

Neither with nor without you: A complex algal control on bacterioplankton in a high mountain lake

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Abstract

The literature proposes that the microbial loop is a key link for ecosystem functioning, particularly in oligotrophic conditions. From original and published data for a period spanning 1986 to 1999, we examined the food web structure of a clear-water, oligotrophic, high mountain lake. The heterotrophic microbial food web was weakly developed in a grazing food chain dominated by copepods and phytoplankton, the latter mainly composed of mixotrophic flagellates. Bacteria constituted a minor component of the plankton community in terms of abundance, biomass, and production, in contrast to the situation usually reported in oligotrophic conditions. Abiotic and biotic factors that regulate bacterioplankton biomass and production were assessed, including the bacterivory capability of mixotrophic flagellates experimentally quantified by using ³H-thymidine as a tracer. Algae were the main factor controlling bacterioplankton. Their regulatory effect has a dual nature: (1) a resource-based control, through the dependence of the bacteria on photosynthetic carbon released by algae, i.e., a commensalistic interaction (“without you I cannot live”) and (2) a predatory control, with bacteria as prey for mixotrophs (“with you I die”). Mixotrophic metabolism can constitute an adaptive strategy for algae to overcome ultraviolet (UV) stress, by using bacteria as a source of carbon and mineral nutrients in conditions of inhibited photosynthesis and mineral nutrient uptake. Mixotrophy acts as a bypass of carbon flux toward the grazing food chain, explaining the scarce development of the heterotrophic microbes in this and other high mountain lakes.

The role of bacteria in ecosystem functioning, as a link between dissolved organic matter (DOM) and higher trophic levels, appears to strengthen in more oligotrophic conditions, where bacterial:algal biomass and bacterial:primary production ratios tend to be higher than in eutrophic ecosystems (Gasol et al. 1997; Biddanda et al. 2001; Cotner and Biddanda 2002).

Within an oligotrophic gradient, high mountain lakes over the tree line are exposed to low temperatures, high ultraviolet irradiation, long ice and snow cover period, and fluctuating hydrology. These abiotic factors may influence the development and dynamics of bacterioplankton (Straskrabová et al. 1999). The generally low concentration of dissolved organic carbon (DOC) in clear-water high mountain lakes (commonly <1 mg L⁻¹, see Laurion et al. 2000), and its potentially low quality or bioavailability due to photoreactivity and photoalteration processes (Benner and Biddanda 1998; Reche et al. 2001), may make carbon the main limiting nutrient to bacterial growth. Moreover, the interaction of low mineral nutrient and/or DOC concentrations with

stressful physical conditions may aggravate their harmful effects on bacteria (Scully et al. 1996; Xenopoulos and Bird 1997). On the other hand, several studies have reported that harsh conditions had no harmful effect on bacteria. Thus, low temperature did not appear to impede bacterial growth in alpine lakes and even within the ice cover of high mountain lakes (Felip et al. 1995; Karlsson et al. 2001). The ultraviolet B (UVB)-inhibition of bacterial growth can be attenuated by DOM photolysis increasing molecules' bioavailability (Lindell et al. 1995; Benner and Biddanda 1998), by ultraviolet A (UVA) and/or photosynthetic active radiation (PAR) due to photorepair mechanisms (Kaiser and Herndl 1997 and references therein), or by the interaction with algae (Aas et al. 1996), which can also stimulate the bacterial growth exposed to full sunlight (Carrillo et al. 2002). It is, therefore, difficult to generalize about the key factor determining the bacterial dynamics in clear-water high mountain lakes.

Biotic factors, such as the commensalistic versus competitive interaction between algae and bacteria or the predatory control of heterotrophic bacterivorous protists, have traditionally been considered responsible for the structure and seasonal succession of bacterioplankton in water ecosystems (Bratbak and Thingstad 1985; Thingstad et al. 1997; Pedrós-Alió et al. 2000 and references therein). However, the predatory control over bacteria of algal assemblages exhibiting a mixotrophic metabolism has seldom been considered a major cause of bacterioplankton structure or activity (e.g., Hitchman and Jones 2000), despite the preponderance of mixotrophs in many high mountain lakes (Straskrabová et al. 1999). This predominance suggests that mixotrophy is an

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advantageous strategy in these environments, despite its higher energetic cost compared with that of obligate autotrophic or heterotrophic metabolism (Rothhaupt 1996; Raven 1997). Besides, it must be borne in mind, from an evolutionary perspective, that phagotrophy in pigmented cells was and is an essential ability that drives the evolution of photosynthetic eukaryotes (Raven 1997).

The immediate benefit of mixotrophy lies in the acquisition of organic C and/or mineral nutrients from prey in order to maintain growth (Caron et al. 1993b; Raven 1997). Because C:N or C:P ratios of prey (mainly bacteria) are usually lower than those of photosynthetic phagotrophs, bacteria are suitable sources of mineral nutrients for mixotrophs in mineral nutrient limiting conditions (Caron et al. 1993b). This scenario also provides a compensatory effect because mixotrophs eliminate their main competitors for mineral nutrients (Thingstad et al. 1996). When light is the main limiting factor of algal growth, the differences in C:N or C:P ratios between prey and mixotrophs can produce a nutrient excess in the latter, which may be released into the environment in dissolved form (Rothhaupt 1997). Jones (1997) proposed that a suitable procedure to identify the resources that mixotrophs require from their prey would be to quantify ingestion rate variations in response to light conditions.

Based on the above information, we hypothesize that mixotrophy may play a key role in the control of bacterioplankton dynamics, with important consequences for carbon flux and structure of plankton community in clear-water high mountain lakes. Furthermore, considering the inhibitory effect of UV radiation on both C fixation (Callieri et al. 2001; Carrillo et al. 2002 and references therein) and dissolved nutrient uptake by primary producers (Hessen et al. 1995; Döhler 1997), we discuss in this paper the possibility that the mixotrophic metabolism is an adaptive strategy to counteract the harmful effect of UV radiation, allowing mixotrophs to dominate phytoplankton in these ecosystems.

In the present work, we assessed the main factors that potentially control the biomass and activity of bacterioplankton, including the photosynthetic C released by algae and the bacterivory ability of mixotrophic algae experimentally quantified under in situ conditions. Our results revealed that phytoplankton have a dual role in controlling bacteria.

Material and methods

Study site—La Caldera is a remote oligotrophic (total phosphorus [TP] < 10 $\mu\text{g P L}^{-1}$, Chlorophyll < 1 $\mu\text{g L}^{-1}$) high mountain lake situated above the tree line. This ecosystem is located in the Sierra Nevada mountain range (South Spain, 36°55'–37°15'N, 2°31'–3°40'W) on siliceous bedrock in a glacial cirque at an altitude of 3050 m. The lake, usually covered by ice from November to mid-July, has a surface area of around 20,000 m² and a mean depth of 4.3 m, with a variable interannually maximum depth (from 2 to 14 m); there are no visible inlets or outlets, and the drainage basin area:lake surface area ratio is low (Villar-Argaiz et al. 2001).

The lake water is highly transparent (>10% PAR [400–700 nm] penetrates to maximum depth) and receives consid-

erable UV irradiance (Carrillo et al. 2002). Inputs of allochthonous organic C are negligible and largely restricted to the ice-melting period. The dissolved organic carbon (DOC) concentration is below 1 mg L⁻¹, and most DOC is of autochthonous origin (Reche et al. 2001). The bacterial/primary production ratio is less than 1 (Carrillo et al. 2002). Macrophytes, littoral vegetation, and fish are absent, and the pelagic community is relatively simple (Carrillo et al. 1996; Villar-Argaiz et al. 2001).

