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# Roles of phosphorus and ultraviolet radiation in the strength of phytoplankton–zooplankton coupling in a Mediterranean high mountain lake

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*Abstract*

Ultraviolet solar radiation (UVR) and atmospheric nutrient inputs associated with aerosols are major worldwide stressors that simultaneously affect species and the interaction among them. A $2 \times 5$ field experimental design was used to determine how variations in light regimes (presence and absence of UVR [+UVR and −UVR]) and nutrients might influence the strength of phytoplankton–zooplankton coupling (PZC). We observed unimodal curves for zooplankton biomass in response to increased food supply from nutrient enrichment. These results challenge the "more is better (or at least never worse)" concept, since high food levels resulted in weakened PZC. The effect of UVR on zooplankton was nutrient dependent, significantly reducing zooplankton abundance at intermediate phosphorus (P) supplied levels but not at the two ends of the trophic gradient generated (control and highest P level). Neither food quantity nor food quality explained observed differences in zooplankton biomass between light treatments, suggesting a deleterious direct effect of UVR on zooplankton at intermediate food ranges, resulting in a weakening of PZC. The location of this lake in the Mediterranean region has shown an increasing intensity and frequency of aerosol depositions over the past three decades (1973–2003), resulting in higher phytoplankton biomass. A combination of these higher atmospheric dust depositions with the high UVR levels characteristic of high mountain lakes might underlie the interannual decoupling between phytoplankton and zooplankton dynamics observed in these oligotrophic ecosystems.

Global change factors, acting at different rates and spatial scales, promote changes in organisms that affect their interactions with other organisms and with the environment. Land use alterations, in combination with global warming, might generate or increase drought and dust emission in many areas and are a major driver of global change (Goudie 2009). Higher dust activity in source areas like the Sahara might be responsible for more intensive nutrient depositions in sink areas (Neff et al. 2008) such as the Mediterranean region (Santese et al. 2007). These depositions are rich in phosphorus (P) (Morales-Baquero et al. 2006) and might influence organisms and ecological interactions, especially in neighboring ecosystems with low nutrient availability. In addition, the interaction between global stressors adds a level of complexity to the study of global change effects. Ultraviolet radiation (UVR) is a major worldwide stressor with far-reaching implications for ecological interactions and an overall negative effect on the survival and growth of organisms (Bancroft et al. 2007; Häder et al. 2007). However, few studies have reported the effects of the interaction between global stressors, such as UVR and nutrient inputs, on trophic interactions (Medina-Sánchez et al. 2006).

One crucial question is how global stressors affect the strength of the coupling between primary producers and herbivorous consumers, which has consequences for food web structure and the efficiency with which energy moves to higher trophic levels. The relevance in the study of the primary producer–consumer interface has increased since the recognition that at this level nutrient imbalances are among the highest in nature (Sterner and Elser 2002). Considerable research efforts have focused on aquatic systems because of the high turnover rates of the primary producers. To date, authors have investigated how the coupling between algae and zooplankton varies across trophic gradients (Elser et al. 1990; Auer et al. 2004; Hessen et al. 2006) or in eutrophic ecosystems (Abrantes et al. 2006). While there is empirical evidence of hump-shaped trends when zooplankton biomass is plotted against total phosphorus (TP) (Carney and Elser 1990; Persson et al. 2007), others have reported logarithmic responses to the increase in nutrients (Hessen et al. 2006). Numerous studies have contributed new data on the mechanisms behind phytoplankton–zooplankton coupling (PZC). Durant et al. (2005) showed that food resources and consumer levels also determine the strength of PZC. Other characteristics that have been identified to qualitatively affect the strength of PZC include food quality (Dickman et al. 2008), phytoplankton taxonomic composition (Auer et al. 2004), zooplankton diversity (McCann et al. 1998), alternative food resources such as microphytobenthos or microbial communities (Rautio and Vincent 2006), and zooplankton predators (Hessen et al. 2006; Dickman et al. 2008). All of these might be influenced by global stressors. While the role of global warming on PZC is well studied (Winder and Schindler 2004; Domis et al. 2007; Sommer et al. 2007), additional work is required to elucidate the contribution of other joint global factors. There has been enormous research interest in developing our understanding of the

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response of organisms and ecosystems to the interaction between UVR and other global stressors (Williamson et al. 2002; Vinebrooke and Leavitt 2005). However, the nature of this interaction is difficult to predict, and effects are not necessarily straightforward. For example, Carrillo et al. (2008) demonstrated that P inputs from dust unmask deleterious UVR effects on algae instead of attenuating these effects, as expected. It was also reported that P inputs increased algal biomass and that both P inputs and UVR exposure reduced the seston carbon : phosphorus (C : P) ratio (Xenopoulos et al. 2002; Carrillo et al. 2008; Hessen et al. 2008). As pointed out by these authors, these effects might positively enhance consumer growth by simultaneously improving food quantity and quality.

The aim of this study was to determine how the interaction between UVR and nutrient availability (mimicking atmospheric dust depositions) might affect the strength of PZC via changes in the quantity and quality of food for herbivore grazers. Our prediction was that plentiful food that had high quality due to combined P fertilization and UVR would promote high herbivore growth and, consequently, the strength of PZC. We tested this hypothesis in an experiment in which large mesocosms were incubated in situ in the presence and absence of UVR across an experimental P gradient in a high mountain lake. High mountain lakes are "sentinels of change" (Williamson et al. 2009), offering ideal natural scenarios for testing the effects and mechanisms of climate change. These types of lakes are ultrasensitive to atmospheric inputs, largely due to the oligotrophy associated with their remote location. Their high altitude exposes them to extreme UVR environments and favors the interception of dust-transporting atmospheric winds, especially when lakes are near major dust-emission sources (Morales-Baquero et al. 2006; Di Ioiro et al. 2009).

To assess whether changes in nutrients and UVR affect the long-term dynamics of phytoplankton–zooplankton populations in natural ecosystems, we analyzed phytoplankton and zooplankton biomass collected in Lake La Caldera from 1973 in relation to long-term aerosol index (AI) data as a proxy for P deposition (Morales-Baquero et al. 2006) and UV irradiances derived from original data of the National Aeronautics and Space Administration (NASA).

## Methods

*Study site*—The study was performed in Lake La Caldera in the National Park of Sierra Nevada (Spain, 36°55′–37°15′N, 2°31′–3°40′W) at an elevation of 3050 m above sea level (asl). La Caldera is a small lake with a surface area of ~ 0.02 km$^2$, maximum depth of < 10 m, and a mean depth of ~ 3 m immediately after ice-out in 2003. The ice-free period usually extends from the middle of June to the end of October, when temperatures fluctuate between 5°C and 15°C. During this season, UVR of considerable intensity penetrates deeply in the lake (Table 1) due to the high transparency of the water (Secchi's disk visibility reaching maximum depth) and low values of dissolved organic carbon (< 1 mg L$^{-1}$ as reported in references in Carrillo et al. 2008). The pelagic community was strongly P limited during 2003, with a dissolved inorganic nitrogen : total phosphorus (DIN : TP) ratio of ~ 100 (by mass) (Carrillo et al. 2008), which is characteristic of this lake (Villar-Argaiz et al. 2001). Its altitude and geographical position very close to Africa make it prone to high allochthonous P inputs from dust, since Saharan dust plumes are deposited within the first 2000 km, largely between 1500 and 4000 m asl (Morales-Baquero et al. 2006).

