

Solar radiation–nutrient interaction enhances the resource and predation algal control on bacterioplankton: A short-term experimental study

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Presentación Carrillo

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Abstract

An in situ experimental assessment was made of the effects of the interaction between spectral composition of solar radiation and the limiting nutrient (phosphorous, P) on the algae–bacteria link, measured over the short term (1.5 h after P enrichment) in an oligotrophic and autotrophic high mountain lake. Variables related to the metabolism of algae (primary production, excretion of photosynthetic carbon) and bacteria (bacterial production, percentages of photosynthetic exudates assimilated and used for bacterial production) were studied. P enrichment suppressed or attenuated the negative effects exerted by ultraviolet radiation on algae when their elemental composition was P deficient. This was reflected in antagonistic interactive effects between P enrichment and solar radiation ($P \times R$), which were triggered by a decrease in primary production and an increase in organic carbon excretion due to metabolic adjustments to growth. P enrichment also suppressed or attenuated the main effects exerted by ultraviolet radiation on bacteria. This was reflected in antagonistic $P \times R$ interactive effects triggered by an enhancement of the dual control (resource and predation) that algae exerted on bacteria and by the growth stimulation of P-deficient bacteria after P enrichment. All of these observed responses contribute to improving the food quality for herbivores and reinforce the flux of carbon and nutrient to the grazing chain, which explains its development in this and other clear-water ecosystems.

Solar radiation and nutrient supply are the main ecological factors determining the structure and functioning of ecosystems (e.g., Xenopoulos et al. 2002 and references therein). Hence, any alteration in either or both of these abiotic variables can affect the ecosystem as a whole. It is known that an increase in ultraviolet B (UVB) fluxes derived from stratospheric ozone depletion can affect basal processes in food webs, such as photosynthesis, nutrient uptake, motility, reproduction, or growth of organisms (Helbling and Zagarese 2003 and references therein). In addition, the ecological effects of an increase in nutrient availability on the structure and dynamics of biota are widely known (e.g., Sterner and Elser 2002). Although study of the ecological effects of nutrient availability has traditionally been associated with anthropogenic eutrophication, research on atmospheric pulses of mineral nutrient input is attracting increasing attention in oligotrophic ecosystems such as oceans (Prospero and Lamb 2003) and high-mountain lakes (Villar-Argaiz et al. 2001). Understanding and predicting the effects of these factors

(i.e., increase of ultraviolet B, pulsed nutrient inputs) on species, communities, and ecosystems is a challenge for ecosystem research and management (Harrington et al. 1999).

Although many studies have focused on individual effects of solar radiation or nutrients on organisms, their interaction can affect species performance and relationships in ways not predictable from single-factor studies because of nonadditive effects. Therefore, multiple-factor analysis performed at different rates and scales has recently become a research topic (Folt et al. 1999; Xenopoulos et al. 2002). Numerous studies have focused on the combined effects of light and nutrients on planktonic communities because of their easy handling and key role in biogeochemical cycles. Thus, the light:nutrient hypothesis (LNH, Sterner et al. 1997) was the first attempt to describe the relationship between primary producers and bacteria within a predictive framework that included light and nutrient, but radiation quality was not considered. Several studies have highlighted the importance of radiation quality, which can interact with dissolved organic matter (DOM), enhancing or decreasing the availability of organic carbon to bacteria (e.g., Benner and Biddanda 1998), or with mineral nutrients, affecting the structure and succession of pelagic communities (Bergeron and Vincent 1997; Xenopoulos and Frost 2003) or the functionality and sensitivity of algal communities (Litchman et al. 2002; Xenopoulos et al. 2002). Nevertheless, studies addressing the interactive effects of radiation quality and nutrient availability on the relationship between primary producers and heterotrophic microbes are scarce, despite these being the link for energy transfer between the two major food chains in aquatic ecosystems, i.e., microbial and grazing food chains.

It has been established that the relationship between algae

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Acknowledgments

We thank María José Villalba, Eloisa Ramos, and José María Conde for assistance in the field, Rafael Morales for comments on statistics, and Richard Davies for English-writing assistance. We are grateful to Félix L. Figueroa and José Aguilera for providing their equipment and expertise. We thank the staff of the Radiopharmacy Department of Granada University for contributing their laboratory and experience. Four anonymous reviewers and the editors are acknowledged for their helpful suggestions.

This study was supported by the Spanish Ministry of Science and Technology Project REN2001-2840HID (to P.C.) and the Spanish Ministry of Environment Project RPN25/2003 (to P.C.).

and bacteria can change from a mutualism based on commensalism–mineralization to a competition for a limiting mineral nutrient in oligotrophic environments (Aota and Nakajima 2001; Joint et al. 2002 and references therein). In fact, nutrient limitation may be strengthened for bacteria because of the high C:N and C:P ratios of organic substrates usually released by autotrophs under oligotrophic conditions, due to the uncoupling between photosynthesis and growth (Berman-Frank and Dubinsky 1999). The underlying physiological mechanisms for this release of carbon range from an end step of alternative catabolic pathways that allow recycling of intracellular limiting nutrient (Plaxton and Carswell 1999) to a more general process that dissipates the excess of reducer power generated during the light phase of photosynthesis (Behrenfeld et al. 2004).

Several studies have underlined that the algae–bacteria relationship can be controlled by the algae themselves, based on (1) their potential ability to rapidly regulate the excretion of organic substrates, dependent on UVR stress (Carrillo et al. 2002), cellular elemental composition (Berman-Frank and Dubinsky 1999; Villar-Argaiz et al. 2002b), or metabolic active processes (Lignell 1990); and (2) the bacterivorous capacity of many nanoalgae, which can be enhanced as an adaptive response to ultraviolet (UVR) stress by the use of bacteria as a source of carbon and mineral nutrients under conditions of inhibited photosynthesis and mineral nutrient uptake. This capacity, alongside the excretion of carbon, constitutes a dual control, supplying carbon for the growth of bacteria on which algae then predate (Medina-Sánchez et al. 2004). Further, radiation quality can directly affect the relationship between algae and bacteria, depending on their sensitivity to sunlight (Carrillo et al. 2002). This sensitivity can be varied by interaction with nutrients, although the direction of this variation remains controversial. Thus, whereas the sensitivity to ultraviolet B (UVB) of P-deficient bacteria can be attenuated by an increase in P availability (Medina-Sánchez et al. 2002; Pausz and Herndl 2002), the sensitivity of algae to UVB appears to increase with nutrient availability, suggesting that nutrient stress may weaken or mask UVB stress (Behrenfeld et al. 1994; Xenopoulos et al. 2002), although the opposite results have also been reported (Cullen and Lesser 1991).

With this background, we hypothesized that the interaction between the quality of radiation and nutrient availability may determine the type of relationship between algae and bacteria. Thus, if nutrient inputs attenuate the negative effects of UVR on algal photosynthesis, a decrease in algal bacterivory and carbon release would be expected, shifting the algae–bacteria relationship from a dual control (with bacterivory) to a C-dependent commensalism. To address this issue, we experimentally assessed in situ the short-term interactive effects of P enrichment and the spectral composition of solar radiation on algal and bacterial activities and their commensal relationship.