Abiotic and biotic structural parameters—Abiotic (temperature, TP, total nitrogen [TN], dissolved inorganic nitrogen:total phosphorus ratio [DIN:TP]) and biotic (chlorophyll *a*, and biomass of obligate autotrophs, mixotrophs, bacteria, heterotrophic nanoflagellates, ciliates, and zooplankton) parameters were original (1986, 1987, and 1999), as well as compiled from previous studies. Sampling and methods were of routine use in high mountain lakes (Straskrabová et al. 1999) and are further described in the referred studies (see Table 1).

Biotic functional parameters—Primary and bacterial productions were determined at different depths throughout the open-water period from July to early November in both 1996 and 1997, yielding a total of 24 measurements (in triplicate). For primary production (PP) measurements, sets of four 70-ml quartz flasks (three clear and one dark), with 0.37 MBq of NaH¹⁴CO₃ (specific activity [SA]: 310.8 MBq mmol⁻¹, NEN Dupont) added to each flask, were incubated in situ at 0.5 m under surface, 0.5 m above the bottom of the lake, and at an intermediate depth, for 4 h symmetrically distributed around noon. All flask sets were horizontally held during the incubations. The laboratory procedure has been described in detail elsewhere (Carrillo et al. 2002). Briefly, it consisted of the determination of total organic ¹⁴C followed by serial filtration through 1- and 0.2- μm filters to segregate the organic ¹⁴C retained in algal (>1 μm), bacterial (0.2–1 μm), and dissolved (<0.2 μm) fractions.

No autotrophic picoplankton were found in lake water samples during the open-water period (see Results), and no significant differences (*t*-test for dependent samples, *t* = 1.40, *df* = 5, *p* = 0.22) were found in bacterial counts between whole and 1- μm filtered water. Both findings imply that the ¹⁴C retained on the 0.2- μm pore-size filters from <1- μm filtrates corresponded to the algal exudates incorporated by heterotrophic bacteria. The algal excretion of organic C (EOC) corresponded to the organic ¹⁴C measured in the <1- μm fraction, i.e., the sum of ¹⁴C in the bacterial and dissolved fractions.

For bacterial production (BP) measurements, a set of 10 (6 + 4 blanks) acid-cleaned and sterilized flasks, each filled with 25 ml of lake water with added [methyl-³H] thymidine (SA: 2.6–3.2 TBq mmol⁻¹, Amersham Pharmacia; 10.9 nmol L⁻¹, saturating final concentration), was incubated in situ in the dark for 1 h at the same depths as were used for PP measurements. The laboratory procedure has been described in detail elsewhere (Carrillo et al. 2002).

Mixotrophy experiments—We experimentally assessed the bacterivory (mixotrophic) ability of algae as the proportion

Table 1. A summary of annual mean values of abiotic and biotic parameters (temperature [T]; total phosphorus [TP]; inorganic dissolved nitrogen : total phosphorus ratio [DIN : RP]; chlorophyll *a* [Chl *a*]; biomass of obligate autotrophs [AUT], mixotrophs [MIX], bacteria [BAC], heterotrophic nanoflagellates [HNF], ciliates [CIL], and zooplankton [ZOO]) in La Caldera Lake. Numbers in parentheses indicate number of sampling days.

Parameters	1986*	1987*	1992†	1993†	1995‡	1996‡	1997‡	1999
T (°C)	10.3 (26)	13.7 (20)	12.2 (11)	14.4 (6)	11.4 (21)	5.2 (15)	7.7 (15)	12.8 (3)
TP ($\mu\text{g L}^{-1}$)	5.1 (26)	3.9 (8)	4.4 (11)	7.6 (6)	7.1 (21)	4.7 (15)	4.4 (15)	9.1 (3)
DIN:TP (by mass)	170.6 (26)	62.0 (8)	48.3 (11)	24.2 (6)	36.9 (21)	65.9 (15)	46.4 (15)	51.1 (3)
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	0.77 (26)	0.64 (11)	n.m.§	n.m.	0.59 (21)	1.37 (15)	0.53 (15)	0.35 (3)
AUT ($\mu\text{g C L}^{-1}$)	1.07 (25)	2.87 (11)	10.50 (7)	5.36 (9)	15.40 (21)	1.32 (15)	8.45 (15)	21.79 (3)
MIX ($\mu\text{g C L}^{-1}$)	3.01 (25)	0.92 (11)	4.88 (7)	5.38 (9)	9.55 (21)	38.67 (15)	23.90 (15)	3.96 (3)
BAC ($\mu\text{g C L}^{-1}$)	n.m.	n.m.	12.68 (11)	11.48 (11)	10.33 (21)	4.88 (15)	1.88 (15)	5.20 (3)
HNF ($\mu\text{g C L}^{-1}$)	n.d.§ (25)	n.d. (11)	n.d. (11)	n.d. (9)	5.15 (21)	0.06 (15)	0.11 (15)	n.d. (3)
CIL ($\mu\text{g C L}^{-1}$)	n.d. (25)	n.d. (11)	n.d. (11)	n.d. (9)	0.67 (21)	3.85 (15)	0.02 (15)	n.d. (3)
ZOO ($\mu\text{g C L}^{-1}$)	26.4 (25)	36.75 (13)	37.47 (6)	37.00 (6)	52.70 (21)	4.04 (15)	28.84 (15)	30.10 (3)

* Echevarría et al. (1990); Carrillo et al. (1995).

† Carrillo et al. (1996).

‡ Villar-Argaiz et al. (2002).

§ n.m., not measured; n.d., not detectable.

of bacterial production incorporated by the algal fraction by means of a single technique (tritiated thymidine [TdR] incorporation). This procedure is based on the incapability of eukaryotic algae and cyanobacteria to uptake dissolved thymidine at the currently employed concentrations (nmol L^{-1}) to measure bacterioplankton secondary production (Fuhrman and Azam 1982; Bern 1985; Robarts and Wicks 1989). Consequently, TdR actively incorporated by algae have to be derived from labeled native bacteria consumption. The advantage of using this technique in contrast to others (e.g., fluorescently labeled bacteria [FLBs], dilution method, etc.) lies in that TdR satisfies the requirements of an ideal tracer to the simultaneous measurement of bacterial production and its consumption by mixotrophs: it offers vitality, "staining" specificity, detection sensitivity, and temporal stability and causes minimal disturbance to bacterial and consumer assemblages and their trophic relationships (Epstein and Rosset 1995; Caron 2001). On the other hand, the epifluorescence microscopy observations revealed that phytoplankton in La Caldera was composed by free algal cells without attached bacteria, therefore avoiding an overestimation of TdR measurement on the algal fraction. Our approach also allowed the evaluation of flagellate bacterivory under in situ conditions, as recommended by Sherr and Sherr (2002), and contributes to the demanded experimental studies on algae-bacteria interactions in different ecosystems (Daufresne and Loreau 2001).

Experiments were conducted on cloudless days in August and September of 1999, usually two distinct periods in the seasonal succession of the pelagic community in La Caldera Lake (Carrillo et al. 1996; Villar-Argaiz et al. 2001). For each experiment, a composite water sample was constructed from equal volumes of water samples obtained with an acid-cleaned 6-liter horizontal Van Dorn sampler from three depths that spanned the entire water column and were affected by >1% of incident UVB (0.5, 3, and 5 m; Carrillo et al. 2002). The water was prescreened through a 40- μm mesh to remove zooplankton.