Table 1. Diffuse attenuation coefficients for downward irradiance ($K_d$) in Lake La Caldera from different sampling days during experimental period in 2003 measured using a LI-8000 spectroradiometer (LI-COR). Days of the experimental period are given in parentheses after dates. $K_d$ were determined from the slope of the linear regression of the natural logarithm of downwelling irradiance vs. depth for each region of the solar radiation spectrum: UVB (300–319 nm), UVA (320–399 nm), and PAR (400–800 nm).

| Date (No. of days in period) | $K_{d\ UVB}$ | $K_{d\ UVA}$ | $K_{d\ PAR}$ |
|---|---|---|---|
| 01 Aug (1) | 0.23 | 0.15 | 0.09 |
| 03 Aug (3) | 0.11 | 0.07 | 0.05 |
| 20 Aug (20) | 0.46 | 0.32 | 0.27 |
| 01 Sep (32) | 0.36 | 0.23 | 0.17 |
| 11 Sep (42) | 0.24 | 0.15 | 0.14 |
| 24 Sep (55) | 0.13 | 0.06 | 0.07 |

The phytoplankton community is mainly represented by *Chromulina nevadensis* (Chrysophyceae), *Dictyosphaerium chlorelloides* (Chlorophyceae), and *Cyclotella* sp. (Bacillariophyceae) (Delgado-Molina et al. 2009). Herbivorous consumers are the highest trophic level in the pelagic community, and the calanoid copepod *Mixodiaptomus laciniatus* is the dominant zooplanktonic species (> 90% in zooplankton biomass). Other zooplankton species such as *Daphnia pulicaria*, linked to littoral areas, or *Hexarthra bulgarica*, are scarce (Carrillo et al. 1995).

*In situ experiment*—An in situ experiment was carried out with a 2 × 5 factorial design: two light treatments (full-sunlight [+UVR] vs. photosynthetic active radiation [−UVR]) and five nutrient treatments. The unreplicated factorial design carried out to test interactive effects (UVR × P in this study) proved useful in previous studies (Carrillo et al. 2008). Each treatment consisted of one mesocosm made of clear polyethylene tubes (0.7 m diameter × 7 m length), closed at the bottom, with a total volume of 2.7 m$^3$. A water pump was used to fill each mesocosm with unfiltered lake water collected from 3-m depth (photic layer affected by > 5% of UVB). The 10 mesocosms were set in two 3 × 3 m$^2$ racks made of 3-cm polyvinyl chloride pipe; each rack contained five enclosures for each light treatment. The two racks were separated by approximately 50 m to avoid shading effects. Both subsets were secured to a buoy attached to an anchored rope.

The +UVR treatment was obtained by using polyethylene plastic that transmits 90% of photosynthetic active radiation (PAR [400–700 nm]) and most of UVR (75% of ultraviolet A [UVA; 320–399 nm] and 60% of ultraviolet B

[UVB; 295–319 nm]). The −UVR treatment was obtained by using a cover of Plexiglas UF3, a long-wave–pass plastic that transmits 90% of PAR but blocks UVR (< 390 nm). Optical properties of the cutoff filters used in light treatments were tested before experiments with a double-beam spectrophotometer (Perkin-Elmer Lambda 40). Further, the rack (subset) containing −UVR enclosures was surrounded by 2-$m^2$ layers of Plexiglas UF3 below the lake surface to prevent incidence of refractory solar UVR. Comparisons between radiation water profiles (affected by > 25% of UVB) within and outside the bags receiving no nutrients showed transmittances of 56% of UVB, 72% of UVA, and 73% of PAR in the +UVR treatments, mimicking natural conditions reasonably well. In the −UVR treatments, attenuations were 82% of UVB, 70% of UVA, and 17% of PAR, i.e., considerably blocking UVR.

The five P-enrichment levels were set by adding a final concentration of 0, 20, 30, 40, and 60 $\mu$g P $L^{-1}$ (as $NaH_2PO_4$). The enclosure with no added nutrient and +UVR served as control for nutrient-enriched enclosures and reproduced the closest conditions to those of the lake, with soluble reactive phosphorus (SRP) concentrations < 1 $\mu$g P $L^{-1}$. The added nutrient concentrations generated the gradient produced by the natural atmospheric depositions of P. Although the 60 $\mu$g P $L^{-1}$ treatment duplicated the maximum dissolved P concentration measured in this ecosystem after an allochthonous input (Villar-Argaiz et al. 2001), it remained below the estimation of 81.4 $\mu$g P $L^{-1}$ for a single event calculated from weekly collected atmospheric inputs in the lake area (Morales-Baquero et al. 2006).

Previous investigations in this lake established clear connections between atmospheric deposition and lake nutrient concentrations (Morales-Baquero et al. 2006). Highly similar concentrations in the P- and N-dissolved and total fractions were described immediately after an allochthonous input (Villar-Argaiz et al. 2002*b*), implying that most of the nutrients that adhered to dust particles are readily available for phytoplankton uptake. Hence, the nutrient pulses in our experiment represented an appropriate simulation of natural inputs. The amount of P to be added was calculated from the dissolved total phosphorus concentration found in the water column on the day before starting the experiment. To ensure that P remained as a limiting nutrient, inorganic nitrogen (as $NH_4NO_3$) was added to reach a nitrogen : phosphorus (N : P) molar ratio of 30, mimicking the mean atmospheric dust TN : TP ratio (10–50, as reported by Morales-Baquero et al. 2006). Nitrate and ammonium are major inorganic N compounds in the water-soluble fraction of aerosol particles (Chen et al. 2007), supporting their use as N sources in the present experiment. After nutrient addition and before taking samples, the water in mesocosms was vigorously mixed with a plastic bucket to avoid problems associated with the patchy vertical and horizontal distribution of organisms. Sestonic and zooplankton samples were taken in triplicate from three randomly chosen mesocosms to determine the initial experimental conditions (*see* sampling methods below). The coefficient of variation (CV = (standard deviation/mean) × 100) for algal biomass and zooplankton abundance never exceeded 3%. Finally, the top of each enclosure was covered (polyethylene for +UVR and Plexiglas UF3 for −UVR) to avoid external nutrient inputs during the incubation period but allow air exchange.

The experiment lasted for 70 d, i.e., most of the ice-free season (from 01 August to 10 October 2003). Sampling was performed on days 1, 3, 11, 20, 32, 42, 55, and 71, after mixing the entire length of the enclosure. The sampling frequency was appropriate for the study of phytoplankton dynamics, and the experiment was sufficiently long to allow calanoid copepods, with their low growth rates, to reach adulthood (Villar-Argaiz et al. 2002*a*).

*Chemical and biological analyses*—Water samples for nutrients (DIN and SRP), sestonic elemental composition (C, N, and P), chlorophyll *a* (Chl *a*), and phytoplankton abundance were taken in triplicate with a plastic bucket after gently mixing the entire length of the enclosure before sampling and prefiltering with a 40-$\mu$m mesh to remove zooplankton. Zooplankton samples to determine abundance and biomass were taken using one vertical tow of a 40-$\mu$m mesh zooplankton net (12.5-cm diameter), which, covering the full depth of the enclosure, sampled 3% of the total enclosure volume. Immediately afterward, samples were preserved in 4% formaldehyde. Additional zooplankton samples were also collected and brought in mesocosm water under dark and cold conditions for C biomass determinations. The above variables were also monitored at three depths (0.5, 4, and 8 m) at a central station of the lake. We used a Van Dorn sampler for nutrients and seston and obtained zooplankton samples for abundance and biomass determinations after sieving 12 liters of water from each depth through a 40-$\mu$m mesh.