Material and methods

Study site—La Caldera is a remote high-mountain lake situated above the tree line (3,050 m above sea level) in the

Sierra Nevada mountain range (S Spain, 36°55′–37°15′N, 2°31′–3°40′W). The lake is oligotrophic (total phosphorus [TP] < 10 $\mu\text{g P L}^{-1}$, chlorophyll *a* < 1 $\mu\text{g L}^{-1}$) and external inputs of mineral nutrient occur during thaw and are associated with sporadic events of Saharan dust deposition (Talbot et al. 1986; Villar-Argaiz et al. 2001). According to the dissolved inorganic nitrogen : total phosphorus (DIN : TP) ratio of >12, the pelagic community is P limited throughout the ice-free period (Villar-Argaiz et al. 2001 and references therein). The lake water is highly transparent (Secchi depth to at least 14 m, the maximum depth of the lake), dissolved organic carbon (DOC) concentration is below 1 mg L^{-1} (Reche et al. 2001), and UV radiation of considerable intensity penetrates deep into the lake (Carrillo et al. 2002). The bacterial/primary production ratio is less than one (Carrillo et al. 2002). Visible inlets or outlets, macrophytes, littoral vegetation, and fish are absent, and the phytoplankton community consists of nanoplankton species (e.g., Villar-Argaiz et al. 2001).

Sampling and structural variables—The sampling and the methods for quantification of structural abiotic (light, temperature, TP, total nitrogen, DIN : TP ratio) and biotic (chlorophyll *a*, taxonomic composition, abundance and biomass of algae and bacteria) parameters were those used in high-mountain lakes and are detailed elsewhere (APHA 1992; Villar-Argaiz et al. 2001, 2002a,b; Carrillo et al. 2002).

For determinations of N:P ratio of algae (N:P_a) and bacteria (N:P_b), a serial filtration at low pressure (vacuum filtration at <13.3 kPa) of 40- μm prescreened lake-water samples was performed through precombusted (1 h at 550°C) 1.0- μm pore-size filters (Whatman GF/B) for the algal fraction and through precombusted (1 h at 550°C) 0.2- μm pore-size filters (Whatman Anodisc) for the bacterial fraction within 3 h of sampling (water preserved under cold and dark). Filters were then either immediately analyzed for P (spectrophotometrically as soluble reactive phosphorus in 10-cm quartz cuvettes, using the acid molybdate protocol, after potassium persulfate and boric acid digestion; APHA 1992) or dried (24 h at 60°C) and kept desiccated until analysis for N using a Perkin-Elmer 2400 elemental analyzer. Blanks and standards were carried out for all procedures. All N:P ratios were calculated on a molar basis.

The assignment of the fraction between 1 and 40 μm to the algal fraction and of that between 0.2 and 1 μm to the heterotrophic bacterial fraction in La Caldera Lake is justified by the lack of major size overlap between organism groups; the negligible abundance of heterotrophic protozoa (1–40 μm size, Table 1); the absence of autotrophic picoplankton (<2 μm size); and the small size of bacteria (<1 μm , see Results), which avoided any significant retention on the 1- μm pore-size filters (*t*-test for dependent samples, *t* = 1.40, *df* = 5, *p* = 0.22).

Experimental design and functional variables—Two experiments were performed in situ at 0.5 m, where UVB radiation was >60% that of surface (Carrillo et al. 2002), on two different periods during the ice-free season (27 August and 10 October 1997, respectively), characterized by a shift

Table 1. Values for several physical, chemical, and biological variables obtained at 0.5-m depth during the experimental dates in La Caldera Lake. T is temperature; PAR, photosynthetic active radiation (400–700 nm); UVB_{300–318}, ultraviolet B radiation measured in the 300–318-nm range (2 nm of interval); UVA_{320–398}, ultraviolet A radiation measured in the 320–398-nm range (2 nm of interval); DIN, dissolved inorganic nitrogen; TP, total phosphorus; Chl *a*, chlorophyll *a*; A-A, autotroph abundance; A-B autotroph biomass; M-A, mixotroph abundance; M-B, mixotroph biomass; B-A, bacterial abundance; HNF-A, heterotrophic nanoflagellate abundance; HNF-B, heterotrophic nanoflagellate biomass; CIL-A, ciliate abundance; CIL-B, ciliate biomass; B-B: bacterial biomass; N:P_{algae}, N:P ratio of algae; N:P_{bact}, N:P ratio of bacteria. All radiation data shown were those measured at 0.5 m at noon.

	Aug experiments	Oct experiments
T (°C)	12.4	7.7
PAR ($\mu\text{mol quanta s}^{-1} \text{ m}^{-2}$)	1,300	1,164
UVB _{300–318} (W m^{-2})*	2.54	2.20
UVA _{320–398} (W m^{-2})*	55.05	52.30
TN ($\mu\text{g N L}^{-1}$)	200	479
TP ($\mu\text{g P L}^{-1}$)	5.50	4.20
DIN ($\mu\text{g N L}^{-1}$)	163	85
DIN/TP	29.6	20.3
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	0.58	0.17
A-A ($\text{cell mL}^{-1} \times 10^3$)	7.5	3.3
A-B ($\mu\text{g C L}^{-1}$)	19.0	11.8
M-A (cell mL^{-1})	4.52	2.30
M-B ($\mu\text{g C L}^{-1}$)	13.7	8.6
HNF-A (cell mL^{-1})	10.4	0.0
HNF-B ($\mu\text{g C L}^{-1}$)	0.107	0.000
CIL-A (cell mL^{-1})	0.025	0.120
CIL-B ($\mu\text{g C L}^{-1}$)	0.0042	0.0201
BA ($\text{cell mL}^{-1} \times 10^5$)	2.63	1.63
BB ($\mu\text{g C L}^{-1}$)	2.53	1.57
N:P _{algae} †	31.2	8.2
N:P _{bact} †	20.2	47.2

* Mean value for midsummer and late summer 2003, $n = 6$, coefficient of variation <25% and <10% for UVB_{300–318} and UBA_{320–398}, respectively.

† Mean value for the water column.

within and between the elemental composition of algae and bacteria (Villar-Argaiz et al. 2002b).

For each experiment, a 3×4 treatment experimental design was performed: unenriched versus P-enriched lake water (two P-enrichment levels) by four light treatments. The two P-enrichment levels were set by adding NaH_2PO_4 to each subsample to reach a molar ratio of either 16 (N:P₁₆ treatments) or 5 (N:P₅ treatments). The molar ratio of unenriched lake water (N:P_c treatment) was >180 for each experiment. Each subsample consisted of a known volume taken from a composite lake sample constructed from equal volumes of lake-water samples obtained with an acid-cleaned 6-liter horizontal Van Dorn sampler from three depths, spaced evenly within the photic layer affected by >1% surface UVB (0–5 m), and prescreened through a 40- μm mesh to remove zooplankton. The required amount of P to add for each N:P treatment was calculated from the total dissolved phosphorus and DIN concentration found in the water column on the day before each experiment. After P addition, the subsamples were vigorously shaken and left for

an acclimation time of 90 min exposed to full sunlight in the lake (up-opened containers) before being used to fill the experimental flasks assigned for each enrichment treatment.

The light treatments were the following: (i) full sunlight, using quartz flasks; (ii) exclusion of UVB (280–320 nm), covering the flasks with Mylar-D foil (Dupont de Nemours); (iii) exclusion of UVB and UVA (320–400 nm), covering the flasks with UF3 Plexiglas (Atohaas North America), and (iv) darkness, covering the flasks with opaque material. Optical properties of the cut-off filters used in the light treatments were tested prior to the experiments with a double-beam spectrophotometer (Perkin-Elmer Lambda 40). Cut-off filters were replaced for each experiment.

