

Resource versus consumer regulation of phytoplankton: testing the role of UVR in a Southern and Northern hemisphere lake

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Abstract Models predict that phytoplankton is bottom-up regulated by resources and top-down controlled by consumers. However, how the strength of these controls varies with UV radiation (UVR) is not well known. In this study, we test the hypothesis that the phosphorus (P) content of phytoplankton affects the role that UVR exerts strengthening or weakening the resource and consumer control on distinct stoichiometric and functional phytoplankton traits. To accomplish this, we coupled field meso- and microcosms in a split-plot design using UVR treatments at the plot level and nutrients and zooplankton (presence/absence) at the subplot level, in two mountain lakes

characterized by their P-sufficient (La Caldera) and P-deficient phytoplankton (Los Cántaros). We found that the addition of nutrients decreased phytoplankton C:P in both lakes, but enhanced primary production and chlorophyll *a* only under UVR in La Caldera. Also, the effects of zooplankton on phytoplankton varied between lakes and UVR conditions, and increased primary production and chlorophyll *a* under UVR in La Caldera, but not in Los Cántaros. These results suggest that differences in the stoichiometric aspects associated with the P content in phytoplankton play a key role in how UVR affects resource and consumer controls, with much weaker effects in P-deficient food webs.

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Andean Patagonian Lakes as Sensor of Global Change

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Introduction

Among the most powerful conceptualizations in Ecology is how species are organized into trophic levels that generate strong interactions between adjacent upper and lower trophic levels (Lindeman, 1942). Most findings to date can be synthesized into models that predict that the food webs are bottom-up regulated by resources and/or top-down regulated by consumers (Carpenter et al., 1985; McQueen et al., 1986). While the trophic cascade is a non-disputed ecological

concept, the ubiquity of its effects has been long debated (Hairston et al., 1960; Shurin et al., 2006). Indeed, it is at the primary producer–consumer interphase where autotrophic and heterotrophic feeding modes interact and where the trophic cascade effects have mostly deviated from theoretical predictions, and basically because the response of phytoplankton and zooplankton to the enrichment with nutrients does not fulfill the expectations based on the length of the food chain (Brett & Goldman, 1997). Elser & MacKay (1998) proposed the key role of the elemental composition of primary producers in the top-down control in aquatic food webs. In their study, they discriminated truncated food webs as those where low food quality, characterized by high phytoplankton C:P ratios, limited the growth of consumers and therefore mitigated the effectiveness of a top-down control. Therefore, any factor that alters the elemental composition of phytoplankton not only shapes their ecology but also the way they interact with other organisms. Thus, Urabe & Sterner (1996) demonstrated the chief role of nutrients and photosynthetic active radiation on the carbon-to-nutrient ratio in phytoplankton, and field and laboratory evidence further determined that temperature (Hutchins et al., 2007) and UVR (Xenopoulos et al., 2002) also affect the composition of autotrophs. We now know that food quality research needs also integrating the consumer as different taxa have different somatic compositions and therefore nutritional needs (Elser et al., 1996; Sterner & Elser, 2002). Thus, as a fundamental principle of ecological stoichiometry, the balance or imbalance between elemental composition of consumers and resources shapes the manifold ecological properties of food webs including organism growth, or the interaction with the biotic and abiotic environment (Sterner & Elser, 2002). Also, several works have reported intraspecific variations in zooplankton nutrient content (DeMott et al., 1998; Villar-Argaiz et al., 2002a). However, while a disproportionate amount of food quality research has focused on cladocerans and specifically on *Daphnia* species, knowledge about the calanoid copepods that dominate zooplankton in many oligotrophic freshwaters is comparatively scarce.

Although much attention has been focused on the numerous biotic features that affect the structure of food webs including trophic position (Hairston et al., 1960), food web length (Drenner & Hambright, 2002),

and diversity (Hillebrand & Cardinale, 2004; Finke & Denno, 2005), still many questions regarding the role of abiotic conditions remain unsolved. Recently, several studies on global change have analyzed how multiple environmental variables alter the way UVR affects organisms (Doyle et al., 2005; Cooke et al., 2006). However, there is still scarce information on how the effects of UVR propagate and affect the fundamental processes behind food web structure and function in nature (Paul & Gwynn-Jones, 2003). Because UV radiation has been identified as an important determinant of phytoplankton elemental content (Xenopoulos et al., 2002; Villar-Argaiz et al., 2002b; Korbee et al., 2012) or zooplankton grazing rates (Williamson et al., 2010), more studies are needed to examine the ultimate impact of UVR controlling food webs by impairing the stoichiometric producer–consumer interphase.

With the purpose of analyzing the role of UVR on top-down and bottom-up control in aquatic food webs, we designed a series of in situ experiments in which we first manipulated light (presence and absence of UVR) and tested how these different light conditions in turn affected the role of nutrients and zooplankton controlling phytoplankton. We deliberately selected two high-mountain lakes located in the Northern and Southern Hemisphere, dominated by calanoid copepods (with similar elemental composition, see Souza et al., 2010) but with strikingly different phytoplankton C:P ratios. Whereas phytoplankton in lakes of Sierra Nevada in the Northern Hemisphere are nutrient rich with characteristically low C:P ratios frequently below 300 (Villar-Argaiz et al., 2002a), phytoplankton in the Andean lakes of Patagonia are frequently nutrient poor with C:P ratios typically above 800 (Balseiro et al., 2007; Laspoumaderes et al., 2013).