Samples for DIN and SRP were analyzed on the same day as their collection. DIN was considered the sum of $NO_3^-$, $NO_2^-$, and $NH_4^+$, which were determined by UV-spectrophotometric screening and sulphanilamide and phenol-hypochlorite techniques, respectively. SRP was analyzed by means of the acid molybdate technique (APHA 1992). For sestonic C, N, and P determinations, samples were filtered through precombusted (1 h at 550°C) 1-$\mu$m glass fiber filters (Whatman GF/B) at low pressure (< 100 mm Hg). Filters were then immediately analyzed for P, dried (24 h at 60°C), and kept desiccated until C and N analysis. Particulate C and N were determined using a Perkin-Elmer model 2400 (Perkin-Elmer Corporation) elemental analyzer. For the analysis of particulate P, filters were introduced into acid-washed vials, digested with a mixture of potassium persulfate and boric acid at 120°C for 30 min, and determined as SRP in 10-cm quartz cuvettes using the acid molybdate technique (APHA 1992). Blanks and standards were performed for all procedures. All C : N, C : P, algal C biomass : N, and algal C biomass : P ratios were calculated on a molar basis. Chl *a* was measured fluorimetrically after grinding filters (Whatman GF/F glass fiber filter, 25-mm diameter) with pigments (concentrated by filtration of up to 300 mL at < 100 mm Hg of pressure) and extracting the pigments in 90% acetone kept in the dark at 4°C for 24 h. A Chl *a* standard (Fluka Chl *a* from

algae) was used to transform the fluorescence data into Chl *a* concentrations.

Phytoplankton was preserved by using Lugol's reagent. Cells were counted in 100 randomly selected fields of view at ×2000 magnification under an inverted microscope (Leitz, Fluovert FS, Leica). Between 20 and 30 cells of each species were measured for each date, using image analysis (Quantimet 500, Leica) to estimate cell volume according to a corresponding geometrical shape. The biovolume density ($mm^3\ mL^{-1}$) for each taxon was determined by multiplying mean cell volume by abundance. Cell volume was converted to C by using specific conversion factors (Carrillo et al. 2008).

For zooplankton C content, copepods from each mesocosm were identified to stage level, measured with the aid of a stereomicroscope, sorted alive into specific Petri dishes containing GF/F-filtered lake water, and then transferred to secondary Petri dishes with deionized water. Between 40 and 70 individuals of the most abundant stages were analyzed for C in triplicate following the methods described for seston. Zooplankton abundance was determined by counting under an inverted microscope at ×100 magnification. For each sample, 20 individuals of each species or copepod stage were measured by image analysis (Quantimet 500, Leica). Subsequently, zooplankton biomass was calculated by using the length–weight relationships specifically developed for the zooplankton species in this ecosystem (Carrillo et al. 2001).

*Long-term biological data series*—Data on phytoplankton and zooplankton are available since 1973 and 1975, respectively. Samples were collected during the ice-free season of the lake at a maximum depth station. Standard collection protocols and original data are in Martinez (1977), Cruz-Pizarro (1981), Carrillo et al. (1995), Villar-Argaiz et al. (2001), Medina-Sánchez et al. (2004), Pulido-Villena (2004), and Delgado-Molina et al. (2009). Briefly, water for phytoplankton abundance and biomass determinations was taken from three or four depths (0.5 m below the surface, 0.5 m above the bottom, and at one or two intermediate points) and, after filtration through a 40-$\mu$m mesh to remove zooplankton, preserved with Lugol's reagent (1% vol/vol). Counts, measurements, and biovolume determinations were made as described above. Biomass ($\mu g$ fresh weight $L^{-1}$) was obtained from biovolume–density conversions, assuming a specific weight of 1. Zooplankton samples to estimate abundance and biomass were obtained after sieving 18 or 24 liters of water from different depths through a 40-$\mu$m mesh, and they were immediately fixed in 4% formaldehyde. Counts and size determinations were done as described above, and biomass was estimated using standard length–biomass relationships (Bottrell et al. 1976) or relationships specifically developed for the species in this system (Cruz-Pizarro 1981; Carrillo et al. 2001). A minimum of four measurements were taken each sampled year, and the biomass values reported are annual averages.

*Remote sensing*—As a measure of aerosol content in the troposphere, we used the AI developed by the Ozone Processing Team (NASA/Goddard Space Flight Center) from measured irradiances by the total ozone mapping spectrometer (TOMS) on board the *Nimbus 7* (1978–1993) and *Earth Probe* (1996–2004) NASA satellites. Aerosol data were successfully used for the study of Saharan dust in previous studies of this ecosystem, as a result of the highly positive correlation of TOMS AI with total phosphorus (TP) and particulate matter linked to dry atmospheric deposition (Morales-Baquero et al. 2006). We used annual averages of weekly TOMS AI data given for 37.5°N, 3.075°W (closest geographic coordinates to the lake) as a measure of the intensity of aerosol deposition. An AI value $> 0.5$ was considered to represent a deposition event, and the annual frequency of these events was calculated as the percentage of days affected by aerosol deposition events.

Average annual UVR values were calculated by using 325-nm wavelength data given for 37.5°N, 3.5°W (closest geographic coordinates to the lake) and only considering the period corresponding to the ice-free season (approximately from 01 June to 15 November). This wavelength represented an intermediate value among the available wavelengths (305, 310, 325, and 380 nm). Original data are available at http://jwocky.gsfc.nasa.gov.

*Statistical analyses*—Simple linear regression analysis was used to test the effects of (1) experimental time on zooplankton biomass; (2) P enrichment on TP; (3) TP on algal standing stock variables (algal biomass; Chl *a*; sestonic C, N, and P); (4) food quality variables (sestonic C : N and C : P, algal C biomass : N, algal C biomass : P, Chl *a* : sestonic C) on zooplankton biomass; (5) time on TOMS AI, annual frequency of aerosol deposition events, and UV 325 nm irradiance; and (6) TOMS AI and annual frequency of aerosol deposition events on phytoplankton biomass.

When the regression was significant, a homogeneity of slopes model (analysis of covariance [ANCOVA]) was used to test the effect of light treatment (categorical factor, +UVR, −UVR) across the continuous predictor variables (covariates, time, P enrichment, and TP) on the response variables (zooplankton biomass, TP, and algal standing stock variables) (Quinn and Keough 2002). When no significant differences were found in the *y*-axis intercepts, UVR effect was graphically checked along the covariate by examining 95% confidence intervals of the regression lines (*see* Urabe et al. 2002 for a similar statistical analysis). When no significant regressions were observed, differences due to UVR were tested by a paired *t*-test. Paired *t*-tests were also used to examine differences in zooplankton abundance, size, and C content due to UVR at each nutrient level.

Polynomial regression models were used to test the effects of TP and food quantity variables (algal biomass; Chl *a*; sestonic C, N, and P) on zooplankton biomass for each light treatment, determining the UVR effect by examining 95% confidence intervals of the regression lines, and the relationship between phytoplankton and zooplankton biomass (long-term biological data series). Statistical analysis of the experimental results, except for zooplankton temporal changes, considered mean values after the second week of the incubation ($n = 5$) to allow for the lag-response