A 2 × 2 treatment experimental design was used: (1) pres-

ence versus absence of algae (namely, $\text{BP}_{\text{A+B}}$ vs. BP_{B} , respectively) and (2) full sunlight versus dark. The BP_{B} treatment constituted a bacterivory control (absence of mixotrophs) and was obtained by filtering a maximum of 100 ml per filter through 1 μm (glass fiber filter Whatman GF/B). Because of their small size, bacteria were not significantly retained on the 1- μm filters (*t*-test for dependent samples, *t* = 1.40, *df* = 5, *p* = 0.22), and the negligible abundance of ciliates and heterotrophic nanoflagellates (HNFs) during the experiments (*see Results*) precluded a bias estimation of the algal fraction.

The light treatments were carried out using sets of five (three plus two blanks) experimental 5-ml quartz bottles horizontally held on a platform, either uncovered (light treatment) or covered with opaque material (dark treatment). In order to determine the proportion of bacterial production incorporated by algal fraction, an additional set of five (three plus two blanks) experimental 35-ml quartz bottles (presence of algae) was added to each light treatment. This set was used to separately quantify the incorporation of TdR in the algal and bacterial fractions.

Incubations with TdR (*see BP measurements*) started immediately after samples were disposed (negligible acclimation time) to evaluate the instantaneous response of the organisms to the experimental treatments. Samples were incubated at 0.5 m depth for a maximum of 90 min, symmetrically distributed around noon. This incubation time minimizes the potential artifact associated with label release and/or recycling (Caron et al. 1993a). After incubations, samples were fixed with neutral formaldehyde (3.4% final concentration), which was also applied to blank bottles before the incubations. These blanks served as controls for the bacterial incorporation of TdR and the passive algal incorporation and adsorption of TdR.

After incubations, the water from the set of 35-ml bottles was filtered by gravity through a 1- μm Nuclepore filter. This filter (after two rinses with 2 ml of 0.2- μm filtered lake water) was used to quantify the bacterial production incorporated by the algal fraction (BP_{A}), whereas the 1- μm filtrate

served to quantify the remnant bacterial production (not incorporated by algal fraction, BP_B). The bacterial production was quantified following the centrifugation protocol developed by Smith and Azam (1992). The bacterial TdR incorporation rate was converted to cell number by using the conversion factor of 0.815×10^{18} cells mol TdR⁻¹ experimentally calculated for this lake. The amount of bacterial C produced was obtained by using the factor of 2×10^{-14} g C cell⁻¹ (Lee and Fuhrman 1987). The bacterivory ability of algae was estimated using the above conversion factors and the abundance and biomass data of mixotrophs as the specific consumption of bacterial production (C_{BP} , bacteria consumed cell⁻¹ h⁻¹, or $\mu\text{g C}_{\text{bac}} \mu\text{g C}_{\text{mix}}^{-1}$ h⁻¹, where C_{bac} is bacterial carbon and C_{mix} is mixotrophic carbon). The bacterivory ability of algae (BA) was also estimated as percentage of bacterial production incorporated by algal fraction:

$$BA = BP_A \times (BP_{A+B})^{-1} \times 100$$

Statistical analysis—Stepwise multiple-regression analyses were carried out to assess the relative influence of potential factors controlling bacterial biomass (temperature, TP, TN, and biomass of mixotrophs, autotrophs, heterotrophic microbacterivorous, and zooplankton) and bacterial production (bacterial and algal biomass, temperature, EOC, TP, TN) in 1996 and 1997. Linearity and multiorthogonality among independent variables were verified by previous correlation analysis and/or controlled by specifying 0.6 as the minimum acceptable tolerance (StatSoft 1997). The *F* values entering the multiple-regression model were established on the basis of the number of independent variables and cases. The normal distribution of residues was checked by Shapiro–Wilks' *W* tests. Interannual variation of algal and bacterial activity variables was tested by *t*-test.

The impact of mixotrophic bacterivory on bacterioplankton was tested by one-way analysis of variance (ANOVA) comparing BP_B to BP_{A+B} data for each light treatment and date. The effects of algae absence (BP_B vs. BP_{A+B}), sunlight (light vs. dark treatments), and their interaction on bacterial TdR incorporation rate were tested by two-way ANOVA for every experimental period. The differences among treatments were examined by contrast analysis (least squares difference [LSD]), adjusting their probabilities using Bonferroni's test. Data were checked for normal distribution with the Shapiro–Wilks' *W* test. Homoscedasticity was verified with the *F*-ratio variance and Levene's tests, and the data were log-transformed when these conditions were not met. Statistics were performed with Statistica 5.1 for Windows (StatSoft 1997).

Results

Abiotic and structural biotic parameters—Table 1 shows the interannual variations in several physical, chemical, and biological parameters (annual means) from 1986 to 1999 in La Caldera Lake. In summary, thermal stratification was generally absent or weakly developed in midsummer. TP values were usually lower than $10 \mu\text{g P L}^{-1}$, although occasional increases were recorded in late summer (e.g., 4 Sep-

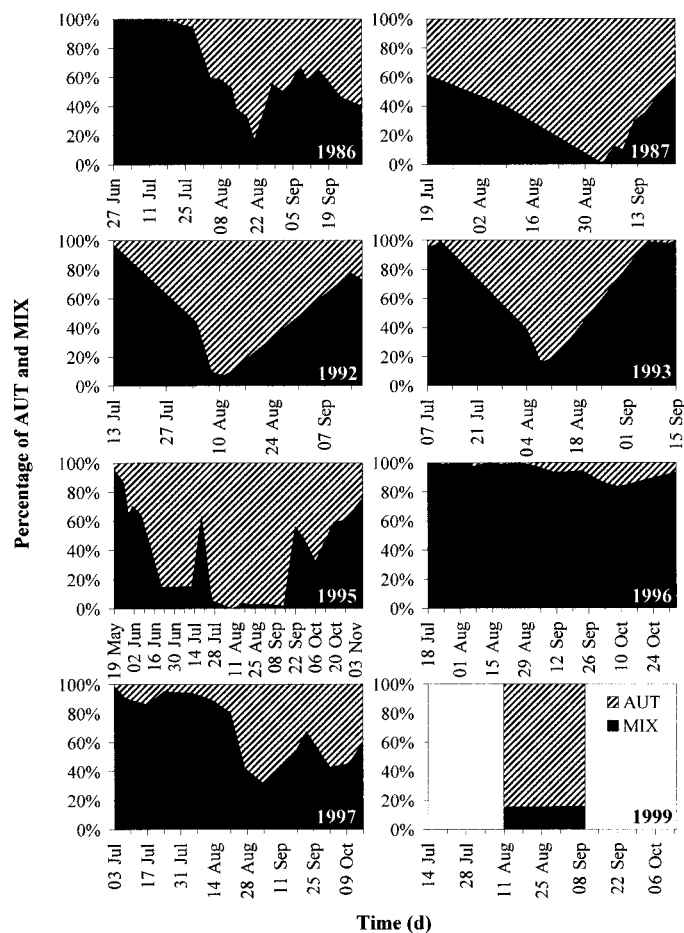


Fig. 1. Seasonal and interannual variations of biomass percentage of obligate autotrophs and mixotrophs in La Caldera Lake. AUT, obligate autotrophs; MIX, mixotrophs.

tember 1996, $TP > 32 \mu\text{g P L}^{-1}$; total dissolved phosphorus [TDP] $> 25 \mu\text{g P L}^{-1}$), associated with dust inputs from the Sahara (Villar-Argaiz et al. 2001). DIN:TP (by weight) ratio values always exceeded 12, implying a strong likelihood of P limitation (Morris and Lewis 1988).