According to the stoichiometric predictions based on the extremely high P-deficient phytoplankton (high phytoplankton C:P) in the Andean relative to the Sierra Nevada lake, we anticipated much weaker bottom-up and top-down effects in response to UVR and nutrient manipulations on the lower trophic level of phytoplankton in this lake. In addition, the experimental manipulation of UVR and nutrients should provide with relevant information on how future global change scenarios might prevent or exacerbate resource and trophic cascade control of food webs.

Materials and methods

Study sites

The experiments were conducted during the mid-ice-free period of 2007 and 2008 in two oligotrophic lakes located in Sierra Nevada (Spain) and Nahuel Huapi National Parks (Argentina), respectively. The Spanish lake La Caldera is a high-mountain lake located above tree line (3050 m a.s.l.) in the Northern Hemisphere (37°03'N, 3°19'W) and has a surface area of approximately 2 ha and a maximum depth of 14 m. The Argentinian lake of Los Cántaros is a mountain lake located at 1000 m a.s.l. in the Southern Hemisphere (41°00'S, 71°49'W) and has a surface area of 22 ha and a similar maximum depth of 14 m.

Mean chlorophyll *a* is frequently $<1 \mu\text{g l}^{-1}$ in both lakes, and dissolved organic carbon does not exceed 1 mg l^{-1} in La Caldera (Carrillo et al., 2015) and 0.7 mg l^{-1} in Los Cántaros (Morris et al., 1995; and unpublished data). The concentrations of nutrients were also low in both lakes, with P values $<10 \mu\text{g l}^{-1}$ as characteristic of oligotrophic lakes. Despite the strong P limitation of lake production, seston C:P ratios are markedly different between lakes with

values frequently above 600 in the Anden lake (e.g., Souza et al., 2010), but rarely above 300 in La Caldera (Villar-Argaiz et al., 2002b), possibly due to the frequent inputs of P-rich Saharan dust in the later Lake (Morales-Baquero et al., 2006; Mladenov et al., 2011). The autotrophic communities of the studied lakes are relatively simple and composed of nanoplankton species as it is characteristic for oligotrophic systems. The quantity of food for zooplankton is frequently $<100 \mu\text{g C l}^{-1}$ in La Caldera (Villar-Argaiz et al. 2002b). Pelagic zooplankton communities are dominated by calanoid copepods in both lakes: *Boeckella gibbosa* [Brehm] in Los Cántaros and *Mixodiatomus laciniatus* [Lilljeborg] in La Caldera, with other zooplankton species present at very low abundances.

Experimental set-up

The experiments, with basically identical designs for both studied lakes, were carried out during the summers of 2007 in La Caldera and 2008 in Los Cántaros. We used a split-plot design with a factorial arrangement of treatments, which consisted of two types of UVR pretreatments (presence and absence of UVR, herein +UVR and -UVR) in mesocosms as

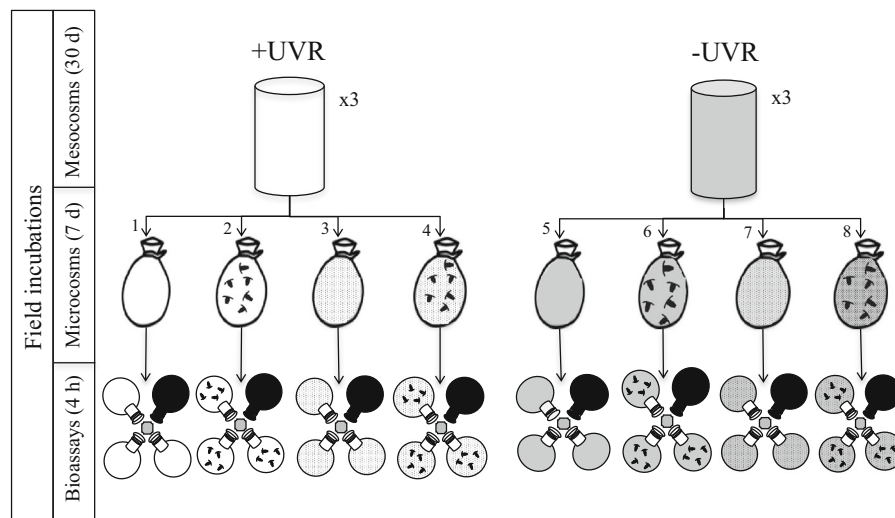


Fig. 1 Scheme illustrating the experiments carried out in La Caldera and Los Cántaros. Field mesocosms are indicated by cylinders and gray color denotes the exclusion of UVR. After one-month incubation, water from each mesocosm was used to fill the microcosms which were either unmanipulated (solid colored bags), received nutrients (dotted bags), zooplankton (copepod bags), or nutrients and zooplankton (copepod-dotted

bags), completing eight treatments indicated by numbers. After 7 days, water from the microcosm was used to estimate PP using four flasks per microcosm bag in controlled bioassays that lasted for 4 h. The field mesocosms were carried out in triplicate, such that the experimental set-up yielded a total of 6 mesocosms, 24 microcosms, and 96 PP flasks