The studied response variables were related to (1) algal photosynthetic metabolism, i.e., primary production (PP), and absolute (EOC) and relative (%EOC) rates of photosynthetic C release by algae; (2) bacterial growth (i.e., bacterial production, BP); and (3) algae-bacteria commensal relationship, i.e., the percentage of photosynthetic exudates assimilated by bacteria (%PEA) and the percentage of carbon from photosynthetic exudates used for bacterial production (%CUEb).

The response variables related to algal photosynthetic metabolism were quantified by means of the ^{14}C method proposed by Steeman-Nielsen (1952). A set of four 70-mL quartz flasks (three clear and one dark) for each light and N:P treatment was disposed. Immediately before incubations, each flask was filled from the corresponding subsample and added with 0.37 MBq of $\text{NaH}^{14}\text{CO}_3$ (specific activity [SA]: 310.8 MBq mmol^{-1} , NEN Dupont). The flask sets, horizontally held, were incubated in situ at 0.5 m under surface for 4 h symmetrically distributed around noon. The laboratory procedure has been described in detail elsewhere (Carrillo et al. 2002). Briefly, it consisted of the determination of total organic ^{14}C (TOC or PP) followed by a serial filtration through 1- and 0.2- μm filters to segregate the organic ^{14}C retained in algal (particulate organic carbon >1 μm , POC₁), bacterial (particulate organic carbon 0.2–1 μm , POC₂), and dissolved (dissolved organic carbon <0.2 μm , DOC) fractions.

Because of the absence of autotrophic picoplankton (<2 μm) and the nonsignificant retention of bacteria in 1- μm filters (see above), the organic ^{14}C retained on the 0.2- μm pore-size filters from <1 μm filtrates corresponded to the algal exudates incorporated by heterotrophic bacteria. Thus, the algal excretion of organic carbon (EOC) corresponded to the organic ^{14}C measured in the <1 μm fraction, i.e., $\text{EOC} = \text{POC}_2 + \text{DOC}$. The percentage of EOC (%EOC) was calculated as $\% \text{EOC} = \text{EOC} \times \text{PP}^{-1} \times 100$.

BP was quantified by using the radiolabeled thymidine-incorporation technique (Fuhrman and Azam 1982; modified following Torretton and Bouvy 1991). A set of 10 (6 + 4 blanks) experimental 25-mL quartz flasks for each light and N:P treatment was disposed. Immediately before incubations, each flask was filled from the corresponding subsample and added with [methyl- ^3H] thymidine (TdR; SA: 2.6–3.2 TBq mmol^{-1} , Amersham Pharmacia; 10.9 nmol L^{-1} , a saturating final concentration). The flask sets, horizontally held, were incubated in situ at 0.5 m under surface for 1 h (TdR uptake linear) around noon. The laboratory procedure

has been described in detail elsewhere (Carrillo et al. 2002). Briefly, it consisted of determining the tritiated thymidine incorporated only in bacterial DNA after removal of bacterial ^3H -RNA by NaOH fixation, and quantifying the difference between the amount of ^3H incorporated in the remaining bacterial macromolecules (picked up on 0.2- μm filters after trichloroacetic acid [TCA] treatment on a 3 + 2 blanks subset) and the amount incorporated in bacterial macromolecules other than DNA (picked up on 0.2- μm filters after TCA + DNase treatment on the other 3 + 2 blanks subset). The incorporated TdR was converted to the number of cells produced by using the conversion factor of 1.07×10^{18} cells mol^{-1} of incorporated TdR experimentally calculated for this system. The amount of bacterial C produced was estimated by using a factor of 2×10^{-14} g C cell^{-1} (Lee and Fuhrman 1987).

With respect to the response variables related to the algae-bacteria commensal relationship, %PEA and %CUEb were calculated as

$$\% \text{PEA} = \text{POC}_2 \times \text{EOC}^{-1} \times 100$$

$$\% \text{CUEb} = \text{BP} \times \text{EOC}^{-1} \times 100$$

Finally, because of constraints imposed by the experimental setup used, photosynthetic carbon required by bacteria (CARB) was estimated as the quotient between the BP and the fraction of photosynthetic exudates assimilated by bacteria:

$$\text{CARB} (\mu\text{g C L}^{-1} \text{ h}^{-1}) = \text{BP} \times (\text{POC}_2 \times \text{EOC}^{-1})^{-1}$$

CARB may be an upper estimate of the actual bacterial demand for photosynthetic carbon because respired carbon is not included in the POC_2 variable and steady state and ^{14}C -isotopic equilibrium with the autochthonous pool is assumed; therefore, the denominator (i.e., $\text{POC}_2 \times \text{EOC}^{-1}$) can be underestimated.

Statistical analysis—Effects of the spectral composition of solar radiation and P enrichment on the response variables were tested by two-way ANOVA. Differences among treatments were checked by Newman–Keuls test (StatSoft 2001). Because the different spectral regions of solar radiation often exert contrasting biological effects, P \times R interactive effects on each response variable were tested for each spectral region of solar radiation (e.g., UVB, UVA, photosynthetically active radiation [PAR], etc.) by the corresponding two-way ANOVA, including in each analysis as radiation factor (R) the two light treatments that differed solely in the tested spectral region, and as P-enrichment factor (P) the three N:P treatments (N:P₅, N:P₁₆ and N:P_{31.2}) as levels of P enrichment. Normality (by Shapiro–Wilks' *W*-test), homoscedasticity (by Cochran's and Levene's tests), and correlation between means and standard deviations were checked for each data group in order to verify the assumptions required by ANOVA. The data were log transformed when these conditions were not met. The statistical analyses were performed using Statistica 6.0 for Windows software (StatSoft 2001).

Results

Limnological variables—Table 1 shows data for various limnological variables at the incubation depth and date when the experiments were performed in La Caldera Lake. Phytoplankton were composed mainly of nanoplankton species, with a remarkable absence of autotrophic picoplankton (< 2 μm) and a codominance of obligate autotrophs (mainly *Dyctiosphaerium chlorelloide*) and mixotrophs (mainly *Chromulina nevadensis* and *Ochromonas* sp.). Values of chlorophyll *a* and algal standing stock variables were lower in October than in August experiments (Table 1), and eukaryotic microheterotrophs (heterotrophic nanoflagellates [HNF] and ciliates) were negligible during the experiments. Bacterioplankton contributed less than phytoplankton did to the microbial food-web biomass and were composed of small cells, mainly free cocoid forms (0.28 μm average diameter, $0.01 \pm 0.004 \mu\text{m}^3$), secondarily rod forms (0.9 μm average length and 0.3 μm average width; $0.05 \pm 0.02 \mu\text{m}^3$), and very scarce filamentous bacteria ($0.18 \pm 0.07 \mu\text{m}^3$).

Stoichiometric analysis indicated qualitative differences in nutrient status between bacteria and phytoplankton and between experimental dates. During the August experiments, the algae were likely to be P limited (N:P_{algae} ratio = 31.2) according to the criteria of Healey and Hendzel (1980), whereas the bacteria were likely to be P sufficient (N:P_{bacteria} = 20.2) according to the criteria of Chrzanowski et al. (1996). Conversely, the algae were likely to be P sufficient (N:P_{algae} ratio = 8.2), whereas the bacteria were likely to be P limited (N:P_{bacteria} ratio = 47.2) during the October experiments (Table 1).

