the maximum values towards the middle of August. Biomass showed a similar seasonal pattern with values from 7 to 38 μg C $l^{-1}$ in 1992 and from 4 to 19 μg C $l^{-1}$ in 1993. Flagellate populations, mostly *Chromulina nevadensis*, developed after ice-melt. In August, *Cyanarcus* sp. (a unicellular, non-N-fixing cyanophycean) proliferated, accounting for >50% of the total abundance. Finally, in September a second *C.nevadensis* population developed.

The bacterial community of La Caldera was composed primarily of free coccus-like forms. Changes in cell abundance (from $0.8 \times 10^6$ to $1.8 \times 10^6$ cells $ml^{-1}$ in 1992 and from $0.6 \times 10^6$ to $1.9 \times 10^6$ cells $ml^{-1}$ in 1993) were accompanied by variations in bacterioplankton biomass (from 16 to 35 μg C $l^{-1}$ and from 12 to 56 μg C $l^{-1}$ in 1992 and 1993, respectively), with a seasonal pattern similar to that of the phytoplankton community. These similar seasonal dynamics implied a significant and positive relationship between bacterial and phytoplankton standing stocks (Figure 3).

Zooplankton community composition was simple, with a calanoid copepod (*Mixodiaptomus laciniatus*) comprising almost 85% of the total zooplankton density. Rotifers (*Hexarthra bulgarica*) and cladocerans (*Daphnia pulicaria*) were present at low abundance. The maximum abundance of this community was reached in midsummer. The biomass changed from 4 to 192 μg dry weight (dw) $l^{-1}$ and from 10 to 112 μg dw $l^{-1}$ in 1992 and 1993, respectively.

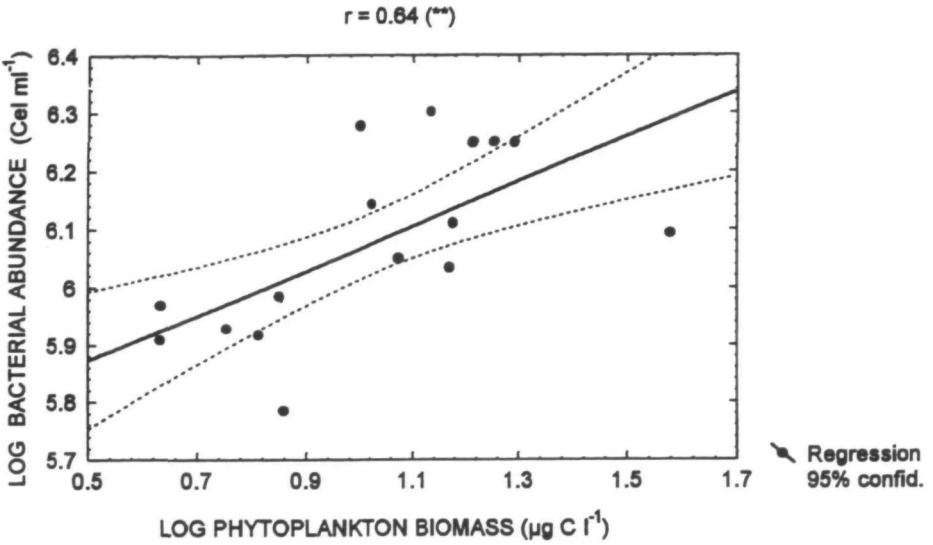


**Fig. 3.** Relationship between phytoplankton biomass and bacterial abundance. Data (log–log transformed) are averages for the water column of 1992 and 1993.

*Analysis of the phytoplankton–bacteria relationship*

The lowest gross primary production values (0.26–0.87 µg C $l^{-1}$ $h^{-1}$) were observed in July, when the algal community was mainly *C.nevadensis*, whereas at the beginning of August, with the development of *Cyanarcus* sp., maximum production occurred (2.46–3.41 µg C $l^{-1}$ $h^{-1}$). Subsequently, there was a decrease of almost 50% (1.1–2.11 µg C $l^{-1}$ $h^{-1}$) as a new population of *C.nevadensis* developed. The gross exudation of compounds of photosynthetic origin (GE) ranged from 0.12 to 0.65 µg C $l^{-1}$ $h^{-1}$, with the highest values at the end of the ice-free period. The NBP ranged between a minimum value of 0.019 and a maximum of 0.145 µg C $l^{-1}$ $h^{-1}$.