main plots, and a 2×2 factorial combination of nutrient and zooplankton as subplots using microcosms, for an overall $2 \times 2 \times 2$ factorial design with three replicates (Fig. 1). For the mesocosms (three per UVR pretreatment), 40 μm filtered water from 3 m depth was pumped into polyethylene bags that contained 2 m^3 . Three UVR-transparent mesocosms received the full spectrum of solar radiation (+UVR treatment), while the other three were either covered by a plexiglas UF3 sheet that extended horizontally over the water surface 2 m beyond the enclosures in La Caldera or made of a distinct polyethylene bag that cut off radiation at 380 nm while allowing 85% transmittance above 400 nm in Los Cántaros (-UVR treatments). The optical properties of polyethylene and plexiglas were checked before the experiments using a double-beam spectrophotometer (Perkin Elmer Lambda 40 in La Caldera and Shimadzu UV2450 in Los Cántaros). Mesocosms were incubated for one month, after which water containing phytoplankton was used to fill 20-L microcosms using the same plastic material as that of the mesocosm of origin, corresponding to the following treatments: control water without zooplankton (N0Z0), control water with zooplankton (N0Z+), nutrient-enriched water without zooplankton (N+Z0), and nutrient-enriched water with zooplankton (N+Z+). Copepods, collected from the lake, were isolated and added to the zooplankton treatments at a final density that doubled that found in the lake. Nutrient treatments received P as Na_2HPO_4 to around double the concentration in the lake (approximately $20 \mu\text{g P l}^{-1}$). To ensure that P remained as the limiting nutrient, inorganic N as NH_4NO_3 was added to reach a N:P molar ratio of 30. After the addition of zooplankton and nutrients, microcosms were immediately incubated for one week at a depth of 0.1 m, corresponding with 75% of surface solar radiation, and under the same light conditions as those of the original mesocosms. This experimental design required a total of 6 mesocosms for the UVR pretreatments and 24 microcosms for the UVR \times nutrient \times zooplankton treatments as shown in Fig. 1.

Underwater irradiance–depth profiles were obtained for 305, 320, and 380 nm and PAR (400–700 nm) using a multichannel radiometer (Biospherical Instrument Compact BIC in La Caldera and a Biospherical Instrument Ultraviolet Radiometer PUV 500B in Los Cántaros). During the experiments, we

measured similar surface PAR irradiances between lakes of 2195 and 2120 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in La Caldera and Los Cántaros, respectively. Diffuse attenuation coefficients for downward irradiance (K_d) calculated from the slope of the linear regression of the natural logarithm of downwelling irradiance versus depth were higher for Los Cántaros relative to La Caldera (k_{305} of 1.74 vs. 0.65; k_{320} of 1.16 vs. 0.51; k_{380} of 0.58 vs. 0.22; and k_{PAR} of 0.29 vs. 0.12). Nonetheless, UVB irradiance at the microcosm incubation depths were not different between lakes due to the 40% higher surface irradiance reaching lake Los Cántaros relative to La Caldera (7 vs. $5 \mu\text{W cm}^{-2} \text{nm}^{-1}$ for 305 nm wavelength) (see Souza et al., 2010 for K_d and irradiance values). We did not measure differences in the temperature between lake and enclosure waters and only slight differences in water temperature were found between lakes ($17 \pm 1^\circ\text{C}$ in La Caldera, and $19 \pm 1^\circ\text{C}$ in Los Cántaros).

With this experimental design, we were able to distinguish between the resource, i.e., nutrient, and consumer, i.e., zooplankton, control on phytoplankton, as well as their interaction. To evaluate the effects of nutrients, we compared experimental treatments with nutrients added against control treatments with no nutrients added (bags 3 and 7 vs. bags 1 and 5 for +UVR and -UVR treatments, respectively in Fig. 1). For the zooplankton effects, we compared treatments with zooplankton against control treatments without zooplankton (bags 2 and 6 vs. bags 1 and 5 for the +UVR and -UVR treatments, respectively in Fig. 1). Finally, the combined effects of nutrients and zooplankton could be tested by adding an extra treatment with zooplankton and nutrients (bags 4 and 8 vs. bags 1 and 5 for +UVR and -UVR treatments, respectively in Fig. 1).

After one-week incubation, the samples were taken for primary production (PP), phytoplankton C:N:P composition, and chlorophyll *a* (Chl *a*). For PP, water from each microcosm bag was used to fill a set of four 250-ml quartz flasks (three clear and one dark). Flasks were immediately added with 1 MBq of $\text{NaH}^{14}\text{CO}_3$ (specific activity = $310.8 \text{ MBq mmol}^{-1}$, NE Dupont) and incubated at 0.1 m depth under the same light conditions as their bags of origin. After incubations, flasks were transported cold and dark to the laboratory within a few hours after collection. In the laboratory, copepods were removed from the zooplankton treatments by filtering the complete volume through a

40- μm mesh. Filtrates were used to calculate the PP by means of the ^{14}C method proposed by Steeman-Nielsen (1952). Briefly, an aliquot of 50–60 ml was filtered through 1- μm Nucleopore filter. Filters were placed in scintillation vials, acidified with 100 μl of 1 N HCl to remove DI^{14}C , and allowed to stand open in a hood for 24 h as recommended by Lignell (1992). Vials were counted with liquid scintillation in a counter equipped with autocalibration (Beckman LS 6000 TA) in La Caldera and a Wallac 1414 scintillation counter (Wallac, Turku, Finland) in Los Cántaros. The total CO_2 in the water was calculated from the measurement of alkalinity and pH (APHA, 1992). In all PP calculations, dark values were subtracted from the corresponding light values. For phytoplankton C, N, P, and Chl *a* determinations, between 100 and 200 ml of water with phytoplankton from the microcosms was filtered through precombusted 1.0- μm glass fiber filters (GF/B, Whatman). Filters were analyzed for P using the acid molybdate technique (APHA, 1992) or for C and N using a Perkin Elmer model 2400 (PerkinElmer Corporation) in La Caldera and a Thermo Finnigan EA1112 (Thermo Scientific) in Los Cántaros. Chl *a* was measured fluorimetrically (LS55 Perkin Elmer fluorometer in La Caldera and Turner AU 10 fluorometer in Los Cántaros) after grinding filters and extracting the

pigments in 90% acetone kept in the dark at 4°C for 24 h. Phytoplankton samples were fixed with Lugol's solution and a 50-ml aliquot counted in Utermöhl chambers at $\times 1000$ magnification using an inverted microscope (Leitz Fluovert FS, Leica).