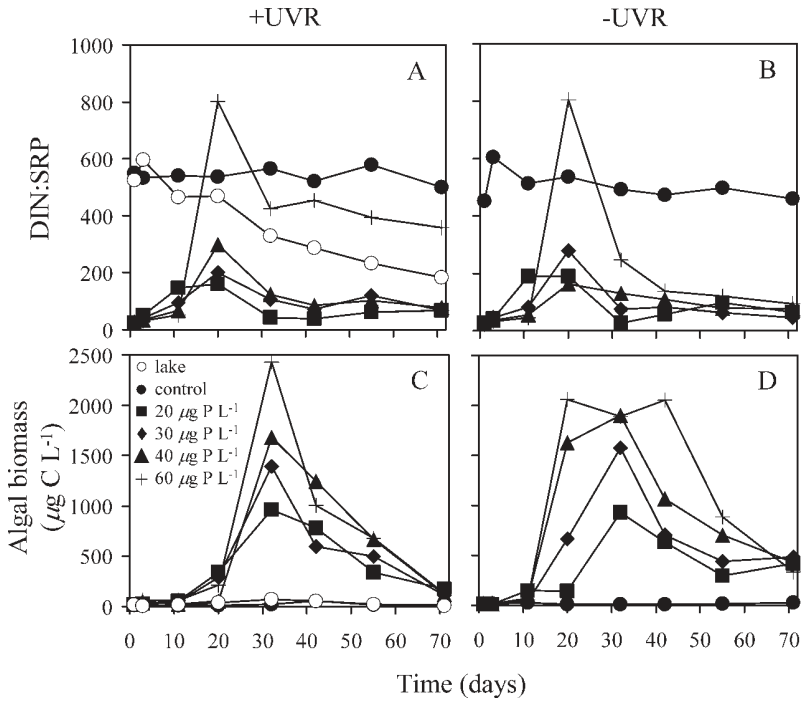


Fig. 1. Temporal dynamics of DIN : SRP and algal biomass under (A,C) +UVR and (B,D) −UVR in the lake, nonenriched (control) and P-enriched (20, 30, 40, and 60 $\mu$g P $L^{-1}$) treatments during the experimental period. Each point represents a single day.

of zooplankton (dominated by a slow-growth copepod species) to experimental manipulation. Analyses of temporal variations in zooplankton biomass considered values for single days ($n = 5$). Normality was tested by Shapiro–Wilks $W$-test and homoscedasticity by Levene's test. Statistica 7.0 for Windows software (Stat Soft 2001) was used for the statistical analyses.

## Results

*UVR and P-enrichment effects on temporal dynamics*—The temporal dynamics of DIN : SRP ratio and algal biomass are depicted in Fig. 1. The ratio followed a similar pattern in the two light treatments. The controls showed few changes in this ratio over the experimental period, with a mean value around 550, similar to the initial lake DIN : SRP ratio. The initial ratio in the nutrient-enriched enclosures was very similar to the experimental DIN : SRP ratio of 30, which increased up to day 20 and then progressively decreased until the end of the experimental period (Fig. 1A,B). No appreciable changes in algal biomass were detected in the control enclosures and the lake, with values remaining low throughout the experimental period. Algal biomass strongly augmented in response to nutrient enrichment up to day 32 and then steadily declined until the end of the experiment (Fig. 1C,D). Patterns were similar for other phytoplankton variables (Chl *a*, sestonic C, N, and P) because of their strong intercorrelations (all correlations $r \geq 0.90$ and $p < 0.05$). Phytoplankton was mainly composed of *C. nevadensis* until day 20, but *D. chlorelloides* dominated the algal community thereafter (Delgado-Molina et al. 2009).

The calanoid copepod *M. laciniatus* represented nearly 100% of the zooplankton biomass in the enclosures. The zooplankton assemblage was initially composed largely of copepodite stage III, with most reaching adulthood by the end of the experiment, i.e., 70 d later. Fig. 2 shows temporal variations in zooplankton biomass for each UVR and nutrient treatment. Zooplankton biomass in the controls was low and did not show major temporal changes, reflecting the lake dynamics. Zooplankton biomass strongly increased in response to nutrient enrichment with the exception of the highest P-enriched treatment, in which the biomass barely changed and eventually decreased to a level lower than the control. Zooplankton biomass decreased after day 20 in enclosures 20 and 30 $\mu$g P $L^{-1}$ and after day 42 in enclosures 40 and 60 $\mu$g P $L^{-1}$. These decreasing trends were linear for +UVR in the 20 $\mu$g P $L^{-1}$ enclosure and for both light treatments in the 30 $\mu$g P $L^{-1}$ enclosures (Table 2).

At the intermediate P-enriched level (30 $\mu$g P $L^{-1}$), zooplankton biomass was significantly higher in the −UVR than in the +UVR enclosure (analysis of covariance, intercept, $F_{1,6} = 282.45$, $p < 0.001$; slope, $F_{1,6} = 4.90$, $p = 0.068$). Treatments that did not show linear temporal trends were compared using $t$-tests, revealing that zooplankton biomass was also consistently higher in the 20 $\mu$g P $L^{-1}$ ($t$-test, $t = -3.80$, df = 4, $p = 0.019$) and 40 $\mu$g P $L^{-1}$ ($t$-test, $t = -2.81$, df = 4, $p = 0.048$) treatments in the absence of UVR vs. presence of UVR.

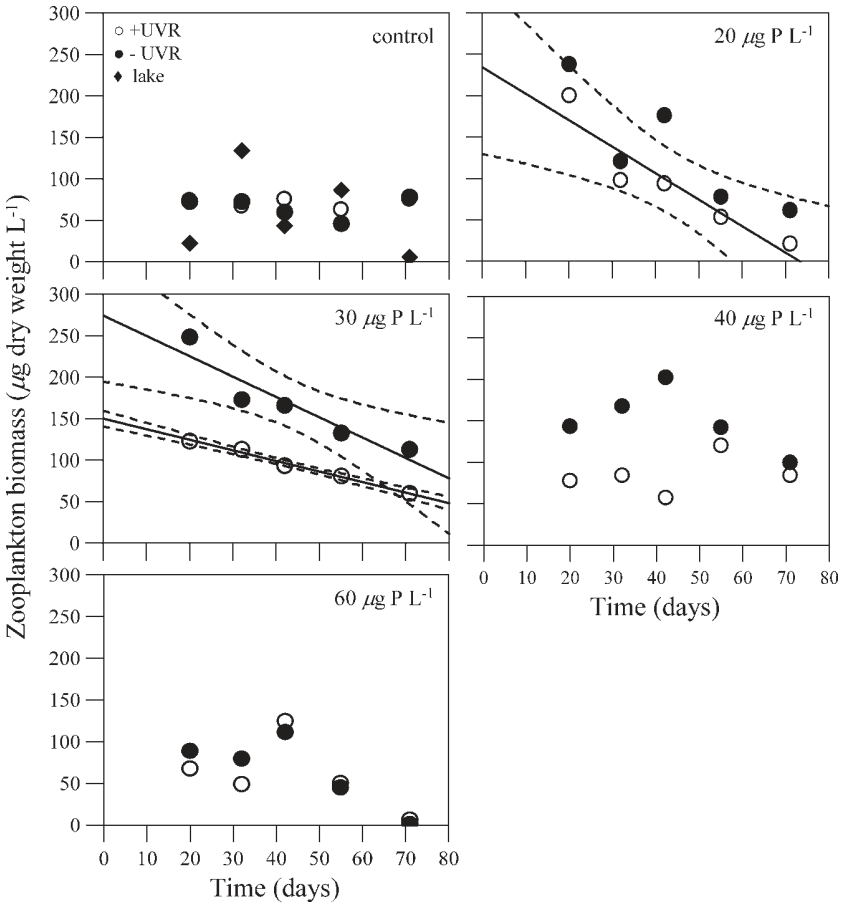


Fig. 2. Relationships between experimental time and zooplankton biomass under +UVR and −UVR for nonenriched (control) and P-enriched (20, 30, 40, and 60 $\mu$g P $L^{-1}$) treatments. Dashed lines indicate 95% confidence intervals around the fitted regression lines (solid lines). Dots represent values for each of the last five sampling days for each light treatment.