The importance of organic C released by phytoplankton for bacterial metabolism was assessed directly by comparing GE values with BCR. The values of BCR calculated were generally lower than the rates of GE, irrespective of the growth efficiency considered. Therefore, if we assume that algal organic C excretion is

**Table I.** Comparison between the organic carbon required by bacteria and the organic carbon supplied by phytoplankton at different phases of plankton succession during the ice-free period of 1993 in Lake La Caldera. Data are mean values ± SE for the water column

| Date | Organic carbon exudation (µg C $l^{-1}$ $h^{-1}$) | Organic carbon requirements (µg C $l^{-1}$ $h^{-1}$) | |
|---|---|---|---|
| | | Growth efficiency = 0.3 | Growth efficiency = 0.5 |
| 7 July | 0.20 ± 0.01 | 0.41 ± 0.04 | 0.24 ± 0.02 |
| 4 August | 0.36 ± 0.04 | 0.28 ± 0.01 | 0.17 ± 0.00 |
| 17 August | 0.35 ± 0.03 | 0.06 ± 0.00 | 0.04 ± 0.00 |
| 7 September | 0.61 ± 0.03 | 0.33 ± 0.03 | 0.20 ± 0.02 |

**Table II.** Comparison between the phosphorus required by algae and bacteria and the phosphorus supplied by metazooplankton in Lake La Caldera during the ice-free period of 1993. Data are averages for the water column

| Date | P released by zooplankton ($\mu g\ P\ l^{-1}\ day^{-1}$) | Minimum algal P requirements ($\mu g\ P\ l^{-1}\ day^{-1}$) | Minimum bacterial P requirements ($\mu g\ P\ l^{-1}\ day^{-1}$) | Minimum requirements ($\mu g\ P\ l^{-1}\ day^{-1}$) | Gross seston requirements ($\mu g\ P\ l^{-1}\ day^{-1}$) |
|---|---|---|---|---|---|
| 7 July | 0.48 | 0.13 | 0.52 | 0.65 | 1.35 |
| 4 August | 0.93 | 0.76 | 0.36 | 1.12 | 3.34 |
| 17 August | 1.36 | 0.33 | 0.10 | 0.43 | 1.31 |
| 7 September | 1.14 | 0.45 | 0.42 | 0.87 | 9.5 |

mainly available for bacterioplankton, there appears to have been sufficient C input from the phytoplankton based on exudation alone to support the bacterioplankton (Table I).

Traditionally, TP concentration has been considered, in large-scale studies, as a good indicator of the P availability to both algae and bacteria (Currie, 1990). However, in Lake La Caldera, on a seasonal scale, there is no significant relationship between the TP concentration and algal and/or bacterial biomass or abundance (all regression analyses $P > 0.05$). The comparison between P available for bacteria and algae through zooplankton release and P requirements shows that the P from zooplankton regeneration can satisfy between 74 and 316% of the minimum P demands (Table II). With respect to the gross P requirements of the seston fraction <40 μm, the P released by zooplankton can satisfy between 12 and 104% of the demands.