Statistical analyses

Differences in phytoplankton response variables (C:N:P ratios and Chl *a*) between mesocosms due to UVR were analyzed using *t* test.

For the microcosm experiments, the effects of UVR, as the main factor, and nutrients and zooplankton as the subordinate factors on phytoplankton response variables were tested by two-way split-plot repeated measures analysis of variance (RM-ANOVA) (Quinn & Keough, 2002). This model contained all terms in the fully crossed factorial UVR, nutrients, and zooplankton. All ANOVAs were followed by Newman–Keuls post hoc test to determine *P* values for pairwise significant differences. Normality (Shapiro–Wilks' *W* test) and homoscedasticity (Levene's test) were checked for each data group in order to verify the assumptions required by the ANOVA. Normalized treatment responses, expressed as effect sizes in Figs. 2–4, were calculated by dividing the variable response mean in the

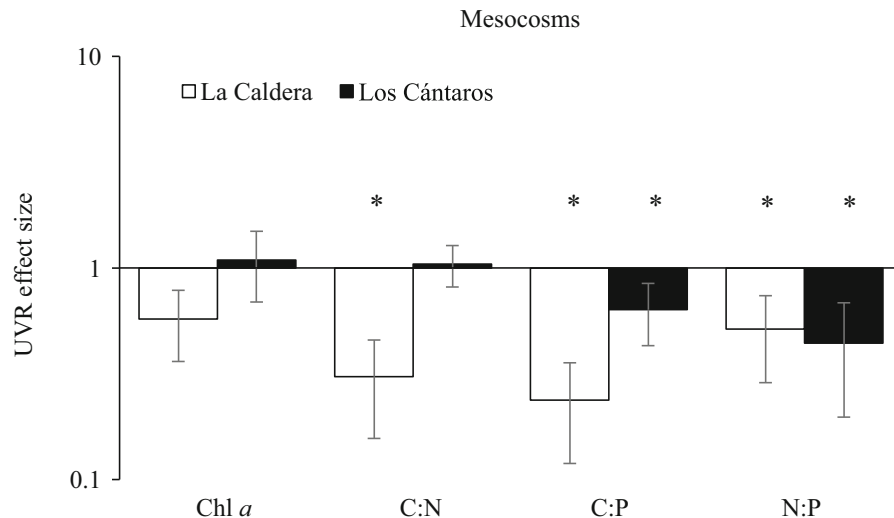


Fig. 2 UVR effect size on phytoplankton Chl *a* and C:N:P ratios in the mesocosms of La Caldera and Los Cántaros. Effect size was calculated by dividing the variable response mean in +UVR by the variable response mean in –UVR treatments. Error bars correspond with the SD calculated following the

error propagation approach. Significant effects of UVR on the response variables tested by *t* tests are denoted by *asterisk*; **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. The absence of *asterisks* indicates non-significance. See absolute values in Souza et al. (2010)

experimental treatment by the variable response mean in the control treatment. The values above one were defined as the cases in which the experimental treatment increased the response variable, whereas the values below one indicated the inhibition of the response variable. All statistical analyses were run using Statistica software (StatSoft, 2013).

Results

The abundance of major algal taxonomical groups was basically unaffected after one-month incubation in the presence and absence of UVR (*t* test, Los Cántaros, *t* = 0.28, *df* = 4, *P* = 0.788; La Caldera, *t* = 1.67, *df* = 4, *P* = 0.170). Phytoplankton in the enclosures was dominated by Chrysophyceae and Dinophyceae in Los Cántaros (88 and 7% in cell abundance, respectively), and by Chlorophyceae, Bacillariophyceae, and

Chrysophyceae in La Caldera (82, 14, and 4% in cell abundance, respectively). *Monoraphidium* sp. in La Caldera and *Chrysochromulina parva* [Lackey] in Los Cántaros were the most abundant phytoplankton species. All algae were nutritionally palatable for herbivorous zooplankton with mean cell biovolumes of 289 μm^3 in Los Cántaros and 92 μm^3 in La Caldera. Ciliates were not detected in La Caldera and were scant in Los Cántaros (<2% algal abundance) with no differences in abundance between UVR treatments (*t* test, *t* = 1.36, *df* = 4, *P* = 0.243).

Also, Chl *a* in the mesocosms did not change in response to UVR in any lake (Fig. 2). Incubations in the mesocosms revealed that phytoplankton C:N decreased in response to UVR, but only in Caldera (Fig. 2). The effect of UVR decreasing phytoplankton C:P and N:P was general for both lakes, although much more pronounced for C:P in La Caldera (from 410 to less than 100) than in Los Cántaros (from 1021

Table 1 Results of the two-way split-plot analysis of variance for the main plot effects of UVR and subplot effects of nutrients and zooplankton and their interaction on phytoplankton C:N:P ratios, Chl *a*, and PP