Effects of P enrichment and spectral composition of solar radiation on algae: Primary production and photosynthetic C release—P enrichment affected PP and excretion of organic carbon (EOC and %EOC) only in August, when algae were P deficient. P enrichment decreased PP in the absence of UVB and even more so in the absence of UVR (Fig. 1a,b; Table 2). Absolute and normalized values of organic carbon excretion (EOC and %EOC) were increased by P enrichment, and these increases were even greater in the absence of UVB or UVR at N:P₁₆ (Fig. 1c–f; Table 2). When excretion was expressed as %EOC, a clear effect of UVR (mainly UVA) emerged because it caused the highest %EOC values. This effect was even more marked in the October than in the August experiments (Fig. 1e,f; Table 3). These effects of P enrichment on PP, EOC, and %EOC in the August experiments led to a suppression or attenuation of the effects that UVB, UVA, and/or UVR exerted on these variables. This was reflected in significant antagonistic P \times R interactive effects that explained a notable percentage of the variance (Tables 4 and 5).

Effects of P enrichment and spectral composition of solar radiation on BP—BP was unaffected (dark treatment in August, UVA + PAR treatments at N:P₅ in October) or even inhibited (all light treatments in August, UVA + PAR treatments at N:P₁₆ in October) by P enrichment, which only enhanced BP under full sunlight and dark treatments in October (Fig. 2a,b; Table 6).

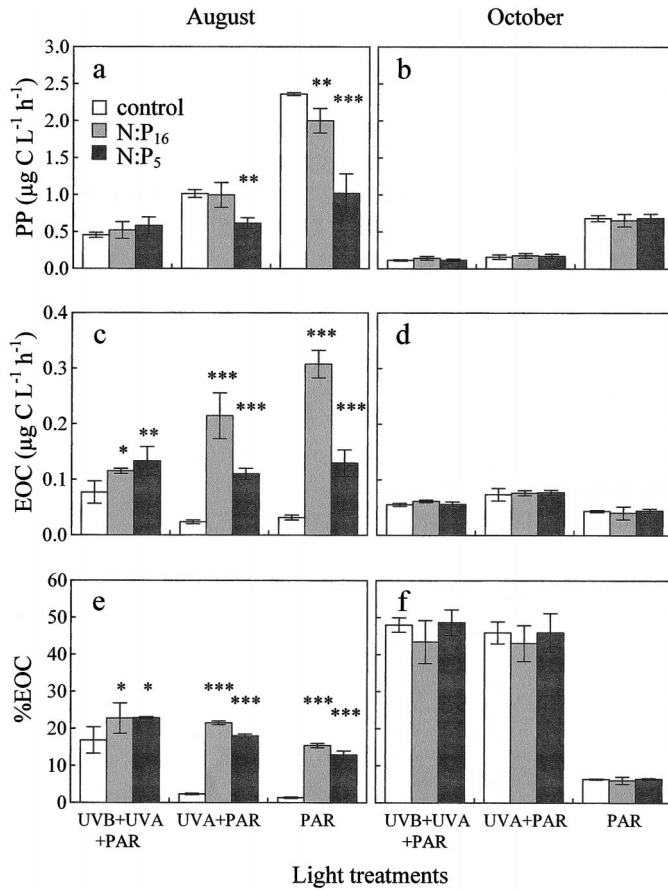


Fig. 1. (a, b) Primary production, (c, d) excretion of organic carbon (EOC), and (e, f) percentage of the excretion to the production of organic carbon (%EOC) measured in the experimental spectral solar radiation and P-enrichment (N:P ratio) treatments during the months of August and October. Error bars: mean \pm SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate the significance levels of the effect exerted by each N:P enriched treatment on the response variable, tested by two-way ANOVA and post hoc Newman–Keuls’ test. The absence of asterisks indicates not significant.

P enrichment suppressed or attenuated the effects exerted by the different spectral regions of solar radiation, thereby generating antagonistic $P \times R$ interactive effects that explained a notable percentage of the variance (see Tables 4 and 5). Nevertheless, after P enrichment, the inhibitory effect of UVB on BP persisted in August, whereas it was suppressed in October (Fig. 2a,b; Table 7).

Effects of P enrichment and spectral composition of solar radiation on algae–bacteria relationship: Assimilation and use of photosynthetic carbon by bacteria—After P enrichment, the fraction of excreted photosynthetic carbon assimilated (%PEA) and used for BP (%CUEb) varied in a similar way to the BP. Thus, both variables were increased only at full sunlight in October and were otherwise unaffected (i.e., UVA + PAR treatments at N:P₅ in October) or inhibited (i.e., all light treatments in August, UVA + PAR treatments at N:P₁₆ in October) after P enrichment (Fig. 2c–f; Table 2). P enrichment also modified the effects exerted by the dif-

Table 2. Effects of the enrichment with P, tested by two-way ANOVA and post hoc Newman–Keuls’ test, on primary production (PP), excretion of organic carbon (EOC), percentage of excretion of organic carbon (%EOC), percentage of photosynthetic exudates assimilated by bacteria (%PEA), and percentage of carbon from photosynthetic exudates used for bacterial production (%CUEb); Δ expresses the magnitude (as percentage) and sign (–, inhibitory; no sign, stimulatory) of each individual effect; p , the significance level (n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Month	Factor	Light treatments	PP			EOC			%EOC			%PEA			%CUEb		
			Δ	p	Δ	p	Δ	p	Δ	p	Δ	p	Δ	p	Δ	p	
Aug	N:P ₁₆	UVB+UVA+PAR	15	n.s.	50	*	35	*	–63	***	–88	***	–88	***	–88	***	
		UVA+PAR	–2	n.s.	801	***	819	***	–74	***	–94	***	–94	***	–94	***	
		PAR	–15	**	866	***	1,042	***	–62	***	–96	***	–96	***	–96	***	
N:P ₅	UVB+UVA+PAR	29	n.s.	73	**	36	*	–51	***	–88	***	–88	***	–88	***		
	UVA+PAR	–39	**	363	***	669	***	–64	***	–92	***	–92	***	–92	***		
	PAR	–57	***	307	***	856	***	–47	***	–90	***	–90	***	–90	***		
Oct	N:P ₁₆	UVB+UVA+PAR	24	n.s.	11	n.s.	–10	n.s.	97	***	46	**	46	**	46	**	
		UVA+PAR	11	n.s.	4	n.s.	–6	n.s.	–42	***	–67	***	–67	***	–67	***	
		PAR	–4	n.s.	–8	n.s.	–5	n.s.	–45	***	–60	***	–60	***	–60	***	
N:P ₅	UVB+UVA+PAR	1	n.s.	1	n.s.	1	n.s.	179	***	332	***	332	***	332	***		
	UVA+PAR	6	n.s.	5	n.s.	0	n.s.	2	n.s.	–22	n.s.	–22	n.s.	–22	n.s.		
	PAR	0	n.s.	2	n.s.	2	n.s.	9	**	13	n.s.	13	n.s.	13	n.s.		

Table 3. Individual effects of spectral regions of solar radiation, tested by two-way ANOVA and post hoc Newman-Keuls' test, on PP, EOC, %EOC, %PEA, and %CUEb. Δ expresses the magnitude (as percentage) and sign (-, inhibitory; no sign, stimulatory) of each individual effect; *p*, the significance level (n.s., not significant; *, *p*<0.05; **, *p*<0.01; ***, *p*<0.001).