The effects of UVR on zooplankton abundance, individual size, and C content were analyzed in order to elucidate the mechanisms responsible for the higher zooplankton biomass accrual in −UVR enclosures at intermediate P-enriched treatments. Mean zooplankton abundance for the last five sampling dates was 27%, 41%, and 61% higher in −UVR vs. +UVR enclosures for the 20 $\mu$g P $L^{-1}$ (*t*-test, $t = -3.81$, df = 4, $p = 0.019$), 30 $\mu$g P $L^{-1}$ (*t*-test, $t = -4.08$, df = 4, $p = 0.015$), and 40 $\mu$g P $L^{-1}$ (*t*-test, $t = -3.25$, df = 4, $p = 0.031$) treatments, respectively (Fig. 3A). However, no UVR-induced size differences were observed (all *t*-tests, $p > 0.05$). The C content of zooplankton was 33% and 29% lower in the absence vs. presence of UVR for the 20 $\mu$g P $L^{-1}$ (*t*-test, $t =$

Table 2. Results of regression analyses of the effect of experimental time (d) ($x$) on zooplankton biomass ($\mu$g dry weight $L^{-1}$) ($y$) for each light treatment at each P-enrichment level ($\mu$g P $L^{-1}$) for the last five sampling days. Regression model was $y=bx+a$. Significant regressions are shown in bold.

| P-enrichment treatment ($\mu$g P $L^{-1}$) | Light treatment | $b$ | $a$ | $R^2$ | $p$ |
|---|---|---|---|---|---|
| 0 | +UVR | −0.01 | 71.43 | 0.001 | 0.953 |
| | −UVR | −0.06 | 68.53 | 0.009 | 0.880 |
| 20 | +UVR | **−3.20** | **234.25** | **0.876** | **0.019** |
| | −UVR | −3.17 | 274.95 | 0.749 | 0.058 |
| 30 | +UVR | **−1.27** | **150.11** | **0.992** | **<0.001** |
| | −UVR | **−2.45** | **274.46** | **0.877** | **0.019** |
| 40 | +UVR | 0.38 | 68.40 | 0.114 | 0.579 |
| | −UVR | −1.02 | 196.18 | 0.291 | 0.349 |
| 60 | +UVR | −1.10 | 108.34 | 0.261 | 0.379 |
| | −UVR | −1.77 | 143.46 | 0.663 | 0.093 |

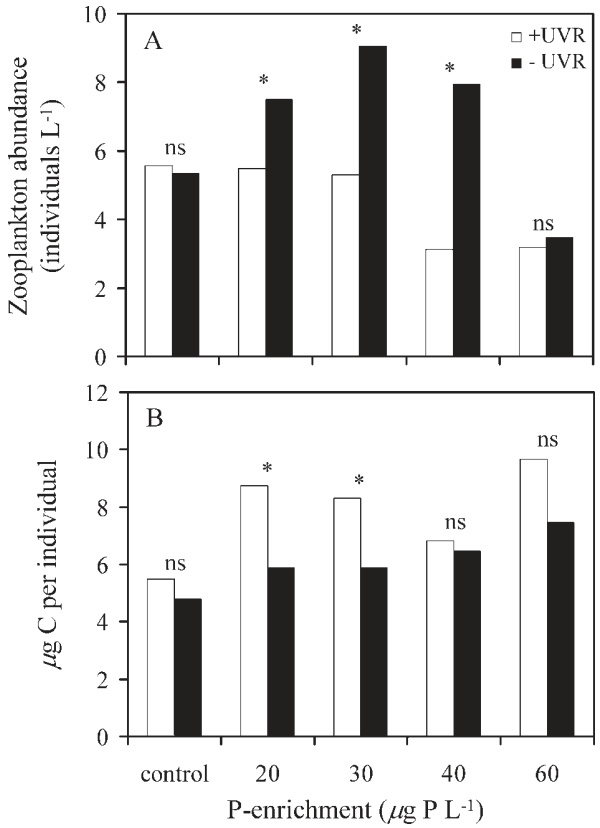


Fig. 3. (A) Zooplankton abundance and (B) C content per individual under +UVR and −UVR treatments in nonenriched (control) and P-enriched (20, 30, 40, and 60 $\mu$g P $L^{-1}$) treatments. Results of paired *t*-test comparisons between light treatments for each phosphorus level: * $p < 0.05$; ** $p < 0.01$; ns, not significant. Values represent the mean for the last five sampling dates.

Table 4. Results of ANCOVA to test the effects of UVR and covariates (P enrichment and total P) on response variables (total P; algal biomass; Chl *a*; sestonic C, N, and P). Units are $\mu$g P $L^{-1}$ for P enrichment and total P; $\mu$g C $L^{-1}$ for algal biomass; and $\mu$g $L^{-1}$ for sestonic C, N, and P. Significant results are shown in bold.

| Response variable | Covariate | Intercept | | Slope | |
|---|---|---|---|---|---|
| | | $F_{1,6}$ | $p$ | $F_{1,6}$ | $p$ |
| Total P | P enrichment | 0.10 | 0.760 | 0.37 | 0.565 |
| Algal biomass | Total P | 1.28 | 0.301 | **9.83** | **0.020** |
| Chl *a* | Total P | 1.87 | 0.221 | **10.24** | **0.018** |
| Sestonic C | Total P | 4.37 | 0.081 | 0.50 | 0.507 |
| Sestonic N | Total P | 6.84 | 0.039 | 0.99 | 0.358 |
| Sestonic P | Total P | 5.18 | 0.063 | 5.24 | 0.062 |

2.89, df = 4, $p = 0.04$) and 30 $\mu$g P $L^{-1}$ (*t*-test, $t = 3.89$, df = 4, $p = 0.018$) enclosures, respectively (Fig. 3B).

*UVR and P-enrichment effects on phyto–zooplankton interface*—A strong relationship was found between P-enrichment level and TP (Table 3), but no systematic differences were detected as a function of light or of the interaction between P enrichment and light (Table 4). We therefore used TP as a predictor of algal and zooplankton biomass. Algal biomass;, Chl *a*; and sestonic C, N, and P were positively correlated with TP in both +UVR and −UVR enclosures (Fig. 4A; Table 3). Although no differences were detected as a result of UVR, we observed significant UVR × P synergistic interactive effects on algal biomass and Chl *a*, but not on sestonic C, N, and P (Table 4). Examination of the UVR × P interaction effect in Fig. 4 showed no differences in algal biomass with the exception of the highest P-enriched level (Fig. 4A; Table 4).

Response of zooplankton biomass to the TP gradient was unimodal for both light treatments (Fig. 4B; Table 3). Differences due to UVR were significant for intermediate P-enriched levels receiving 20, 30, and 40 $\mu$g P $L^{-1}$

Table 3. Results of regression analyses of the effect of P enrichment on total P and of total P on algal biomass; Chl *a*; sestonic C, N, P; and zooplankton biomass in both light treatments (+UVR, −UVR). Units are $\mu$g P $L^{-1}$ for P enrichment and total P; $\mu$g C $L^{-1}$ for algal biomass; $\mu$g $L^{-1}$ for sestonic C, N, and P; and $\mu$g dry weight $L^{-1}$ for zooplankton biomass. Regression model was $y=cx^2+bx+a$. Significant regressions are shown in bold.