Lake	Main/subplot effects	Phytoplankton C:N		Phytoplankton C:P		Phytoplankton N:P		Chl <i>a</i>		PP	
		F _{1,4}	<i>P</i>	F _{1,4}	<i>P</i>	F _{1,4}	<i>P</i>	F _{1,4}	<i>P</i>	F _{1,4}	<i>P</i>
La Caldera											
	Main plot effect										
	UVR	1.68	0.265	66.97	0.001	0.49	0.486	4.18	0.110	210.79	<0.001
	Subplot effect										
	Nutrients	8.16	0.046	39.55	0.003	5.09	0.032	10.85	0.030	479.16	<0.001
	Nutrients × UVR	0.22	0.661	17.96	0.013	2.51	0.124	0.53	0.509	281.92	<0.001
	Zooplankton	1.25	0.326	7.83	0.049	3.30	0.080	10.10	0.034	48.06	0.002
	Zooplankton × UVR	4.36	0.105	6.88	0.059	2.79	0.106	1.64	0.269	261.37	<0.001
	Nutrients × Zooplankton	0.13	0.737	1.06	0.362	1.09	0.306	22.72	0.009	43.82	0.003
	Nutrients × Zooplankton × UVR	4.11	0.113	1.08	0.357	1.01	0.325	74.55	0.001	157.34	<0.001
Los Cántaros											
	Main plot effect										
	UVR	0.14	0.726	35.01	0.107	0.084	0.775	1.42	0.319	2.08	0.223
	Subplot effect										
	Nutrients	0.30	0.615	1110.6	0.019	6.93	0.015	0.01	0.920	292.21	<0.001
	Nutrients × UVR	2.87	0.165	24.58	0.127	0.289	0.596	5.17	0.108	20.17	0.011
	Zooplankton	0.09	0.783	4.35	0.105	3.46	0.075	6.70	0.081	12.89	0.023
	Zooplankton × UVR	1.62	0.272	25.59	0.124	0.04	0.851	1.90	0.262	399.79	<0.001
	Nutrients × Zooplankton	3.53	0.133	90.21	0.067	4.76	0.039	2.26	0.230	10.95	0.030
	Nutrients × Zooplankton × UVR	0.002	0.966	3.91	0.298	0.01	0.983	2.90	0.187	33.72	0.004

Numbers in bold indicate significant single or interactive effects

Table 2 Individual effects of nutrients and zooplankton on phytoplankton C:N:P ratios, Chl *a*, and PP in La Caldera and Los Cántaros

Lake	Factor	Treatment	UVR treatment	Exp./Cont.	Phytoplankton C:N		Phytoplankton C:P		Phytoplankton N:P		Chl <i>a</i>		PP	
					Δ	P	Δ	P	Δ	P	Δ	P	Δ	P
La Caldera	Nutrients	Z0	+UVR	3/1	-18	*	-38	*	-33	n.s.	935	**	2041	***
		Z+	+UVR	4/2	4	n.s.	16	n.s.	14	n.s.	-62	*	199	**
		Z0	-UVR	7/5	-2	n.s.	-35	*	-34	*	18	n.s.	-53	n.s.
	Zooplankton	Z+	-UVR	8/6	-17	n.s.	-46	*	-44	*	170	*	335	*
		N0	+UVR	2/1	-7	n.s.	-26	n.s.	-24	n.s.	832	**	168	*
		+N	+UVR	4/3	17	n.s.	37	n.s.	30	n.s.	-66	*	-63	***
		N0	-UVR	6/5	-3	n.s.	-25	n.s.	-25	*	-13	n.s.	1	n.s.
Los Cántaros	Nutrients	+N	-UVR	8/7	-18	n.s.	-39	*	-36	n.s.	100	*	844	*
		Z0	+UVR	3/1	-20	n.s.	-59	*	-58	*	38	n.s.	64	n.s.
		Z+	+UVR	4/2	87	n.s.	-33	n.s.	-33	n.s.	77	n.s.	1028	**
	Zooplankton	Z0	-UVR	7/5	-59	n.s.	-65	*	-64	**	-56	n.s.	172	*
		Z+	-UVR	8/6	-1	n.s.	-47	*	-46	*	-7	n.s.	163	*
		N0	+UVR	2/1	-22	n.s.	-35	n.s.	-34	n.s.	-56	n.s.	-43	n.s.
		+N	+UVR	4/3	82	n.s.	6	n.s.	5	n.s.	-43	n.s.	290	**
Zooplankton	N0	-UVR	6/5	-52	n.s.	-26	n.s.	-25	n.s.	-42	n.s.	-52	n.s.	
	+N	-UVR	8/7	17	n.s.	-12	n.s.	11	n.s.	22	n.s.	-54	*	

Exp./Cont. indicates the experimental (Exp.) and the control (Cont.) treatments used in the calculation of the effects as numbered in Fig. 1. Δ expresses the magnitude (as percentage) and sign (-, inhibitory; no sign, stimulatory) of the effect tested by ANOVA followed by post hoc Newman-Keuls test; P, the significance level (n.s. not significant; * P < 0.05; * P < 0.01; *** P < 0.001)

to 650) (Fig. 2). Thus, phytoplankton at the start of the microcosm experiments was P-sufficient only in the presence of UVR in La Caldera, but P-deficient in the rest of the conditions.

The experimental approach employed using microcosms in this study allowed us distinguished whether the observed response on phytoplankton corresponded with the individual or combined effects of nutrients and zooplankton, therefore providing valuable insights into the nature of the resource vs. consumer control on food webs. The statistical analysis of the split-plot experiments indicated that UVR main plot effects were significant for PP and seston C:P in La Caldera (Table 1). Because of the numerous interactive effects found in the subplot effects, differences in the response variables among treatments were further analyzed using post hoc tests (Table 2). Also, to visually depict the magnitude, sign, and significance of these effects we graphically represented the quotient between the experimental treatment mean and the respective control mean for each of the response variables in the presence (Fig. 3) and absence of UVR (Fig. 4). Phytoplankton C:N was basically unaffected by nutrients and zooplankton with the exception of nutrients that decreased this ratio by 18% under UVR in La Caldera (Figs. 3, 4; Table 2). In contrast, phytoplankton C:P strongly decreased in response to the single addition of nutrients under both UVR treatments in both lakes (Figs. 3, 4; Table 2). Zooplankton offset the effect of nutrients decreasing phytoplankton C:P under UVR in La Caldera (Fig. 3; Table 2).