Autotrophic picoplankton (APP $< 2\text{-}\mu\text{m}$ diameter) were absent. Phytoplankton were composed mainly of nanoplankton species, with a general dominance of mixotrophs (*Chromulina nevadensis* and *Ochromonas* sp., i.e., *Chrysothrix*) over obligate autotrophs (*Chlorella* sp., *Cyanarcus* sp., or *Cyclotella* sp.) except for midsummer (Fig. 1). During the mixotrophy experiments, algal abundance and biomass were dominated by *Chlorella* sp. (*Chlorophyceae*); other species, such as *Chromulina nevadensis* (*Chrysothrix*) in August and *Cyclotella* sp. (*Bacillariophyceae*), *C. nevadensis*, *Ochromonas* sp., and *Rhodomonas* sp. (*Chrysothrix*) in September, comprised less than 20% of phytoplankton biomass (Table 2). Eukaryotic microheterotrophs, constituted by HNFs plus ciliates, were a minor component of the plankton community ($< 10\%$ of plankton biomass) and were absent during the mixotrophy experiments. Zooplankton community was simple and dominated by *Mixodiatomus laciniatus* (copepod), which comprised $> 80\%$ of the total zooplankton biomass. Rotifers (mainly *Hexarthra bulgarica*) and cladoc-

Table 2. Taxonomic and trophic composition of algae community during mixotrophic experiments in 1999 in La Caldera Lake. Chr, chrysochyta; Din, dinophyta; Cry, cryptophyta; Cya, cyanophyta; Bac, bacillariophyta; Chl, chlorophyta; Mix, mixotrophs; Aut, obligate autotrophs.

Month	Concept	Taxonomic groups						% Trophic groups	
		Chr	Din	Cry	Cya	Bac	Chl	Mix	Aut
Aug	Abundance (cell ml ⁻¹)	1,385	209	0	58	0	11,384	12	88
	Biomass (μg C L ⁻¹)	3.66	1.54	0	0.11	0	29.03	15	85
Sep	Abundance (cell ml ⁻¹)	893	17	25	0	309	3,438	20	80
	Biomass (μg C L ⁻¹)	2.32	0.12	0.28	0	5.66	8.77	16	84

erans (*Daphnia pulicaria*) were generally scarce, although the former showed wide interannual variations. The heterotrophic bacterial community of La Caldera was composed of small cells, mainly free coccoid forms (0.28 μm average diameter, 0.01 ± 0.004 μm³) and secondarily rod forms (0.9 μm average length and 0.3 μm average width; 0.05 ± 0.02 μm³), whereas filamentous bacteria (0.18 ± 0.07 μm³) were negligible. Bacterial abundance ranged from 1.9 × 10⁵ to 1.3 × 10⁶ cells ml⁻¹, and biomass ranged from 1.88 to 12.68 μg C L⁻¹ (mean annual values, Table 1). The main plankton structure trait was the dominance of the classical grazing chain (i.e., phytoplankton and zooplankton) compared to the microheterotrophic community, and particularly the bacterioplankton, which constituted a minor fraction (Table 1).

Potential factors controlling bacterial biomass—In 1996, bacterial biomass was inversely correlated to mixotrophs, explaining 79% of bacterial variance, whereas zooplankton contributed to an additional 15% of this variance. In 1997, bacterial biomass was positively correlated to zooplankton, explaining 31% of bacterial variance. Interestingly, when data from the late ice-free period (October 1997) were excluded, both obligate autotrophs and mixotrophs explained 82% of bacterial variance, showing a positive and a negative relationship to bacterial biomass, respectively (Table 3, Fig. 2). The exclusion of data from the late ice-free period data is justified by (1) a deviation from regression line between

bacteria and mixotrophs (Fig. 2A) and (2) an inverse tendency with respect to the regression line between bacteria and obligate autotrophs (Fig. 2B).

Functional biotic parameters and mixotrophic experiments—Primary production (PP) values ranged from 0.26 to 1.91 μg C L⁻¹ h⁻¹ in 1996 and from 0.12 to 1.76 μg C L⁻¹ h⁻¹ in 1997, with no significant interannual variation ($t = 0.59$, $df = 22$, $p = 0.56$). The EOC ranged from 0.17 to 0.48 μg C L⁻¹ h⁻¹ in 1996 and from 0.06 to 0.33 μg C L⁻¹ h⁻¹ in 1997, yielding significantly higher values in 1996 than in 1997 ($t = 4.29$, $df = 22$, $p = 0.0003$). EOC showed a direct relationship with PP in both years (1996, $r = 0.98$, $p < 0.001$, $n = 12$; 1997, $r = 0.95$, $p < 0.001$, $n = 12$).

Bacterial production (BP) ranged from 1.57 × 10⁻³ to 4.58 × 10⁻³ μg C L⁻¹ h⁻¹ in 1996 and from 2.96 × 10⁻³ to 11.51 × 10⁻³ μg C L⁻¹ h⁻¹ in 1997, yielding significantly higher values in 1997 than in 1996 ($t = 4.12$, $df = 20$, $p = 0.0005$). Bacterial specific productivity (sBP) ranged from 3.7 × 10⁻⁴ to 17.7 × 10⁻⁴ h⁻¹ in 1996 and from 17.8 × 10⁻⁴ to 77.0 × 10⁻⁴ h⁻¹ in 1997, yielding significantly higher values in 1997 than in 1996 ($t = 6.38$, $df = 20$, $p < 0.0001$). BP and sBP variances were significantly explained only by the EOC because neither bacterial biomass, nutrients (TN, TP), nor temperature entered the stepwise regression analyses for these 2 yr (Table 3). Bacterial activity measurements using the two methodological approaches of photosynthetic

Table 3. Results of multiple stepwise regression analysis for bacterial biomass, production, and specific production during 1996 and 1997.

Year	Dependent variable	Independent variables entered*	Beta*	Multiple R ² *	R ² exchange*	df1*	df2*	F _{df1,df2} *	p
1996	BB	MIX	-0.76	0.79	0.79	1	10	40.2	<0.001
		ZOO	0.40	0.93	0.15	1	10	20.9	0.0010
	BP	EOC	0.94	0.88	0.88	1	9	66.5	<0.001
	sBP	EOC	0.92	0.85	0.85	1	9	49.6	<0.001
1997	BB	ZOO	0.55	0.31	0.31	1	13	5.8	0.0322
		AUT†	0.59	0.72	0.72	1	9	25.4	0.0007
		MIX†	-0.41	0.82	0.11	1	9	5.3	0.0463
	BP	EOC	0.87	0.76	0.76	1	9	28.8	<0.001
sBP	EOC	0.81	0.66	0.66	1	9	17.6	0.0023	

* Beta, standardized regression coefficient; Multiple R², coefficient of multiple determination; R² exchange, change in multiple R² caused by entering a new variable in a single step (hierarchical analysis); df1, df2, degrees of freedom; F_(df1,df2), F-test results of the relationship between the dependent variable and the set of independent variables entered in the analysis. Abbreviations: AUT, autotrophic biomass; MIX, mixotrophic biomass; ZOO, zooplankton biomass; BB, bacterial biomass; BP, bacterial production; sBP, specific bacterial productivity; EOC, excretion of organic C by algae.