| Dependent variable ($y$) | Independent variable ($x$) | Light treatment | $c$ | $b$ | $a$ | $R^2$ | $p$ |
|---|---|---|---|---|---|---|---|
| Total P | P enrichment | +UVR | — | **0.65** | **0.18** | **0.988** | **<0.001** |
| | | −UVR | — | **0.61** | **0.52** | **0.984** | **<0.001** |
| Algal biomass | Total P | +UVR | — | **20.95** | **141.69** | **0.856** | **0.024** |
| | | −UVR | — | **40.11** | **17.20** | **0.978** | **0.001** |
| Chl *a* | Total P | +UVR | — | **2.21** | **8.73** | **0.932** | **0.008** |
| | | −UVR | — | **3.70** | **5.88** | **0.979** | **0.001** |
| Sestonic C | Total P | +UVR | — | **33.79** | **388.76** | **0.770** | **0.050** |
| | | −UVR | — | **45.20** | **387.84** | **0.821** | **0.034** |
| Sestonic N | Total P | +UVR | — | **5.18** | **57.55** | **0.834** | **0.003** |
| | | −UVR | — | **6.85** | **43.20** | **0.941** | **0.006** |
| Sestonic P | Total P | +UVR | — | **0.55** | **1.48** | **0.991** | **<0.001** |
| | | −UVR | — | **0.64** | **0.48** | **0.996** | **<0.001** |
| Zooplankton biomass | Total P | +UVR | **−0.07** | **2.71** | **68.03** | **0.950** | **<0.050** |
| | | −UVR | **−0.32** | **12.28** | **46.10** | **0.990** | **<0.050** |

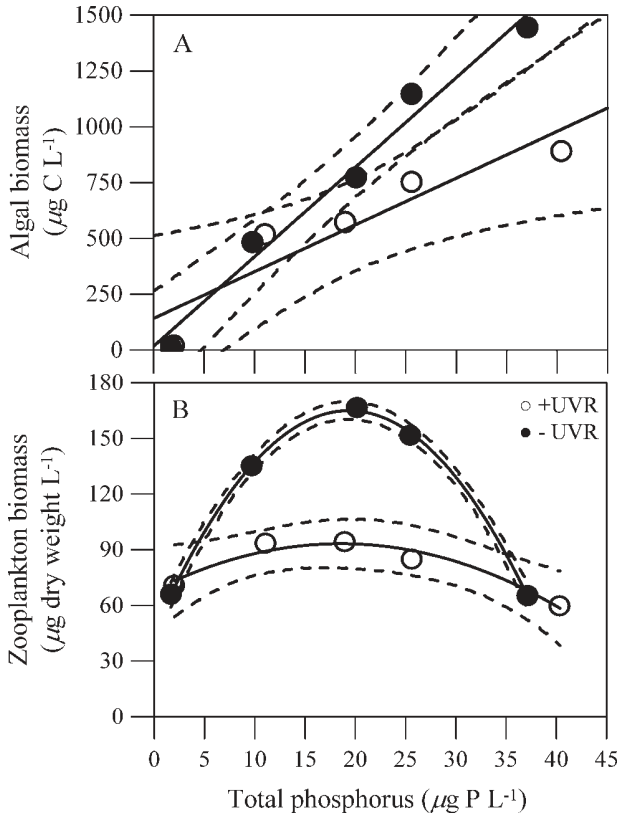


Fig. 4. Response of (A) algal and (B) zooplankton biomass to the total phosphorus trophic gradient under +UVR and −UVR treatments. Dashed lines indicate 95% confidence intervals around the fitted models (solid lines). Values represent the mean for the last five sampling dates.

(Fig. 4B). Mean zooplankton biomass in the 20, 30, and 40 $\mu$g P $L^{-1}$ treatments was 31%, 44%, and 44% lower, respectively in +UVR vs. −UVR enclosures. The unimodal fits of zooplankton biomass were also significant for algal biomass, Chl *a*, and sestonic P (Fig. 5; Table 5).

We then examined whether food quality variables explained the differences observed in zooplankton biomass at intermediate P-enriched levels. Food quality in terms of the sestonic C : N molar ratio ranged from 6.0 to 8.5 in +UVR and from 6.1 to 9.7 in −UVR enclosures. Sestonic C : P ratios ranged from 159.4 to 289.5 in +UVR and from 184.6 to 339.6 in −UVR enclosures (Table 6). The only significant relationships between zooplankton biomass and seston food quality variables were for sestonic C : N in the −UVR treatment and sestonic C : P in the +UVR treatment, which showed positive regression slopes (Table 7).

*Long-term observational study*—Data on aerosol depositions over the past three decades reveal a tendency for an increase in the magnitude and occurrence of these events, especially after 1990. Over this period, there was a more than fivefold increase in TOMS AI ($r = 0.81$, $p < 0.001$; Fig. 6A) and a more than threefold increase in the annual frequency of aerosol deposition events ($r = 0.83$, $p < 0.001$; Fig. 6B), whereas the UV irradiance did not show significant temporal changes ($r = 0.10$, $p > 0.050$; Fig. 6C). The intensity and frequency of aerosol depositions during this time correlated positively with the increase in phytoplankton biomass ($r = 0.78$, $p < 0.05$, *see* Fig 6A inset; $r = 0.86$, $p < 0.01$, *see* Fig. 6B inset, respectively).

To assess whether our experimental results were representative of the natural plankton dynamics of the lake, we plotted phytoplankton and zooplankton biomass over the past three decades (Fig. 6D). The biomass of zooplankton was higher than that of phytoplankton until the beginning of the 1990s, when there was a clear trend to a lower accrual of zooplankton coinciding with the increase in phytoplankton biomass. As a result, zooplankton biomass was unimodally related to phytoplankton ($y = -0.009x^2 + 2.285x$, $p < 0.05$; *see* inset in Fig. 6D).

## Discussion

Our experimental results show that the zooplankton response to increasing nutrients fits a UVR light-specific unimodal curve. Thus, zooplankton proved to be constrained by food at both ends of the nutrient gradient and by UVR at intermediate nutrient inputs. These findings do not support our hypothesis that zooplankton biomass accrual would be increased and PZC strengthened by the higher food quantity and quality from P fertilization and UVR. However, this response of zooplankton is consistent with reports that zooplankton growth, and therefore transfer of energy and nutrients from primary producers to herbivore consumers, is highest at intermediate mesotrophic conditions and decreases toward both ends of the trophic gradient, resembling a unimodal function (Elser et al. 1990; Persson et al. 2007). This decoupling of the predator–prey relationship was recently described in long-term nutrient enrichments in stream ecosystems, reducing the overall food web efficiency (Davis et al. 2010).

In the low seston range, the increase of zooplankton biomass up to an intermediate P-enriched level of 30 $\mu$g P $L^{-1}$ indicates a strong effect of food quantity on zooplankton biomass, in full agreement with the observation that food availability is the dominant constraint on zooplankton growth in poor nutrient conditions (Persson et al. 2007). Thus, the strength of PZC was maximal at intermediate trophic conditions (Fig. 5) but decreased with higher food quantity conditions. In other words, further increases in food quantity not only failed to yield higher zooplankton biomass but in fact had a detrimental effect. The cause of reduced zooplankton performance at high nutrient levels is a question of great interest. It has traditionally been associated with a wide range of negative conditions, including proliferation of inedible algae (Elser et al. 1990; Auer et al. 2004) or decreased food quality in terms of elemental content (Sterner and Elser 2002) or biochemical composition (Brett et al. 2006; Persson et al. 2007). However, phytoplankton in this study was dominated by the edible algae *D. chlorelloides*, and no changes in species composition were observed during the study period (Delgado-Molina et al. 2009). The concentration of other essential biochemicals in seston, including total fatty acids