Month	Factor	N:P treatments	PP			EOC			%EOC			%PEA			%CUEb		
			Δ	<i>p</i>		Δ	<i>p</i>		Δ	<i>p</i>		Δ	<i>p</i>		Δ	<i>p</i>	
Aug	UVB	N:Pc	-55	***	224	***	620	***	-69	***	-81	***					
		N:P ₁₆	-48	**	-46	n.s.	6	n.s.	-57	***	-59	***					
	UVA	N:P ₅	-5	n.s.	21	n.s.	27	n.s.	-59	***	-71	***					
		N:Pc	-57	***	-25	*	73	***	19	***	20	n.s.					
	UVR	N:P ₁₆	-50	***	-30	*	40	*	-20	***	66	**					
		N:P ₅	-40	**	-15	n.s.	40	*	-18	***	-1	n.s.					
Oct	UVB	N:Pc	-81	***	142	***	1,149	***	-63	***	-77	***					
		N:P ₁₆	-74	***	-63	***	48	**	-65	***	-31	*					
	UVA	N:P ₅	-43	**	3	***	77	***	-66	***	-72	***					
		N:Pc	-28	n.s.	-25	n.s.	4	n.s.	-65	***	-77	***					
	UVR	N:P ₁₆	-20	n.s.	-20	*	1	n.s.	19	**	0	n.s.					
		N:P ₅	-32	n.s.	-28	**	6	n.s.	-4	n.s.	25	n.s.					
Aug	UVB	N:Pc	-76	***	69	***	619	***	-7	n.s.	-21	*					
		N:P ₁₆	-73	***	89	***	610	***	-2	n.s.	-35	**					
	UVA	N:P ₅	-75	***	74	***	607	***	-13	***	-46	***					
		N:Pc	-83	***	26	n.s.	651	***	-67	***	-82	***					
	UVR	N:P ₁₆	-78	***	52	***	616	***	16	**	-35	**					
		N:P ₅	-83	***	26	n.s.	648	***	-17	***	-32	*					

ferent spectral regions of solar radiation, thereby generating mostly antagonistic P × R interactive effects that explained a notable percentage of the variance (Tables 4 and 5). As observed for BP, the inhibitory effect of UVB on %PEA and %CUEb persisted after P enrichment in August, whereas this effect was suppressed in October (Fig. 2c-f; Table 3).

EOC was always significantly higher than CARB under full sunlight in the nonenriched treatments. After P enrichment, EOC was significantly higher than CARB under all light treatments in August experiments, whereas they were similar in the October experiments except under full sunlight and UVA + PAR at N:P₁₆ (Fig. 3).

Discussion

P × R interactive effects on algae: The role of algal elemental composition—This study responds to the current demand for research on interactive effects between multiple stressors (e.g., UV radiation and nutrient limitation), and it found that the elemental composition of primary producers appears to play a key role in determining their biological and ecological response to variations in both abiotic variables. Thus, antagonistic P × R interactive effects on primary production and photosynthetic carbon excretion by algae were found (Table 5) when they were P limited (Table 1), led by the a priori counterintuitive effects of P enrichment on PP and EOC. We explain these effects by the documented metabolic adjustments of P-limited algae to an increase of P availability (Gauthier and Turpin 1997), which involve a decrease in the carbon fixation (PP) rate and an increase in the EOC. The decrease in carbon fixation rate is a consequence of the activation of chloroplast triose-P/Pi antiport translocator, which decreases ribulose-1,5-bisphosphate regeneration and hence CO₂ fixation (Gauthier and Turpin 1997; Behrenfeld et al. 2004). Likewise, the increase in EOC is a consequence of the increase in cytosolic triose-P, which is converted to hexoses (gluconeogenic pathway) and organic acids (catabolic pathway) (Gauthier and Turpin 1997), compounds that are commonly present in organic substrates excreted by algae (Sundh 1992).

These metabolic adjustments could be observed in our study because the incubations for measuring PP and EOC were performed a short time (1.5 h) after the P enrichment, within the usual lag time prior to the growth phase of P-limited phytoplankton (e.g., Spijkerman and Coesel 1998). The results indicate that a P enrichment at a level of N:P₁₆ was sufficient to drive the metabolic adjustments, whereas the differences between the responses of PP and EOC to N:P₁₆ versus N:P₅ treatments (Fig. 1; Table 2) might indicate a different sensitivity to P level of these variables involved in metabolic adjustments. The fact that the decrease in PP and increase in EOC were observed mainly in the UVA + PAR and PAR treatments (Fig. 1a,c,e; Table 2) indicates that the metabolic adjustments were more accentuated or detectable in the absence of UVB, which is often a more favorable light condition for P uptake and algal growth (e.g., Hessen et al. 1995).

The lack of P × R interactive effects on algae in the October experiments (Tables 4 and 5) may be explained by

Table 4. Effects of solar radiation (1) and P-enrichment (2) on the response variables tested by two-way ANOVA model. df1, df2 are degrees of freedom; PV, percentage of explained variance calculated as sum of squares of treatment/total sum of squares.

Variable	Factor	df1	df2	Aug			Oct		
				F	p	PV	F	p	PV
PP	1	2	18	217	<0.0001	76.6	358	<0.0001	99.2
	2	2	18	41	<0.0001	14.4	1.0	>0.37	0.28
	1×2	4	18	24	<0.0001	8.7	0.84	>0.51	0.23
	Error					0.35			0.28
EOC	1	2	18	7.2	<0.0050	2.7	64	<0.0001	97.1
	2	2	18	231	<0.0001	85.8	0.25	>0.78	0.38
	1×2	4	18	30	<0.0001	11.1	0.63	>0.64	0.96
	Error					0.37			1.5
%EOC	1	2	18	232	<0.0001	25.7	1,270	<0.0001	99.7
	2	2	18	577	<0.0001	63.8	2.2	>0.13	0.17
	1×2	4	18	94	<0.0001	10.4	0.08	>0.98	0.01
	Error					0.11			0.08
BP	1	3	24	51	<0.0001	47.6	24	<0.0001	25.4
	2	2	24	51	<0.0001	47.0	53	<0.0001	55.1
	1×2	6	24	4.8	<0.0025	4.4	18	<0.0001	18.5
	Error					0.92			1.0
%PEA	1	2	18	2,056	<0.0001	35.2	108	<0.0001	22.9
	2	2	18	3,397	<0.0001	58.2	261	<0.0001	55
	1×2	4	18	380	<0.0001	6.5	103	<0.0001	21.7
	Error					0.02			0.21
%CUEb	1	2	18	136	<0.0001	18	85	<0.0001	35.0
	2	2	18	632	<0.0001	81.1	119	<0.0001	48.8
	1×2	4	18	9.5	<0.0005	1.2	38	<0.0001	15.8
	Error					0.13			0.41

the initially P-enriched elemental composition of algae in this period (Table 1), which would preclude metabolic adjustments to P addition. Thus, the experimental variation in PP, EOC, and %EOC observed in the October experiments

were solely due to ultraviolet radiation (Fig. 1b,d,f; Table 4). The insight that the elemental composition of algae (N:P ratio) determined their response to P enrichment × spectral solar radiation based on pre-growth metabolic adjustments is

Table 5. Interactive effects of P addition (N:P) and the different spectral regions of solar radiation on response variables (see text) by two-way ANOVA model. A, antagonistic; S, synergistic; –, no effect.