Chl *a* increased in response to either nutrients or zooplankton only under UVR in La Caldera (Fig. 3; Table 2). Remarkably, the simultaneous effect of the two factors in this lake caused the decrease of Chl *a* in the presence of UVR (Fig. 3; Table 2), but the increase in Chl *a* when UVR was absent (Fig. 4; Table 2). Contrariwise, nutrients and zooplankton had little effect on Chl *a* in Los Cántaros.

As for Chl *a*, also nutrients and zooplankton enhanced PP only under UVR in La Caldera, being the effect particularly marked for nutrients (~21- vs. threefold increase due to nutrients vs. zooplankton, respectively; Fig. 3; Table 2). Only nutrients in the absence of UVR stimulated PP in Los Cántaros (Fig. 4; Table 2). Although the combination of nutrients and zooplankton enhanced PP under UVR in both lakes, the comparison between the combined and

Fig. 3 Effects of nutrients and zooplankton on phytoplankton C:N:P ratios, Chl *a*, and PP effect sizes for the microcosm experiments conducted under UVR in La Caldera and Los Cántaros. Effect size was calculated by dividing the variable response mean in the experimental treatment by the variable response mean in the control treatment (see treatment description in Table 2). *Error bars* correspond with the SD calculated following the error propagation approach. *Asterisks* denote significant effect sizes as shown in Table 2. Significant differences among treatments are denoted by *different case letters* according to *t* tests

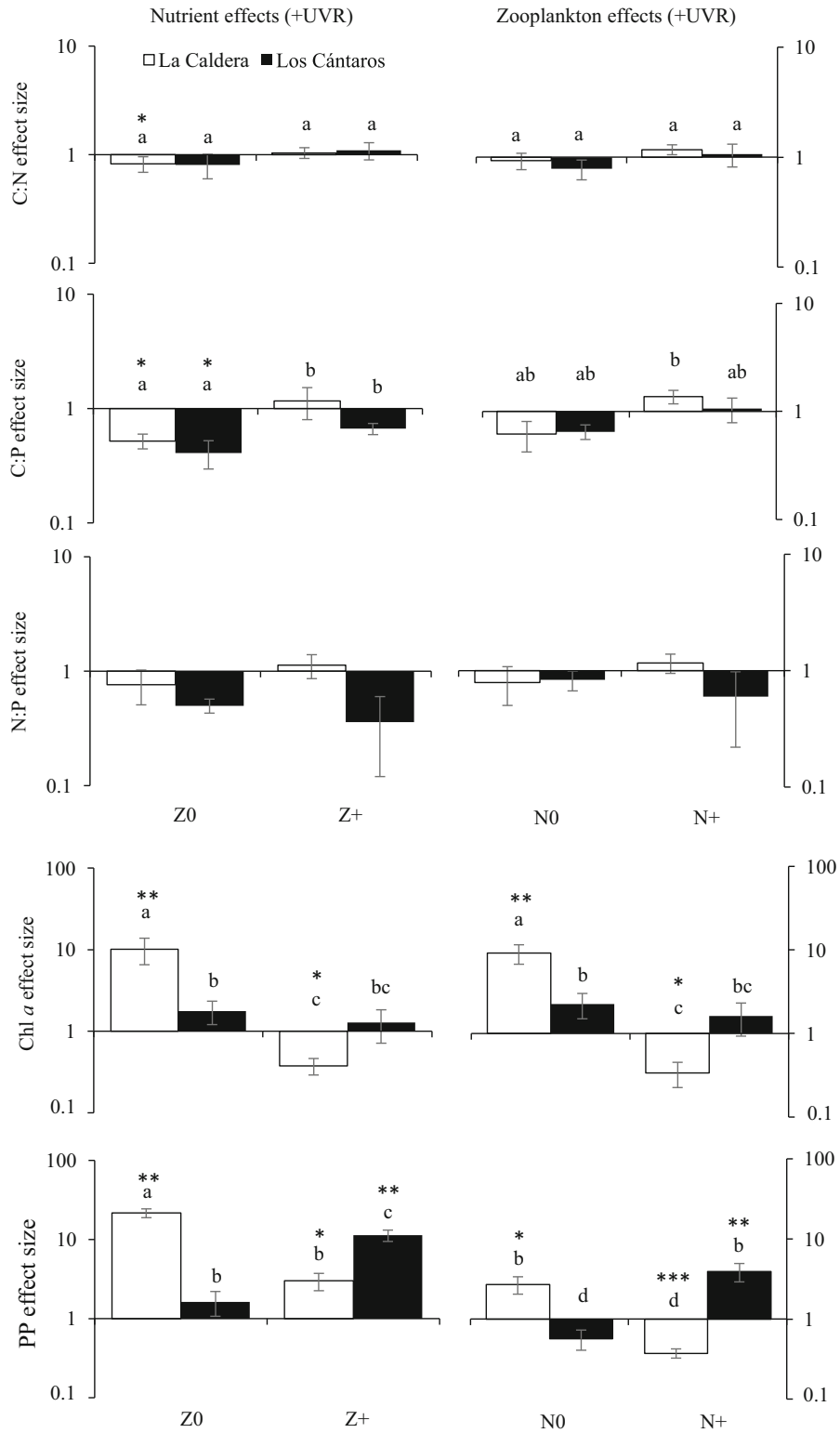
single responses yielded different responses between lakes. Thus, while the combined effect of nutrients and zooplankton on PP in La Caldera was lower than the single effect on nutrients and higher than the single effect of zooplankton, PP, which did not respond to the single addition of nutrients or zooplankton in Los Cántaros, remarkably increased by more than tenfold with the combined effect of these two factors (Fig. 3).