### *Experiments on the effects of the metazooplankton on phytoplankton and bacteria interactions*

Increased zooplankton biomass stimulated primary production (Figure 4, Table III). Analysis of the partitioning of $^{14}C$ among the different plankton size fractions revealed that the increase in the total organic C production in the Z treatments

**Table III.** Results of ANOVAs to determine the effect of high zooplankton biomass on algal and bacterial production and on the organic exudation rate. Values are expressed in $\mu g\ C\ l^{-1}\ h^{-1}$. $F$ is the variance ratio

| Date | Treatments | Algal production | Bacterial production | Organic carbon excretion |
|---|---|---|---|---|
| 8 August 1993 | Control | 1.65 ± 0.14 | 0.081 ± 0.013 | 0.38 ± 0.04 |
| | Zooplankton | 2.05 ± 0.13 | 0.157 ± 0.016 | 0.14 ± 0.00 |
| | $F$ | 4.3 n.s. | 13.5* | 42.7* |
| 17 August 1993 | Control | 0.95 ± 0.03 | 0.020 ± 0.002 | 0.32 ± 0.03 |
| | Zooplankton | 1.60 ± 0.12 | 0.051 ± 0.002 | 0.16 ± 0.04 |
| | $F$ | 27.2** | 84.6*** | 9.6* |
| 15 September 1993 | Control | 1.03 ± 0.03 | 0.012 ± 0.003 | 0.40 ± 0.04 |
| | Zooplankton | 1.35 ± 0.08 | 0.030 ± 0.006 | 0.35 ± 0.03 |
| | $F$ | 14.8* | 8.3* | 0.7 n.s. |

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

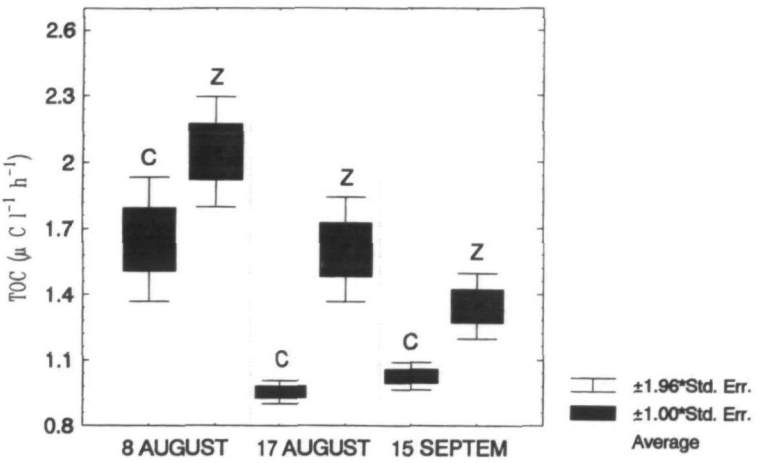


**Fig. 4.** Values of the total organic carbon production (TOC) in the treatments control (C) and zooplankton (Z). The points represent averages, the boxes represent the standard errors (SE) and the error bars represent 1.96 SE.

was completely incorporated into the zooplankton biomass in all the experiments (Figure 5).

Net bacterial production was also significantly stimulated in the Z treatments (Figure 6, Table III). This stimulation of the bacterial production could have been promoted either directly by an increase in the P or organic substrates via zooplankton, or indirectly by an increase in the organic C supply due to increased algal production. However, the inputs of dissolved organic C were significantly reduced in the Z treatments of the experiments performed in August (Figure 7, Table III), a time when the autotrophic community was definitely limited by inorganic P (Figure 2). Therefore, the algal community reduced the organic C

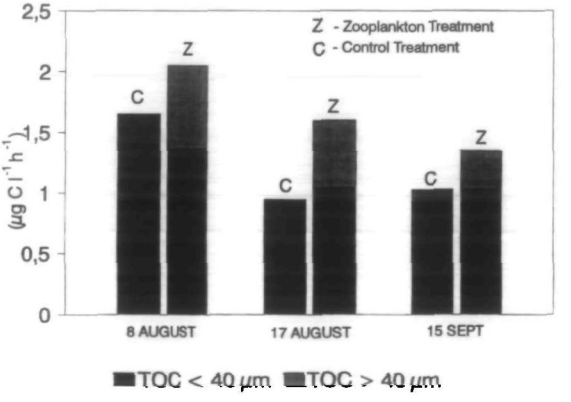


**Fig. 5.** Partitioning of total organic carbon produced between zooplankton and seston <40 µm in the experiments. Data are averages for three replicates.