† Data from late ice-free period excluded.

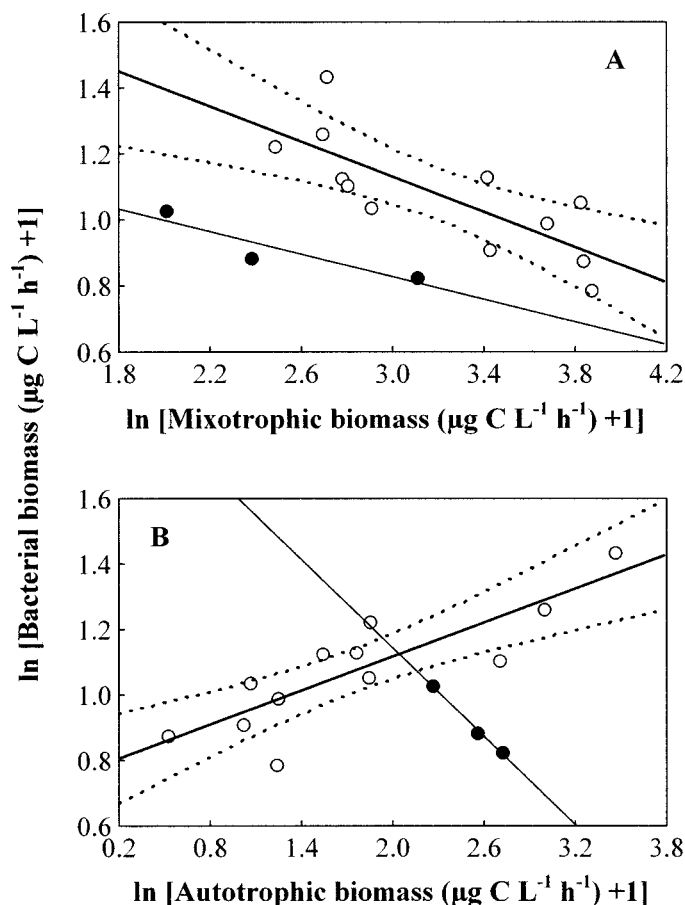


Fig. 2. Relationship of bacterioplankton with (A) mixotrophic or (B) autotrophic algae during 1997. Dashed lines indicate 95% confidence intervals around the fitted regression line (thick solid line). Solid points were not included in this regression analysis, and a thin solid line indicates their tendency line.

C incorporation and TdR incorporation in DNA (*see materials and methods*) were tightly and positively correlated in each year (1996, $r = 0.95$, $p < 0.001$; 1997, $r = 0.82$, $p = 0.002$; Fig. 3A).

Although bacterial production was coupled to primary production through excretion of organic C (1996, $r = 0.93$, $p < 0.001$, $n = 11$; 1997, $r = 0.92$, $p < 0.001$, $n = 11$), bacterial production was on average a small fraction of primary production (mean of 0.37% in 1996 and 1.1% in 1997, Fig. 3B). These values are >10-fold lower than the mean value reported by Cole et al. (1988).

In order to experimentally assess the predatory and resource control of algae on bacterial activity, we quantified bacterial production in the presence versus absence of algae and determined the fraction of bacterial production incorporated in the algal community. Surprisingly, the absence of algae caused a significant decrease in bacterial TdR incorporation rates (BP_B^\bullet), more pronounced in the light treatments (Fig. 4A₁, A₂), leading to a significant synergetic light \times algae interaction (Table 4). The algal fraction showed TdR incorporation values >0 under all experimental conditions (BP_A , Fig. 4B₁, B₂), and the proportion of bacterial production incorporated by algal fraction (BA) ranged between 25% and

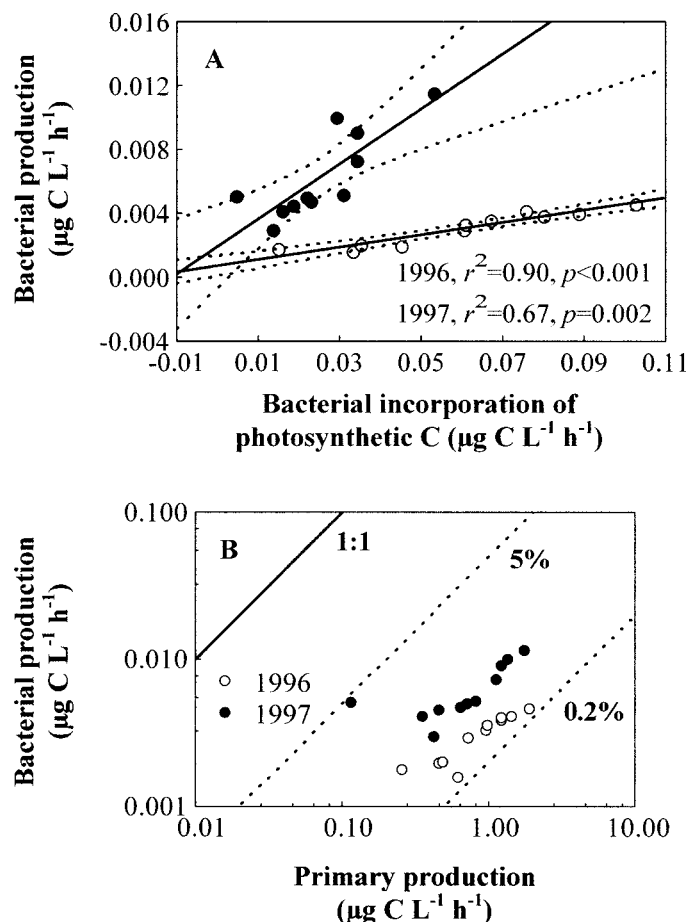


Fig. 3. (A) Annual relationship between bacterial production and bacterial incorporation of photosynthetic carbon. Dashed lines indicate 95% confidence intervals around the fitted regression line (solid line). (B) Log plot of BP-PP relationship in 1996 and 1997. Lines indicate 1:1, 5%, and 0.2% BP:PP relationship.

37% (Table 5). These values implied a specific consumption of bacterial production (C_{BP}) ranging from 0.07 to 1.19 bacteria consumed cell⁻¹ h⁻¹, and 0.5×10^{-3} to 8.2×10^{-3} $\mu\text{g } C_{\text{bac}} \mu\text{g } C_{\text{mix}}^{-1} \text{ h}^{-1}$ (Table 5). Interestingly, the impact of algal bacterivory on bacterial activity was only significant in the light treatments (Table 5).

Discussion

The planktonic structure of La Caldera Lake deviated from the general tendency of a high heterotrophic bacteria: phytoplankton biomass ratio in oligotrophic ecosystems (Gasol et al. 1997; Biddanda et al. 2001; Cotner and Biddanda 2002). In fact, bacteria that yielded abundance, biomass, cell size, and production values at the lower end of the range reported for other high mountain lakes (Thomas et al. 1991; Straskrabová et al. 1999) constituted a minor fraction of the entire plankton biomass. This structural trait also was found in other high altitude/intermediate latitude lakes (Thomas et al. 1991; Straskrabová et al. 1999).