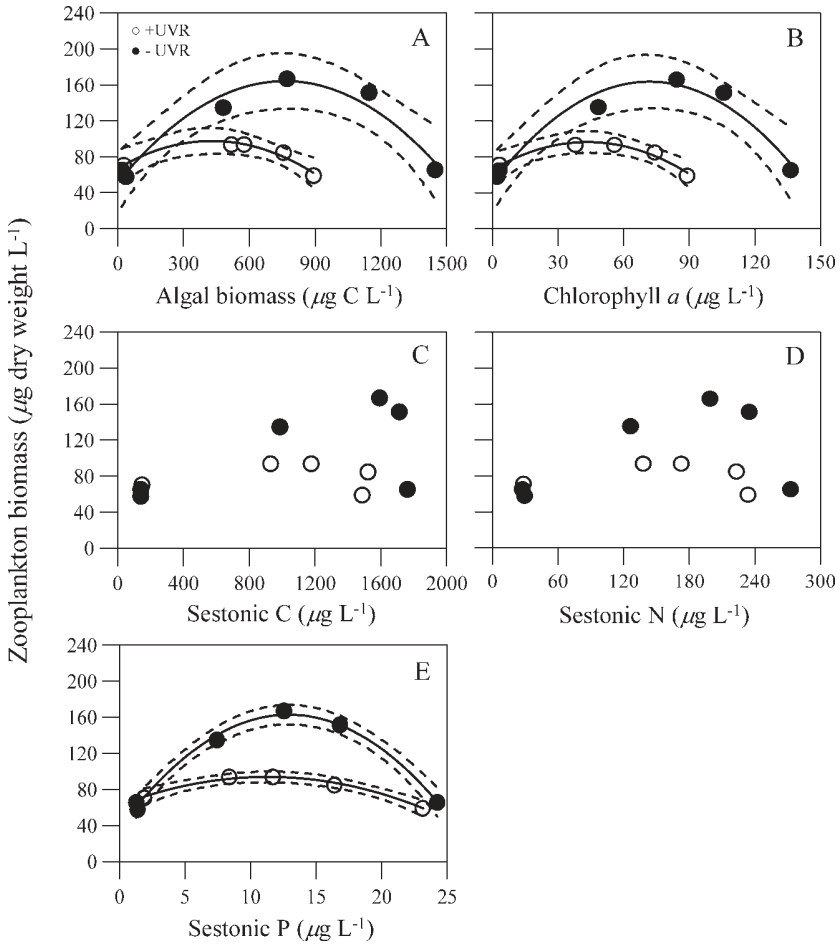


Fig. 5. Response of zooplankton biomass to (A) algal biomass, (B) Chl *a*, (C) sestonic C, (D) sestonic N, and (E) sestonic P under +UVR and −UVR treatments. Dashed lines indicate 95% confidence intervals around the fitted models (solid lines). Values represent the mean for the last five sampling dates.

and $\omega$3-polyunsaturated fatty acids, was also high in P-enriched enclosures (Villar-Argaiz et al. 2009). The decrease in zooplankton biomass observed at the highest end of the trophic gradient may have various explanations. Thus, food "in excess" may have a detrimental effect, since secretions of polysaccharides in high algal populations (Delgado-Molina et al. 2009) may have a clogging effect on copepods by saturating their filtering capacity, as previously observed in large nonedible algae (Gliwicz 2004). An alternative explanation derives from the "stoichiometric knife-edge" hypothesis, which predicts a decline in herbivore performance and therefore biomass accrual from P-

Table 5. Effects of different food quantity predictors on zooplankton biomass under each light treatment. The dependent variable ($y$) is the zooplankton biomass ($\mu$g dry weight $L^{-1}$). Independent variables ($x$) include algal biomass, Chl *a*, sestonic C , sestonic N, and sestonic P. Regression model, $y=cx^2+bx+a$. Significant regressions are shown in bold. ns, not significant.

| Independent variable | Light treatment | $c$ | $b$ | $a$ | $R^2$ | $p$ |
|---|---|---|---|---|---|---|
| Algal biomass ($\mu$g C $L^{-1}$) | +UVR | **−0.00017** | **0.14** | **67.21** | **0.96** | **<0.05** |
| | −UVR | **−0.00019** | **0.29** | **55.52** | **0.94** | **<0.05** |
| Chl *a* ($\mu$g $L^{-1}$) | +UVR | **−0.01643** | **1.43** | **65.56** | **0.96** | **<0.05** |
| | −UVR | **−0.02201** | **3.17** | **49.94** | **0.94** | **<0.05** |
| Sestonic C ($\mu$g C $L^{-1}$) | +UVR | −0.00005 | 0.09 | 59.65 | 0.54 | ns |
| | −UVR | −0.00009 | 0.21 | 34.28 | 0.48 | ns |
| Sestonic N ($\mu$g N $L^{-1}$) | +UVR | −0.00261 | 0.68 | 53.15 | 0.76 | ns |
| | −UVR | −0.00617 | 1.95 | 9.77 | 0.83 | ns |
| Sestonic P ($\mu$g P $L^{-1}$) | +UVR | **−0.24767** | **5.59** | **62.27** | **0.99** | **<0.05** |
| | −UVR | **−0.73859** | **18.94** | **41.02** | **0.99** | **<0.05** |

Table 6. Mean values of sestonic C : N and C : P molar ratios for each light ×P-enrichment treatment.

| P enrichment treatment | Sestonic C : N +UVR | Sestonic C : N −UVR | Sestonic C : P +UVR | Sestonic C : P −UVR |
|---|---|---|---|---|
| 0 | 5.96 | 6.07 | 211.66 | 305.87 |
| 20 | 7.88 | 9.05 | 289.48 | 339.61 |
| 30 | 8.51 | 9.66 | 262.02 | 329.31 |
| 40 | 7.92 | 8.76 | 234.05 | 264.06 |
| 60 | 7.44 | 7.57 | 159.38 | 184.63 |

rich food with a low C : P ratio (Elser et al. 2005). Accordingly, and contrary to long-held beliefs, seston values much lower than the C : N and C : P thresholds proposed by Urabe and Watanabe (1992) (22.5 and 300, respectively) could hamper the performance of zooplankters that have much lower mineral nutrient demands. Our results therefore question the classical hypothesis that "more is better (or at least never worse)" (Boersma and Elser 2006), since they reveal a strong mismatch situation, with high algal biomass and low zooplankton biomass accumulation. On the other hand, these findings are consistent with natural observations in the studied lake that a higher copepod biomass is not promoted by primary producer blooms from strong atmospheric nutrient loads (Villar-Argaiz et al. 2001).

Few studies have considered the effects of increased nutrient availability and hence trophic status together with other potentially relevant environmental factors such as UVR. The present study shows that UVR is a major determinant of PZC, exerting the greatest constraint on zooplankton biomass accumulation at intermediate P-enriched levels. This result implies that UVR effects can prevail against the above-reported effects of food, reducing zooplankton biomass at intermediate food levels. Following this observation of nutrient level–specific UVR damage, a new intriguing question is whether UVR exerts a direct effect on consumer survival or rather an indirect effect on PZC via food quantity or quality regulation.

The absence of differences in food concentrations between UVR treatments, for a given intermediate P-enriched level, implies that food quantity played no role in the UVR-related differences in the biomass of herbivores. We found no major changes in algal taxonomy, and the chlorophyte *D. chlorelloides* dominated all enclosures (Delgado-Molina et al. 2009); therefore variations in food quality cannot be attributed to differences in algal communities. With regard to the elemental content of the algae, sestonic ratios within the range reported for zooplankton in this experiment (C : N = 8–11, unpubl. data from Bullejos et al.; C : P = 269–381, original data from Bullejos et al. 2008) indicate high-quality food for this species. In addition, concomitant analysis of the experimental mesocosms revealed that the fatty acid content was equally high under both UVR regimes in the P-enriched treatments (Villar-Argaiz et al. 2009). Therefore, our results indicate that the parameters of food quality measured had no influence on the negative effect of UVR at intermediate food levels, pointing out some direct deleterious effect of UVR on zooplankton populations. Our results further support that the mechanism behind the detrimental UVR was organism death. However, the large C content per individual under UVR suggests the storage of lipids associated with carotenoid pigmentation (Hylander et al. 2009), which may be interpreted as a tolerance mechanism against UVR-induced stress.