Variable	Factor	Aug			Oct		
		F _{2,12}	p	Effect	F _{2,12}	p	Effect
PP	N:P×UVB	10	<0.005	A	0.38	>0.69	–
	N:P×UVA	15	<0.001	A	0.44	>0.65	–
	N:P×UVR	41	<0.001	A	2.2	>0.15	–
EOC	N:P×UVB	38	<0.001	A	0.64	>0.54	–
	N:P×UVA	0.86	>0.44	–	0.26	>0.77	–
	N:P×UVR	42	<0.001	A	1.5	>0.26	–
%EOC	N:P×UVB	108	<0.001	A	0.12	>0.88	–
	N:P×UVA	4.1	<0.050	A	0.02	>0.98	–
	N:P×UVR	112	<0.001	A	0.10	>0.90	–
BP	N:P×UVB	1.9	>0.19	–	30	<0.001	A
	N:P×UVA	1.4	>0.27	–	0.80	>0.47	–
	N:P×UVR	3.0	>0.088	–	25	<0.001	A
	N:P×PAR	4.2	<0.050	A	21	<0.001	A
	N:P×UVA+PAR	2.4	>0.12	–	20	<0.001	A
%PEA	N:P×full sunlight	25	<0.001	A	18	<0.001	A
	N:P×UVB	14	<0.001	A	171	<0.001	A
	N:P×UVA	59	<0.001	A	1.7	>0.22	–
%CUEb	N:P×UVR	0.76	>0.40	–	179	<0.001	A
	N:P×UVB	5.9	<0.020	A	69	<0.001	A
	N:P×UVA	7.8	<0.010	S	2.3	>0.14	–
	N:P×UVR	14	<0.001	A	55	<0.001	A

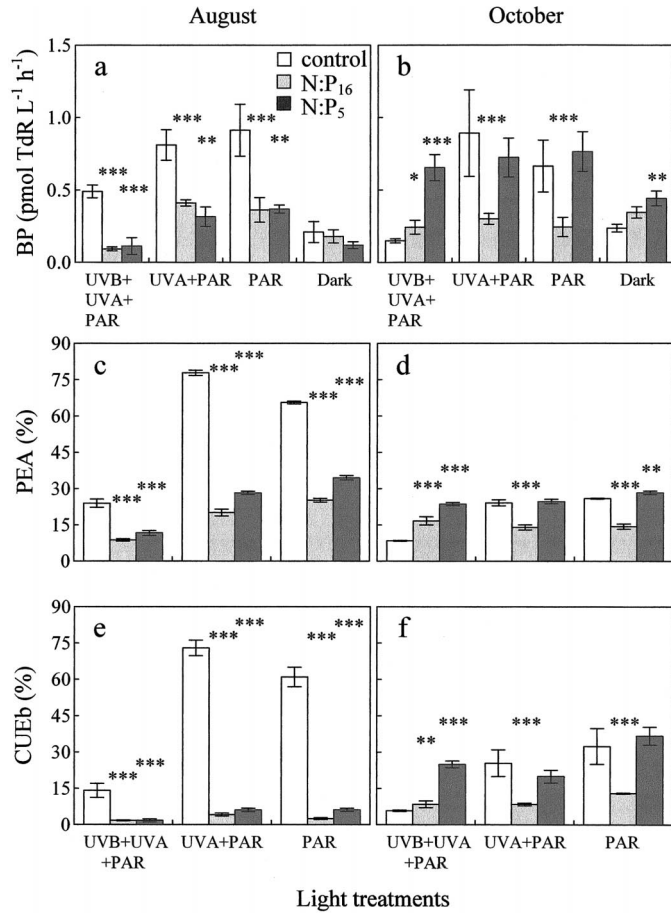


Fig. 2. (a, b) BP, (c, d) percentage of photosynthetic exudates assimilated by bacteria (%PEA) and (e, f) percentage of carbon from photosynthetic exudates used for BP (%CUEb) measured in the experimental spectral solar radiation and P-enrichment (N:P ratio) treatments during the months of August and October. Error bars: mean \pm SD. Asterisks as in Fig. 1.

also supported by the widely observed finding that the elemental stoichiometry (N:P ratio) is a good predictor of algal growth (Sterner and Elser 2002; Villar-Argaiz et al. 2002b).

Most of the significant P \times R interactive effects on algal response variables were antagonistic because the main ef-

Table 7. Individual effects of spectral regions of solar radiation, tested by two-way ANOVA and post hoc Newman-Keuls' test, on bacterial production (BP). Δ expresses the magnitude (as percentage) and sign (–, inhibitory; no sign, stimulatory) of each individual effect; p , the significance level (n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Factor	N:P treatments	Aug		Oct	
		Δ	p	Δ	p
UVB	N:Pc	–39	*	–82	***
	N:P ₁₆	–77	***	–19	n.s.
	N:P ₅	–64	***	–10	n.s.
UVA	N:Pc	–11	n.s.	34	n.s.
	N:P ₁₆	13	n.s.	23	n.s.
	N:P ₅	–14	n.s.	–5	n.s.
UVR	N:Pc	–46	*	–78	***
	N:P ₁₆	–74	***	0	n.s.
	N:P ₅	–69	***	–14	n.s.
PAR	N:Pc	334	***	181	***
	N:P ₁₆	102	*	–29	n.s.
	N:P ₅	208	***	73	*
UVA + PAR	N:Pc	286	***	278	***
	N:P ₁₆	128	**	–13	n.s.
	N:P ₅	163	***	64	*
Full sunlight	N:Pc	133	**	–37	**
	N:P ₁₆	–48	*	–29	n.s.
	N:P ₅	–6	n.s.	48	n.s.

fects exerted by UV radiation on PP and %EOC were attenuated or suppressed after P enrichment (Table 5). Nevertheless, our interpretation is that this attenuating effect was only apparent and does not imply a change in algal sensitivity to UVR after P enrichment because the metabolic adjustments became progressively less intense in the presence of UVA and UVR. Furthermore, UVR negatively affected P-sufficient algae (October) both with and without P enrichment.

P \times R interactive effects on algae–bacteria interaction—Surprisingly, a consistent decrease was observed in all bacterial response variables (BP, %PEA, %CUEb) after P enrichment (Fig. 2) despite being obtained by different methodological approaches (i.e., ³H-thymidine and H¹⁴CO₃ as tracers), suggesting that algae were indeed able to hamper bacterial growth, consistent with previous BP results and in-

Table 6. Effects of the enrichment with P, tested by two-way ANOVA and post hoc Newman-Keuls' test, on bacterial production (BP). Δ expresses the magnitude (as percentage) and sign (–, inhibitory; no sign, stimulatory) of each individual effect; p , the significance level (n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Factor	Light treatments	Aug		Oct	
		Δ	p	Δ	p
N:P ₁₆	UVB+UVA+PAR	–81	***	63	*
	UVA+PAR	–49	***	–66	***
	PAR	–60	***	–63	***
	Dark	–14	n.s.	46	n.s.
N:P ₅	UVB+UVA+PAR	–77	***	338	***
	UVA+PAR	–61	**	–19	n.s.
	PAR	–60	**	15	n.s.
	Dark	–43	n.s.	87	**