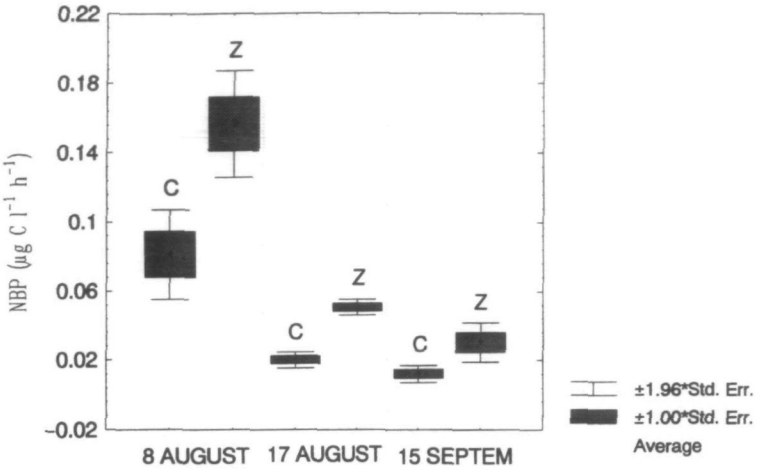


**Fig. 6.** Values of the net bacterial production (NBP) in the treatments control (C) and zooplankton (Z). The points represent averages, the boxes represent the standard errors (SE) and the error bars represent 1.96 SE.

exudation when it was limited by P and an increase in P availability (via zooplankton remineralization) took place.

## Discussion

*Phytoplankton–bacteria relationship under natural conditions*

The values for abundance and biomass of the phytoplanktonic and bacterial communities in Lake La Caldera are within the range characteristic of oligotrophic

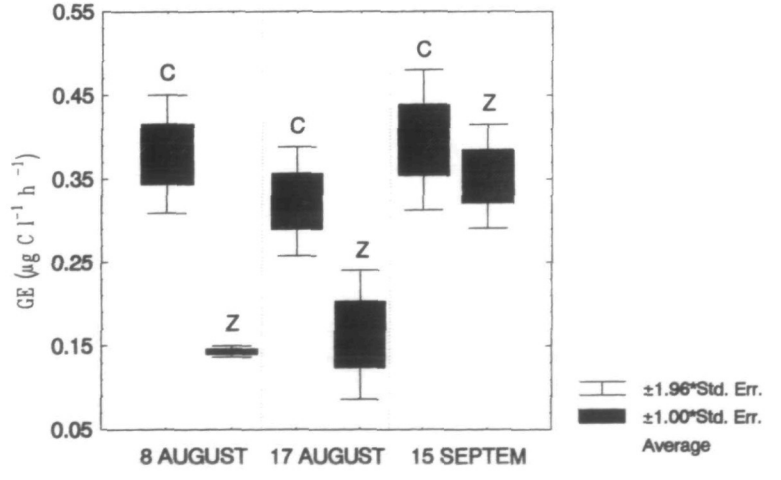


**Fig. 7.** Values of the organic carbon gross exudation (GE) in the treatments control (C) and zooplankton (Z). The points represent averages, the boxes represent the standard errors (SE) and the error bars represent 1.96 SE.

high-mountain lakes (Capblancq, 1972; Thomas *et al.*, 1991a,b; Reche *et al.*, 1994). The primary production estimates do not deviate markedly from prior observations (Capblancq, 1972; Thomas *et al.*, 1991a,b). Nevertheless, the bacterial production values were at the lower limit of those found in the literature for these systems (Borsheim and Andersen, 1987; Thomas *et al.*, 1991a,b).

The direct and significant relationship between the bacterial and phytoplankton standing stocks (Figure 3) can be interpreted either as the dependence of the bacterioplankton on the energy resources supplied by algae, or as a covariation of the two communities with respect to a third factor (Cole *et al.*, 1988; Currie, 1990).