Abiotic stressing factors such as low temperature or high ultraviolet irradiation may be responsible for the scarce de-

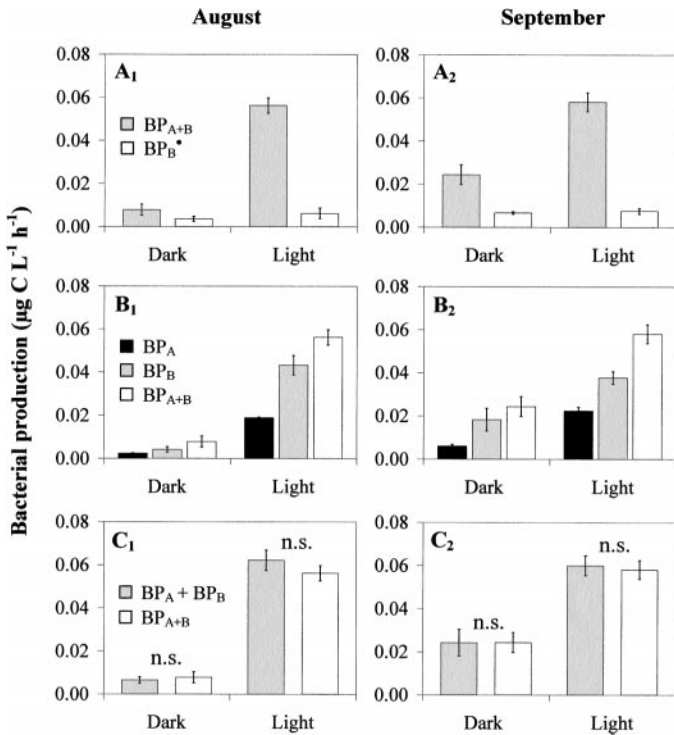


Fig. 4. Bacterial production measured as BP_{A+B} and BP_B^* under dark and full sunlight treatments in (A₁) August and (A₂) September. See text for nomenclature and Table 4 for statistical results. Bacterial production measured as BP_A , BP_B , and BP_{A+B} under dark and light treatments in (B₁) August and (B₂) September. See Table 4 for statistical results. Bacterial production measured as BP_{A+B} and sum of BP_A and BP_B under dark and light treatments, and the ANOVA test results in (C₁) August and (C₂) September. Error bars, mean \pm SD; n.s., not significant.

velopment of bacteria and microheterotrophs in some clear-water high mountain lakes such as La Caldera. However, as in some Arctic alpine lakes (Karlsson et al. 2001), temperature was not a main factor explaining seasonal and/or interannual variations in the bacterial biomass of La Caldera Lake. In spite of the markedly negative effect of UVB on bacterial assemblages (e.g., Medina-Sánchez et al. 2002 and references therein), the beneficial effects of UVA and PAR through photorepair mechanisms (e.g., Kaiser and Herndl 1997 and references therein) indicate that bacteria might be well adapted to UV radiation stress in La Caldera Lake (Carrillo et al. 2002; Medina-Sánchez et al. 2002). The low concentration of mineral nutrients (particularly P) was not a first-order factor constraining bacterial biomass or activity, at least on a seasonal scale. In fact, the elemental composition of the bacteria (i.e., cellular N:P ratio) was P sufficient at ample periods of the open-water phase and did not predict bacterial productivity, indicating that an element other than P (see below) could primarily limit bacterial growth (Villar-Argaiz et al. 2002).

Biotic factors were found to be the main controllers of bacterioplankton in La Caldera Lake. Thus, the positive relationship of bacteria with zooplankton may be related to the ability of the latter to act as source of recycled carbon and/or nutrients available to bacteria (Hygum et al. 1997; Reche

Table 4. Effects on BP of (1) solar radiation, i.e., light versus dark treatments and (2) algae removal, i.e., BP_B^* versus BP_{A+B} , tested by two-way ANOVA. See text for details.

Month	Effect	$F_{1,8}$	p	PV*
Aug	1	289.5	<0.0001	34.06
	2	326.7	<0.0001	38.44
	1 \times 2	232.7	<0.0001	27.38
	Error			0.12
Sep	1	84.0	<0.0001	17.12
	2	329.5	<0.0001	67.15
	1 \times 2	76.2	<0.0001	15.52
	Error			0.20

* PV, Percentage variance calculated as sums of squares of treatment/total sums of squares.

et al. 1997). Nevertheless, algae were shown to be the main factor controlling bacterioplankton in La Caldera Lake. On one hand, algae exert an important bottom-up control on bacteria through their commensalistic interaction, since the release of photosynthetic C is a main constraint on bacterial production (Medina-Sánchez et al. 2002; this paper). On the other hand, mixotrophs exerted a severe top-down control on bacterioplankton that may explain not only the low development of bacterioplankton but also the structural traits of plankton (low development of eukaryotic microheterotrophs, predominance of grazing chain vs. microbial loop). This top-down control is evidenced by indirect and experimental results. The indirect evidence includes the following:

(1) The taxonomic composition of the phytoplankton, with the presence and even dominance of pigmented species of *Chrysophyceae*, *Dinophyceae*, and *Cryptophyceae*, potentially mixotrophic groups (Sherr and Sherr 2002)

(2) The negative relationship between bacterioplankton and mixotrophs (Table 3), particularly strengthened in 1996 (when the plankton community was more simplified)

(3) The absence of relationship between bacterial production and biomass, interpreted in the literature as the result of a control by predation on bacteria (Billen et al. 1990). Furthermore, by applying Billen's model to algae, no significant relationship between PP and algal biomass was found in 1996, when phytoplankton were dominated by mixotrophs and zooplankton was scarce (i.e., weak top-down

Table 5. Algal bacterivory ability quantified as percentage of bacterial production incorporated into algae fraction (BA) and as specific consumption of bacterial production (C_{BP} , see text for details).

Month	Light treatment	BA (%)	C_{BP}	C_{BP} (μ g	p^*
			(bacteria cell ⁻¹ h ⁻¹)	C_{bac} μ g C _{mix} ⁻¹ h ⁻¹)	
Aug	Light	30.5	0.59	3.6×10^{-3}	0.0135
	Dark	36.1	0.07	0.5×10^{-3}	0.0924
Sep	Light	37.2	1.19	8.2×10^{-3}	0.0021
	Dark	24.6	0.32	2.2×10^{-3}	0.1888

* p indicates statistical significance of ANOVA between BP_B^* versus BP_{A+B} for each light treatment.

control). This result indicates that the algal biomass was not directly controlled by autotrophic metabolism (measured as PP) and suggests that algal biomass can be supported by other organic resources such as bacterioplankton.

Finally, our experimental results show that the algal community in La Caldera lake consumed a noticeable fraction of the bacterial production (25–37%) in a period when mixotrophs accounted for a minor fraction of the total algal biomass (Fig. 1). In addition, the contribution of HNFs and ciliates to the consumption of bacterial production was negligible because these organisms were undetectable during the experiments (Table 1). The reliability of our results is supported by the facts that algal consumption of bacteria values (C_{BP}) fell within the range reported in literature (Nygaard and Tobiesen 1993; Havskum and Riemann 1996; Roberts and Laybourn-Parry 1999) and TdR incorporation rates measured as BP_{A+B} were not significantly different (t -test, $df = 4$, $p > 0.2$) from the sum of BP_A and BP_B for each experiment. The latter guarantees a suitable segregation of algae and bacteria fractions (Fig. 4C₁,C₂). If the same bacterivory ability of mixotrophs is assumed during periods when they dominate phytoplankton biomass (e.g., in 1996, when *Chrysothryx* were >90% of algal biomass), it can be concluded that most bacterial production may be consumed by algae. Higher bacterial consumption than production has been reported in marine ecosystems where mixotrophs dominate the flagellate community (Havskum and Riemann 1996).