With regard to the effects of zooplankton on PP, these were much more complex as they varied with nutrients and UVR conditions yielding opposed responses between the two experimental lakes. Thus, in the presence of UVR, zooplankton decreased PP when nutrients were added in la Caldera, but strongly enhanced PP when nutrients were added in Los Cántaros (Fig. 3; Table 2). Remarkably, the reverse was true in the absence of UVR, where zooplankton enhanced PP by more than eightfold after nutrient enrichment in La Caldera, but decreased PP by more than half when nutrients were added in Los Cántaros (Fig. 4; Table 2).

Discussion

The finding of this study that UVR alters the stoichiometric composition of phytoplankton by reducing C:nutrient ratio has been well documented in the literature (Xenopoulos et al., 2002; Carrillo et al., 2008; Villar-Argaiz et al., 2009; Korb et al., 2012). However, UVR reduced phytoplankton C:P to a much larger extent in La Caldera relative to Los Cántaros (~75 and ~25% reduction, respectively) indicating a higher UVR sensitivity of phytoplankton in the former lake. As a consequence, phytoplankton in the UVR mesocosms after 30 days was still markedly P-deficient in Los Cántaros (mean phytoplankton C:P ~650), but P-sufficient in La Caldera with a C:P ratio of 100. The substantial differences in

Microcosms



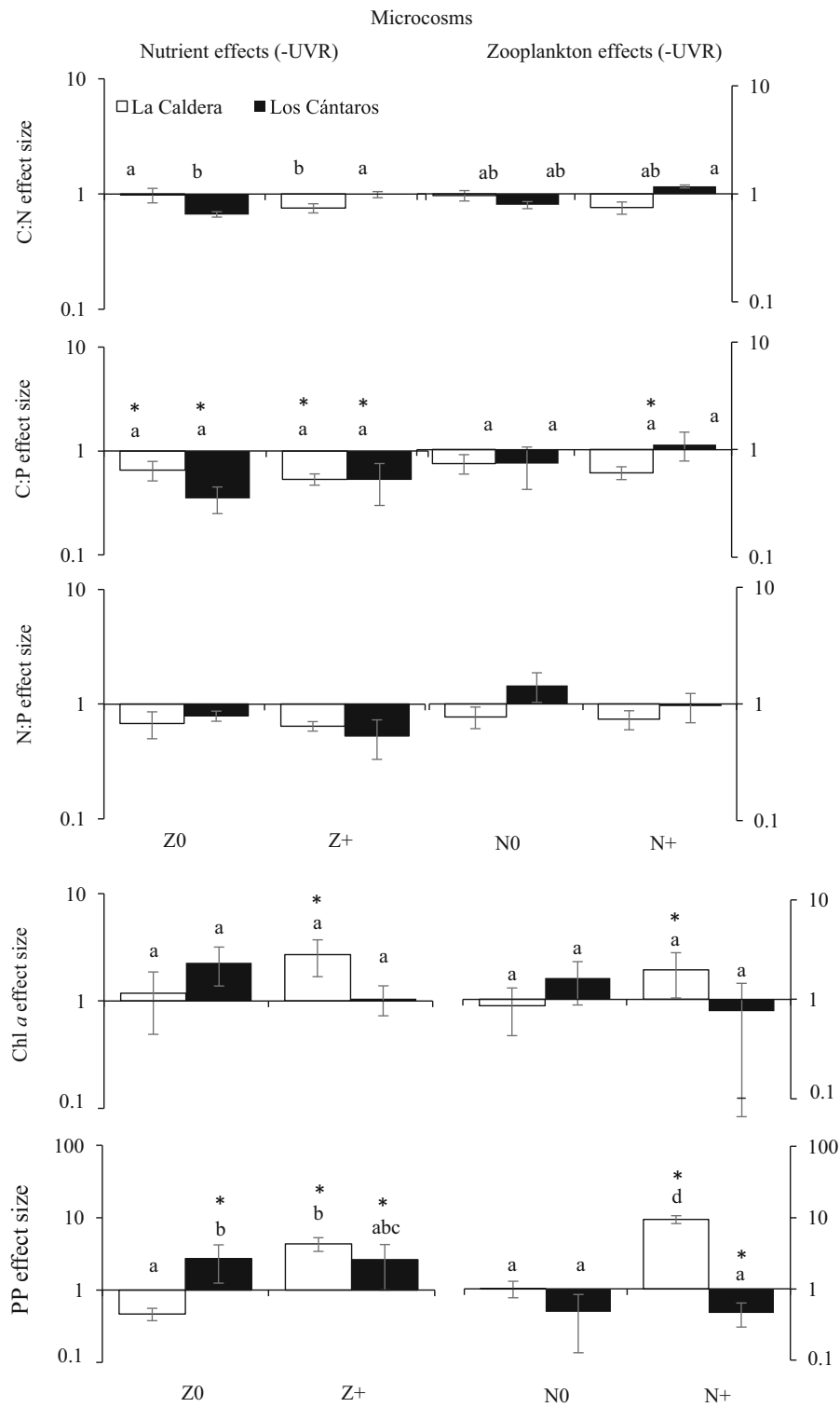


Fig. 4 Effects of nutrients and zooplankton on phytoplankton C:N:P ratios, Chl *a*, and PP effect sizes for the microcosm experiments conducted in the absence of UVR in La Caldera and Los Cántaros. Effect sizes, error bars, asterisks, and case letters as in Fig. 3

the elemental composition of phytoplankton between the two lakes served our purpose of testing the general prediction that resource and consumer effects on phytoplankton after UVR manipulations would be much weaker in the Andean lake due to stoichiometric side-effects of its much more P-deficient phytoplankton. While we realize that there are other determinants of algal food quality besides P content (e.g., essential fatty acids, digestion resistance, or algal taxonomy), the importance of P limitation is widely accepted, whether this is a direct effect or simply a covariation effect with some other unmeasured limiting feature or constituent of the algae (Ravet & Brett, 2006).