The fact that autotrophic production does not seem to be a good predictor of the bacterial production in Lake La Caldera (Reche *et al.*, 1996) and that the ratio of bacterial C requirements to organic C exuded by algae was typically less than one (Table I) suggests a weak dependence of bacterioplankton on organic C released by autotrophs. The absence of decisive methodological errors in the bacterial C demands and/or the gross exudation measurements have been considered in making these assertions (Reche *et al.*, 1996).

Phosphorus availability, which controls autotrophic production in Lake La Caldera (Carrillo, 1989; Reche, 1995), can also control bacterioplankton. An appropriate estimate of the immediate availability of P, during the ice-free period, in this system would be the P input from zooplankton remineralization, as a highly significant fraction of the P demand by algae and bacteria is supported by the zooplankton (Table II). Moreover, Carrillo *et al.* (1995) have shown that in Lake La Caldera the P released by zooplankton, in spite of the concentration of total P, is clearly involved in the pattern of phytoplankton succession. Therefore, it is plausible that a competitive relationship could exist between bacteria and algae for this remineralized P.

### *Influence of metazooplankton on bacteria and algae in the experimental conditions*

Autotrophic production was stimulated under high zooplankton pressure (Figure 4, Table III). This response is called 'compensatory growth' and plays an essential role in supporting algal biomass in resource-limited environments where zooplankton act as a net nutrient regenerator (Redfield, 1980; Elser and MacKay, 1989; Pérez-Martínez *et al.*, 1994).

Bacterial production increased in the Z treatments (Figure 6). This effect could also be called 'compensatory growth' if this growth is the direct result of zooplankton activity. Two processes, nutrient (mineral and/or organic) excretion and sloppy feeding (breakage of prey during feeding), have been proposed as possible direct mechanisms (Riemann *et al.*, 1986; Güde, 1988; Peduzzi and Herndl, 1992; Chrzanowski *et al.*, 1995). We believe that the stimulus of bacterial production in the experiments performed was related to P zooplankton remineralization.

Sloppy feeding and organic C excretion from zooplankton did not seem to be important mechanisms in controlling bacterial production in the experiments performed. Most organic C from zooplankton excretion or from sloppy feeding

should have been measured as part of the [$^{14}$C]DOC pool. Practically all the new TOC produced within Z treatments was ingested by zooplankton during the incubation period (Figure 5). On the other hand, during sloppy feeding, it seems that the animals themselves do not contribute significantly to the ambient organic substrates—e.g. dissolved free amino acids (DFAA)—otherwise a more likely source of DFAA is from damaged phytoplankton (Riemann *et al.*, 1986) and they have also been measured as [$^{14}$C]DOC. Peduzzi and Herndl (1992) noted the highest stimulus on bacterial abundance in treatments which included both phytoplankton and zooplankton communities. The organic C availability for bacterial growth even decreased in Z treatments performed in August (when P was the nutrient limiting), irrespective of whether its origin was from algae or from zooplankton. Therefore, the P released by zooplankton in Lake La Caldera appears to be essential in regulating both bacterial and phytoplankton growth. Similar results have been obtained by Chrzanowski *et al.* (1995) and Sterner *et al.* (1995), but the most important contribution of our experimental approach was that the organic C availability was measured. This has allowed us to elucidate the actual origin of the bacterial growth.

Several studies have shown that bacteria generally dominate in the uptake of inorganic P at ambient concentrations typical of P-limited habitats (Currie *et al.*, 1986; Cotner and Wetzel, 1992; Thingstad *et al.*, 1993). The algal community, on the other hand, has different mechanisms, such as a high storage capacity, phosphatase enzymes, and/or grazing on bacteria to compensate for the lower P-uptake capability (Chrost and Overbeck, 1987; Nygaard and Tobiesen, 1993). Lignell (1990), in the same way, observed a reduction in the algal organic C excretion under mineral enrichments. In this context, we have interpreted the surprising reduction in organic C exudation from algae, under increased P availability, as an alternative strategy which can modulate the phytoplankton–bacteria interactions by changing the initial P competition to a C commensalism when the organic C supply has been reduced. This shifting interaction may be an important feature of phytoplankton–bacteria relationships, especially in clear-water oligotrophic systems.

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