We found that bacterivory had a more significant impact under sunlight than under dark conditions, consistent with other studies (Caron et al. 1993b; Li et al. 2000; Urabe et al. 2000; but see Skovgaard 1996). A positive relationship between light intensity and phagotrophy has been interpreted as a dependency on photosynthetic activity for energetic requirements to support phagotrophy, or as a strategy for supplementing nutrients that are needed for photosynthetic carbon assimilation (Li et al. 2000). It has also been interpreted as the result of daily physiological rhythms, which would allow mixotrophs to accumulate nutrients via phagotrophy during light hours, when C is obtained from photosynthesis, in order to balance their stoichiometry for growth (Urabe et al. 2000). In this context, we attempted the extrapolation of our experimental results to study the potential role of bacterivory in the lake for the years when functional, environmental, and food web structural parameters were simultaneously measured. For this propose, we calculated the potential mixotrophic consumption of bacterial production (PMC_{BP}) as the experimental mean C_{BP} data multiplied by the biomass of potential mixotrophs present in the lake. To mimic the light-dependent vertical gradient of bacterivory activity found in this and other studies (see above), and because of the significant differences of BP measured under light versus dark treatments at upper layers (Carrillo et al. 2002), we applied the C_{BP} values obtained under light treatments to upper layer samples, those obtained under dark treatments to bottom layer samples, and the average light-dark C_{BP} values to intermediate layer samples. Because algae can not only act as predators, but at the same time constitute a source of C for bacteria (which they are dependent on), we estimated the relative importance of predatory versus source control of algae on bacteria by defining a new vari-

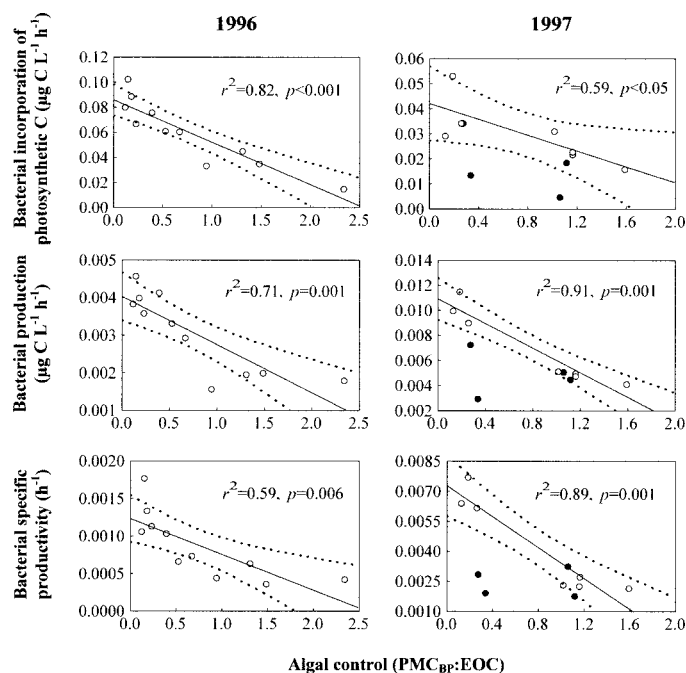
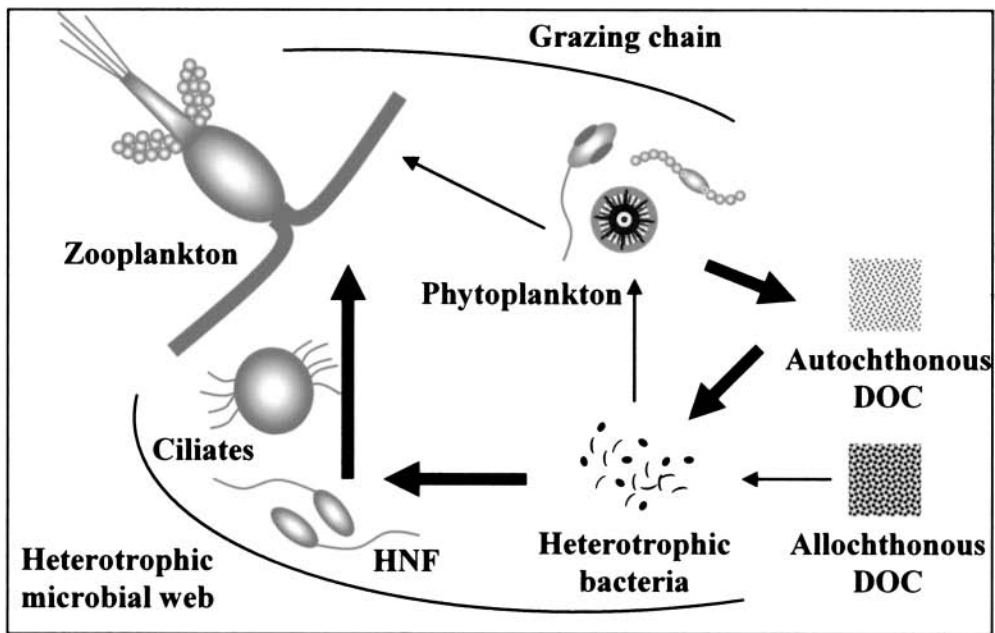


Fig. 5. Intraannual relationship between bacterial activity measured either as bacterial incorporation of photosynthetic C, bacterial production, or specific bacterial productivity and algal control measured as the ratio between potential mixotrophic consumption of bacterial C and photosynthetic C supplied by algae (i.e., $PMC_{BP}:EOC$). Dashed lines indicate 95% confidence intervals around the fitted regression line (solid line). Solid circles, which indicate data from 1997 when strict autotrophs and mixotrophs codominated phytoplankton, were excluded from the regression analysis.

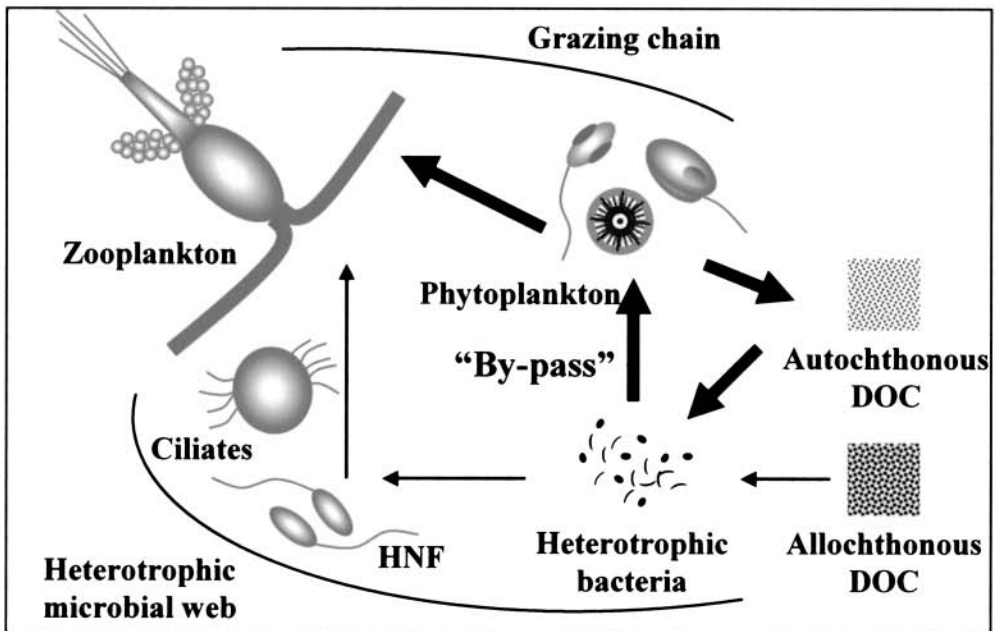
able. This variable, namely, algal control, was the ratio between the potential mixotrophic consumption of bacterial production and the photosynthetic C supplied (i.e., $PMC_{BP}:EOC$, Fig. 5). By plotting bacterial activity either measured as bacterial incorporation of photosynthetic C, production (BP), or as specific productivity (sBP), against the algal control, a strong inverse tendency was found in 1996 (Fig. 5) and for the period when mixotrophs dominated phytoplankton in 1997 (data from thaw to midsummer, Fig. 5).