Although copepods have traditionally been regarded as more resistant to UVR than cladocerans (Hylander

Table 7. Effects of different food quality predictors on zooplankton biomass in both light treatments. The dependent variable ($y$) is the zooplankton biomass ($\mu$g dry weight $L^{-1}$). Independent variables ($x$) are sestonic C : N, sestonic C : P, algal C biomass : N, algal C biomass : P, and Chl *a* : sestonic C. Regression model, $y=bx+a$. Significant regressions are shown in bold.

| Independent variable | Light treatment | $b$ | $a$ | $R^2$ | $p$ |
|---|---|---|---|---|---|
| Sestonic C : N | +UVR | 9.87 | 6.28 | 0.401 | 0.251 |
| | −UVR | **30.72** | **−135.78** | **0.826** | **0.033** |
| Sestonic C : P | +UVR | **0.29** | **13.51** | **0.928** | **0.008** |
| | −UVR | 0.41 | −1.26 | 0.295 | 0.344 |
| Algal C biomass : N | +UVR | 3.34 | 69.11 | 0.092 | 0.620 |
| | −UVR | 9.59 | 76.31 | 0.165 | 0.500 |
| Algal C biomass : P | +UVR | 0.22 | 56.75 | 0.462 | 0.207 |
| | −UVR | 0.58 | 37.71 | 0.493 | 0.186 |
| Chl *a* : sestonic C | +UVR | −258.56 | 90.62 | 0.027 | 0.791 |
| | −UVR | 380.38 | 97.57 | 0.021 | 0.817 |

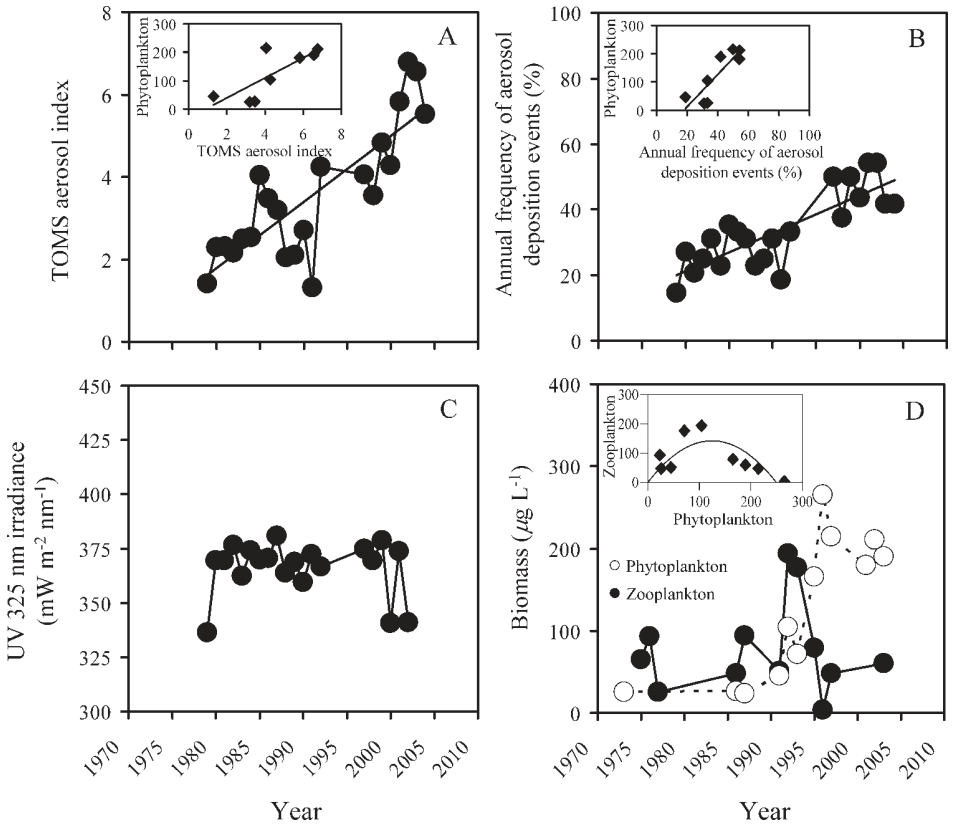


Fig. 6. Interannual trends in (A) TOMS aerosol index, (B) annual frequency of aerosol deposition events, (C) UV 325 nm irradiance, and (D) phytoplankton and zooplankton biomass. Insets represent (A) the relationships of TOMS AI and (B) annual frequency of aerosol deposition events to phytoplankton biomass, and (D) the relationship between phytoplankton and zooplankton biomass in years when mean values were available for both. Lines represent the best fits from least-square regressions. Biomass units are $\mu$g fresh weight $L^{-1}$ for phytoplankton and $\mu$g dry weight $L^{-1}$ for zooplankton.

et al. 2009), they showed strong deleterious UVR effects in this study. Interestingly, the negative effects of UVR were only observed at intermediate food levels, when biomass accumulation was highest. These findings are surprising, since zooplankton can migrate at depth (Rhode et al. 2001) and display antioxidant enzymes (Souza et al. 2010). Moreover, reduced UVR would be expected in P-enriched enclosures as a result of the high algal biomass. Although these counterintuitive results warrant further investigation, they find support in previous studies by Carrillo et al. (2008) in which the deleterious effects of UVR on nutrient limited algae were only observed after moderate nutrient inputs. Regardless of the reasons for these effects of UVR, our results, taken together, imply that the potential benefits of enhanced nutrients linked to moderate aerosol inputs are largely offset by the deleterious effect of UVR.

*Ecological implications*—The long-term record reported in this study, in agreement with future projections for the Mediterranean region (Escudero et al. 2005), indicates an increase in the magnitude and frequency of atmospheric dust aerosols responsible for the increased phytoplankton biomass between 1978 and 2003. These patterns are consistent with previous reports that nutrients from Saharan atmospheric dust deposition are important sources of P, increasing the Chl *a* in these high mountain lakes (Morales-Baquero et al. 2006) and enhancing the primary production of the mixed surface layer of the Western Mediterranean Sea (Ridame and Guieu 2002).

However, the higher phytoplankton biomass was not followed by enhanced zooplankton biomass from the beginning of the 1990s, resulting in decoupled long-term dynamics. This pattern is analogous to our experimental observations of negligible zooplankton biomass augmentation with increasing seston after P pulses applied in the presence of UVR. Our results also match observations of phytoplankton blooms after strong occurrences of atmospheric P inputs in these lakes, which did not translate into higher zooplankton accrual (*see* fig. 6 in Villar-Argaiz et al. 2001). Hence, the combination of greater atmospheric depositions (favoring phytoplankton development) and high UV irradiance (constraining zooplankton growth) in Mediterranean high mountain lakes would not positively

affect the development of herbivores, consequently weakening PZC.

High mountain lakes are climatically sensitive indicators useful in predicting global change (Williamson et al. 2009). Although extrapolation of our experimental results to the full-scale system is limited, since natural conditions are not completely reproduced by mesocosms closed at the bottom (e.g., by preventing nutrient sedimentation and constraining zooplankton migration), both experimental and long-term approaches can help to qualitatively explain the phytoplankton–zooplankton relationship in Lake La Caldera. Thus, the relationships reported here became uncoupled in the presence of UVR in both experimental and long-term observations. However, in the absence of UVR, from low to moderate P enrichments benefited zooplankton performance and reinforced PZC, while stronger P enrichment impaired growth and disrupted PZC. These findings suggest that UVR makes a key contribution to the shape of the phytoplankton–zooplankton relationship. The intensity of the P- and UVR-induced decoupling effect may therefore depend on the magnitude and frequency of the atmospheric inputs, the exposure of lakes to UVR and atmospheric dust depositions, and the specific structure of the zooplankton communities in these high mountain lakes.

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