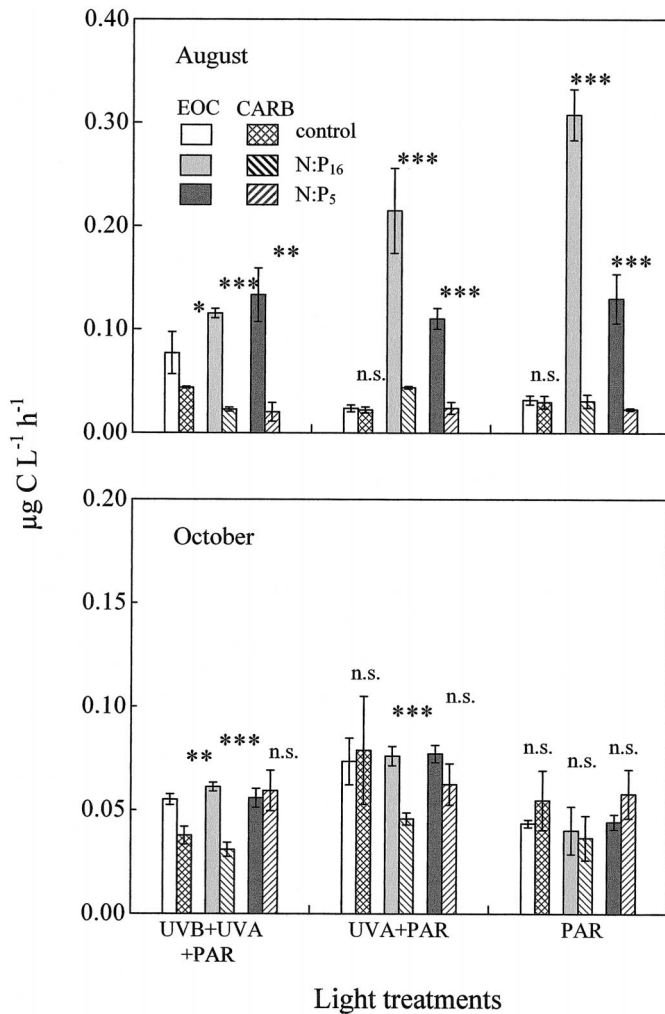


Fig. 3. Algal supply (EOC) versus bacterial demands (CARB) for photosynthetic carbon measured in the experimental spectral solar radiation and P-enrichment (N:P ratio) treatments. Error bars: mean \pm SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate the significance levels of the comparison between both variables tested by one-way ANOVA. n.s., not significant.

interpretations (Medina-Sánchez et al. 2002). Our results may be explained by a competitive advantage of algae over bacterioplankton after a P enrichment, as proposed by Bergeron and Vincent (1997). However, if the competition between algae and bacteria for P was solely based on a predominant flux of the added P to algae rather than to bacteria, we would not expect bacterial activity to be inhibited after P enrichment; at most, it would be nonstimulated, unless P uptake by algae stimulated their transport of other nutrients to depletion. However, this possibility remains speculative. In their preliminary study, Medina-Sánchez et al. (2002) postulated that the negative effect of algae on bacteria may be due to a shortage of photosynthetic carbon released as a result of improved coupling between photosynthesis and algal growth after P addition. This is relevant because bacteria depended on fresh carbon supplied by algae, as indicated by the decrease in bacterial activity after the removal of algae, whereas carbon from aged DOM appeared less bioavailable

for bacteria (Medina-Sánchez et al. 2002), probably due to its high photoreactivity in La Caldera Lake (Reche et al. 2001). However, in the present study, carbon supply (EOC) was found to exceed or at least meet bacterial demands for photosynthetic carbon (CARB, Fig. 3) in the enriched treatments where bacterial response was negative (Fig. 2), even though CARB may be an upper limit estimate (see Material and Method). A qualitative shift in the composition of photosynthetic exudates due to UVR-stressed primary producers (e.g., Goes et al. 1995), making EOC less available or even harmful (if inhibitory compounds were produced) for bacteria, should not be disregarded. However, the decrease in bacterial activity was observed in both the presence and absence of UVB and UVR. Moreover, as discussed above, the increase in EOC linked to the metabolic adjustments of P-limited algae (Gauthier and Turpin 1997) is mainly driven by metabolic pathways that yield compounds commonly present in algal exudates and usable by bacteria (i.e., hexoses, organic acids). All of the above supports the idea that the decrease in bacterial activity after P enrichment was not primarily due to a decreased availability of photosynthetic carbon.

As proposed by Medina-Sánchez et al. (2002), an alternative explanation might be the bacterivorous capacity shown by mixotrophic algae present in the lake, enhanced under light exposure (Medina-Sánchez et al. 2004). This decrease in bacterial activity could be explained by the combined effects of the bacterivory rates measured in La Caldera Lake (Medina-Sánchez et al. 2004) and the high abundance and biomass of mixotrophs observed in the present experiments. In fact, bacterivores, by selectively grazing larger and growing cells, consume newly produced bacterial cells rather than simply the standing stock of bacterial cells (Sherr and Sherr 1994). In support of our interpretation, Sanders et al. (2001) found that mineral nutrient acquisition by mixotrophs was mainly met by consumption of bacteria. Although bacterivory rates can vary in response to mineral nutrient availability, there is no consensus on the direction of these variations. Thus, bacterivory rates can decrease with higher nutrient supply (e.g., Urabe et al. 1999) but can also increase as an indirect effect of bacteria stimulation after mineral nutrient enrichment (e.g., Isaksson et al. 1999). It is possible that the phagotrophic capability of mixotrophs is related to their physiological cell stage (Isaksson et al. 1999) and linked to their elemental composition (Urabe et al. 2000). This proposition is supported by the fact that the negative effect of algae on bacteria appeared more stringent when the elemental composition of algae was P limited (August, Table 1) than when it was P sufficient (October). Thus, in the latter case, bacteria (P deficient) were able to positively respond to P enrichment only under the light conditions that are often less favorable to algal P uptake and growth (i.e., +UVB, dark; Hessen et al. 1995). Moreover, the fact that the decrease in bacterial activity was higher under light than dark conditions (Fig. 2a; Table 6) would be consistent with the view of mixotrophy as an advantageous algal strategy in ecosystems stressed by harmful intensity and/or quality of solar radiation, as discussed by Medina-Sánchez et al. (2004). Therefore, we conclude, contrary to our hypothesis, that the most plausible explanation of our results is an en-