How did UVR exposure affect the bottom-up processes that regulate trophic structure and dynamics in both ecosystems? While our findings add evidence for the unequivocal role of bottom-up factors like nutrients in controlling phytoplankton stoichiometry, the effects did not necessarily transfer into higher Chl *a* or PP. In fact, consistent with our hypothesis we found that under UVR the resource nutrient effects strongly stimulated PP or Chl *a* in La Caldera but not in Los Cántaros with P-deficient phytoplankton. The a priori contra-intuitive result of PP stimulation under UVR (Doyle et al., 2005) in La Caldera is consistent with previous reports of maximum C uptake for photosynthesis near the blue and UVA regions of the solar spectrum in certain phytoplankton species (Gao et al., 2007), and with the combined effect of UVR and nutrients on total production/respiration ratios in a recent study in oligotrophic marine ecosystems (Cabrerizo et al., 2016). In addition, recent food web manipulations in mesocosms in this lake provide support for the idea of an evolutionary adaptation of algal communities to the intensive UVR irradiance of this lake located at a relatively low latitude and high altitude (Bullejos et al., 2010). We hypothesized that the specific reasons behind algal acclimation to UVR in La Caldera include (i) the decrease in seston C:P by UVR due to uptake of P rather than the loss of C (Souza et al., 2010) and (ii) the stimulatory effect of UVR on extracellular alkaline phosphatase which enhances the release of inorganic P from particulate and dissolved fractions available to algae in oligotrophic ecosystems (Sereda et al., 2011 in a boreal lake; Korbee et al., 2012 in La Caldera; Carrillo et al., 2015 in a marine ecosystem, Modenutti et al., 2016 in Andean-Patagonian lakes). On the other hand, the result that nutrients enhanced Chl *a* and PP when

phytoplankton was protected from UVR in Los Cántaros is consistent with previous studies in which subsurface phytoplankton of Andean lakes was heavily photoinhibited by both UVR and PAR (Callieri et al., 2007; Modenutti et al., 2013a).

We have shown that the single effect of nutrients did not affect Chl *a* and PP under UVR in Los Cántaros. Interestingly, despite the amelioration in P deficiency after the addition of nutrients (60% reduction in phytoplankton C:P, see Table 2), phytoplankton still remained P-deficient in this lake. However, this condition changed when zooplankton was added, which resulted in a significant increase in PP in this lake (Fig. 3). Zooplankton affect the phytoplankton through grazing and nutrient release, but the net effect of grazing and recycling combined is not straightforward (Sommer, 1988). Because cladocerans retain more P compared to calanoid copepods (Balseiro et al., 1997), daphnids should increase N:P ratio over the season (Hessen & Andersen, 1992). Consequently, cladoceran zooplankton can enhance P limitation for phytoplankton (Balseiro et al., 1997). However, based on the dominance of calanoid copepods in the studied lakes with less P demands relative to cladocerans, we expect that nutrients released by zooplankton would have alleviated the strong P-deficit of phytoplankton while augmenting PP in Los Cántaros.

In addition, the rapid response of La Caldera phytoplankton to a nutrient pulse may be associated with the frequent atmospheric inputs from the nearby Saharan desert that contribute to explain the population dynamics in this lake (Carrillo et al., 2008; Bullejos et al., 2010). While the long distance from the Sahara desert could prevent Los Cántaros from receiving large dust emissions, recent evidence from observational data and atmospheric models indicate that P depositions might become a major driver of alpine lake trophic status in the Southern Hemisphere (Brahney et al., 2015), and eventual pulses of P can be expected from volcanic eruptions (Modenutti et al., 2013b). While the low frequency of the latter events (one every 50 years) could explain why phytoplankton did not respond to sudden inorganic nutrient pulses, the possibility that humic substances in Los Cántaros affected the chemical speciation and bioavailability of P for phytoplankton should not be disregarded (Hessen & Tranvik, 1998).

Was top-down control affected by UVR? Evidence for consumer top-down control was obtained by direct comparison between microcosms with and without

zooplankton and no nutrients added. We found strong differences in the effects of zooplankton on phytoplankton PP and Chl *a* between lakes. While zooplankton increased Chl *a* and PP in the presence of UVR in la Caldera with low C:P phytoplankton ratio, no zooplankton effect was found in Los Cántaros. That zooplankton did not affect phytoplankton in Los Cántaros is consistent with our hypothesis of weaker consumer trophic effects (grazing) in the most P-deficient lake, and further suggests a higher recycling effect.

While this study supports the top-down and bottom-up control hypothesis of McQueen and colleagues (McQueen et al., 1986), it also provides with evidence to the considerable variations found in trophic cascade experiments between habitats and within systems (Shurin et al., 2002; Borer et al., 2005). Here we suggest that differences in the stoichiometric aspects associated with P content in phytoplankton play a key role in how UVR affects clear water ecosystems. We have reasoned that nutrients enhanced Chl *a* and PP under UVR only in the P-sufficient phytoplankton lake, but not in the P-deficient lake. In addition, only under UVR in the P-sufficient lake did zooplankton inhibit Chl *a* and PP. These currently unappreciated differences in phytoplankton P composition between ecosystems could help explain the role that UVR exerts strengthening or weakening resource and consumer controls in the lower trophic levels of food webs.

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