In summary, the indirect evidence, experimental results, and extrapolations are all consistent with the hypothesis of a strong predatory control of mixotrophs over bacterioplankton in La Caldera Lake. We propose that the stimulus of phagotrophy under full sunlight conditions in La Caldera Lake may be the result of an adaptive strategy of mixotrophs. This strategy would allow algae to gain access to an organic C and mineral nutrient source (bacteria) under stressing light conditions that inhibit photosynthetic fixation of C (Carrillo et al. 2002 and references therein) and uptake and assimilation of dissolved mineral nutrients (Hessen et al. 1995; Döhler 1997). Supporting this interpretation, experiments performed in La Caldera Lake in periods when algae were more P deficient than bacteria showed that bacterial activity decreased at high light intensities (i.e., upper layers), particularly at full sunlight (i.e., in presence of UVB and UVA) after a P enrichment (Medina-Sánchez et al. 2002). This effect could be the result of enhanced algal phagotrophy

Oligotrophic lake



Oligotrophic high mountain lake



→
Organic C flux

Fig. 6. Proposed diagram of carbon flux through microbial food web and grazing chain in oligotrophic lakes versus oligotrophic clear-water high mountain lakes. Thickness of arrows indicates the relative importance of C flux.

preferentially consuming the most active bacteria (Simek and Chrzanowsky 1992; González et al. 1993). In fact, a competition for P alone could not explain the decrease in bacterial activity, since a shortage of organic C supplied by algae was not observed in the same experimental conditions (Medina-Sánchez et al. unpubl. data). The above arguments support the direct effect of UV radiation enhancing phagotrophy in mixotrophs, which acquire mineral nutrient mainly through predation on bacteria (Sanders et al. 2001).

Hence, we found a bacterial regulation paradox (“neither with nor without you”). Bacteria depend on photosynthetic C release by algae (Carrillo et al. 2002; Medina-Sánchez et al. 2002) (“without you I cannot live”) but are simultaneously preyed upon by algae (“with you I die”). Thus, as pointed out by Thingstad et al. (1996), mixotrophs can take advantage of a scenario where phytoplankton provide organic substrates that bacteria depend on, whereas bacteria can favorably compete with algae for mineral nutrients (Currie and Kalff 1984; Cotner and Wetzel 1992), and, finally, mixotrophs consume enriched bacterial cells. Thus, when sunlight is abundant and mineral nutrients are scarce, algae would grow bacteria by feeding them with “cheap” organic C and would harvest the “expensive” packet mineral nutrients (sensu Thingstad et al. 1996). This scenario is consistent with (1) the ability of algae to regulate photosynthetic C release depending on their elemental N:P ratio (Villar-Argaiz et al. 2002), (2) the ability of bacteria to grow under stressing sunlight conditions (i.e., at upper layers, Carrillo et al. 2002; Medina-Sánchez et al. 2002), and (3) the decrease in bacterial production in the presence of algae after P enrichment (Medina-Sánchez et al. 2002). Furthermore, mixotrophs can retain or release bacterial P depending on their predominant nutrition mode (autotrophic vs. phagotrophic) and nutrient requirements (Rothhaupt 1997). These abilities may constitute mechanisms that can modulate the competition with bacteria for mineral nutrients, suggesting a further complexity of the algae–bacteria relationship, with algae exerting the main control. In this way, a mutualistic interaction is established, comparable to other widespread terrestrial trophic mutualisms in nature (e.g., *Attine* ants and fungi), ultimately allowing the coexistence of both communities and ecosystem persistence (Daufresne and Loreau 2001).

Implications: pelagic community structure—The ability to uptake organic carbon and mineral nutrients via phagotrophy would allow mixotrophs to dominate phytoplankton in this kind of ecosystem. Thus, mixotrophs may displace obligate autotrophs, less competitive at low concentrations of dissolved mineral nutrients (Rothhaupt 1996; Christaki et al. 1999), and autotrophic picoplankton, more sensitive to UV radiation stress (Callieri et al. 2001). Nevertheless, mixotrophs have lower growth rates compared with obligate autotrophs because of their higher metabolic costs. This has consequences for the coexistence and competition between the two kinds of organism, depending on limiting nutrient conditions (Rothhaupt 1996). Thus, obligate autotrophs developed when P availability in La Caldera Lake increased, either from sediment resuspension, allochthonous inputs, or zooplankton recycling (Carrillo et al. 1995, 1996; Villar-Argaiz et al. 2002). Other authors found higher increases in

obligate autotrophs compared to mixotrophs after nutrient (N + P) enrichments in mesocosms initially dominated by mixotrophs (Jansson et al. 1996; Isaksson et al. 1999).

Mixotrophs can also displace obligate HNFs, which depend on a minimal threshold of bacterial abundance to satisfy their C requirements (Havskum and Riemann 1996). Although this threshold can be variable (0.5×10^6 to 2×10^6 cells ml⁻¹, Zubkov and Sleigh 1995), mixotrophs, which combine phagotrophy with photosynthesis, can thrive with lower bacterial densities than can HNFs. Thus, several studies have reported the dominance of mixotrophs against HNFs with low bacterial densities (e.g., Havskum and Riemann 1996) and the stimuli of bacteria and HNFs versus mixotrophs after nutrient enrichments (Jansson et al. 1996). This evidence may explain the development of HNFs in 1995, when sediment resuspension events occurred (Villar-Argaiz et al. 2002).

Our results show an important net predatory control exerted by mixotrophs over bacterioplankton in La Caldera Lake, despite the enhancing effect of photosynthetic C release on bacterial growth. This implies a deviation from the established pattern for a trophic gradient of a predatory control over bacteria in eutrophic systems and a resource-based control in oligotrophic systems (Cotner and Biddanda 2002, but see Gasol et al. 2002). With respect to the carbon cycle, mixotrophic bacterivory would act as a bypass of C flux toward the grazing chain, preventing the development of the heterotrophic microbial food web (Fig. 6). In this way, mixotrophs would occupy the niche of microheterotrophs, implying a simplification of planktonic structure. We believe that this scenario may improve the energetic transfer efficiency in high mountain lakes because of the reduction in the number of trophic levels and the consequent loss of energy (about 40–50%, Jahnke and Craven 1995) of each trophic link.

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