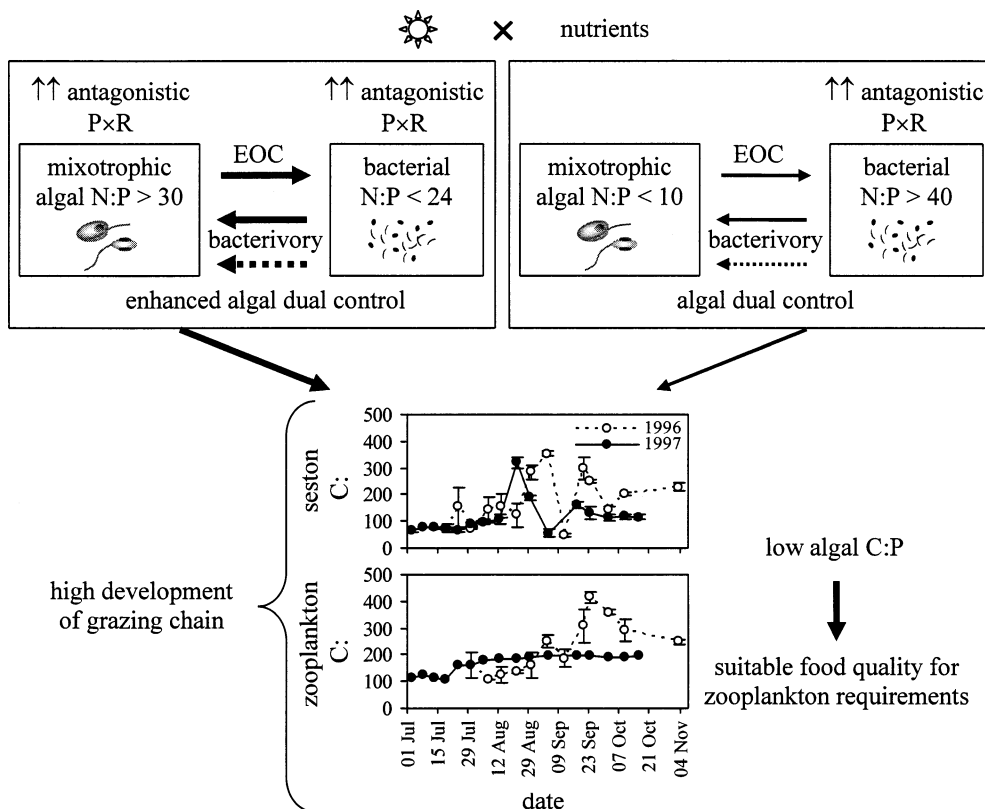


Fig. 4. Conceptual diagram of the antagonistic $P \times R$ interactive effects on the algae and bacteria link and their ecological implications. Data for seston and zooplankton C:P ratios were redrawn from Villar-Argaiz et al. (2002a).

hancement of the dual control (resource and predation) exerted by algae on bacteria (Medina-Sánchez et al. 2004) after pulsed P enrichment (see Implications).

Similar to the algal response, most of the significant $P \times R$ interactive effects on bacterial response variables were antagonistic (Table 5), and they explained a higher percentage of the variance (Table 4) when elemental composition of bacteria was P deficient (October, Table 1). Considering the bacterial response variables overall, the net result of the antagonistic interactive effects was the attenuation, after P addition, of the harmful effects of UVB on bacteria. This attenuation resulted from the dual control exerted by algae in both experimental periods and from the ability of P-deficient bacteria (October) to positively respond to P enrichment under +UVB radiation. Therefore, in accordance with other findings (Medina-Sánchez et al. 2002; Pausz and Herndl 2002), we interpret that P-deficient bacteria became less sensitive to UVB after P enrichment. The frequent pulsed nutrient inputs in La Caldera Lake, which counteract situations of bacterial P deficiency (Villar-Argaiz et al. 2001), and the ability of bacteria to counteract harmful effects of UVB radiation by photorepair mechanisms (Carrillo et al. 2002; Medina-Sánchez et al. 2002) would explain the good adaptation of bacterioplankton in La Caldera Lake to solar radiation (Carrillo et al. 2002).

Implications—The present study showed that a main effect of a limiting nutrient (P) input was an increase in the

excretion of produced carbon (EOC and %EOC) by P-limited algae exposed to high solar radiation. This increase is a short-term transient response linked to the metabolic adjustments described above, and hence expected to persist for no more than a few hours. This is offset by the high frequency of algal P limitation and of pulsed nutrient inputs from atmospheric deposition in this ecosystem (Villar-Argaiz et al. 2001). Therefore, the increase in carbon excretion may enhance the cascade of longer term ecological consequences triggered by carbon excretion, usually high in these UVR-stressed ecosystems (Carrillo et al. 2002; this study). In fact, carbon excretion, evolutionarily considered as a functionally neutral process, acquires various secondary functions with consequences for (i) primary producers and (ii) interspecific interactions (Berman-Frank and Dubinsky 1999).

(i) For primary producers, carbon excretion is a mechanism to protect cells from excess of reducer power during exposure to environmental stresses such as high UVR and P limitation (Berman-Frank and Dubinsky 1999; Behrenfeld et al. 2004) or when pregrowth metabolic adjustments occur after P addition to P-limited algae (Gauthier and Turpin 1997; Behrenfeld et al. 2004). This protective mechanism would give algae an advantage in these stressed environments, explaining their high development in La Caldera Lake (Medina-Sánchez et al. 2004) and the higher carbon excretion (EOC and %EOC) found under high UVR exposure (i.e., at upper layers; Carrillo et al. 2002), especially after a P enrichment (this study). Furthermore, the high ex-

cretion of carbon may explain the low sestonic C:P ratio values found in this clear-water ecosystem (Fig. 4; Villar-Argaiz et al. 2002a), which were unexpected based on previous studies (Sterner et al. 1997; Berman-Frank and Dubinsky 1999; but see Katechakis et al. 2005). Xenopoulos et al. (2002) found that UVR lowered the algal C:P ratio and proposed various scenarios to deduce the underlying mechanisms, given the absence of experimental data. From our results, we propose that these mechanisms are a combination of processes that are determined by high UVR and enhanced after P enrichment, including (a) impairment of photosynthetic carbon acquisition, (b) increase in percentage of carbon excretion, and (c) enhancement of bacterivory by mixotrophic algae that increases their intracellular content of bacteria-derived phosphorous.

(ii) For interspecific interactions, photosynthetic carbon excretion from algae provides an energy source for bacteria, their main competitors for nutrients in oligotrophic environments (Bratbak and Thingstad 1985), even after a P input. The solution to this paradox lies in the regeneration of the mineral nutrient contained in bacteria by their predators, mainly ciliates and HNF (Bratbak and Thingstad 1985). The development of bacterivorous mixotrophic algae compensates for the scarceness of heterotrophic microbial community in La Caldera Lake by occupying its ecological niche (Medina-Sánchez et al. 2004). Hence, the paradox is solved by mixotrophic algae, which release cheap carbon usable by bacteria (EOC), taking advantage of the higher efficiency of bacteria to uptake dissolved mineral nutrient, and which harvest the expensive packets of mineral nutrients (Thingstad et al. 1996). Because this dual control is intensified after P enrichments when algae are P deficient (Fig. 4), it constitutes an adaptive feedback control exerted by algae that allows their dominance in many clear-water and oligotrophic ecosystems (Fig. 4; Medina-Sánchez et al. 2004).

Another consequence for interspecific interactions of the decrease in algal C:P ratio is an improvement in food quality for herbivores (Sterner and Elser 2002; Xenopoulos et al. 2002; Katechakis et al. 2005). This indirect role of UVR in benefiting herbivores (Xenopoulos et al. 2002) is supported by the high development of the grazing chain found in this and other clear-water ecosystems (Cruz-Pizarro et al. 1994; Callieri et al. 1999; Medina-Sánchez et al. 2004), probably based on a balanced C:P ratio between the primary producers and the consumers (Fig. 4).

Finally, at the ecosystem level, the dual control of mixotrophic algae on bacteria, enhanced after P enrichment, would act as a bypass to transfer mineral nutrients and carbon to higher trophic levels (Fig. 4), improving the efficiency of this transference by reducing the number of trophic steps.

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Received: 26 April 2005

Accepted: 21 October 2005

Amended: 16 November 2005