Submitted to Freshwater Biology as an original Research Article

UV radiation and phosphorus interact to influence the biochemical composition of phytoplankton

Manuel Villar-Argaiz*[1], Juan M. Medina-Sánchez[1], Francisco J. Bullejos[1], José A. Delgado[1], Olga Ruíz Pérez[2], Juan C. Navarro[2] and Presentación Carrillo[3]

[1]Departamento Ecología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain

[2]Instituto de Acuicultura de Torre de la Sal, CSIC, Ribera de Cabanes, Castellón, Spain

[3]Instituto del Agua, Universidad de Granada, 18071 Granada, Spain

Abbreviated title: Effects of UV radiation and phosphorus on phytoplankton composition

Key words: food quality, essential fatty acids, seston, UV radiation, phosphorus

* Author to whom proofs should be sent. E-mail: mvillar@ugr.es. Fax: +34 958 246166

SUMMARY

1. Numerous laboratory studies have shown that food quality is suboptimal for zooplankton growth. However, little is know about how food quality is affected by the interaction of potential global change factors in natural conditions. Using field enclosures in a high altitude Spanish lake, seston was exposed to increasing phosphorus (P) concentrations in the absence and presence of UV radiation (UVR) to test the hypothesis that interactions between these factors affected the biochemical and stoichiometric composition of seston in ways not easily predicted from studies of single factors.
2. Phosphorus enrichment increased the content of total fatty acids (TFA), ω3-polyunsaturated fatty acids (ω3-PUFA) and Chlorophyll a:carbon (Chl a:C) and C:N ratios in seston. The pronounced increase in ω3-PUFA was largely explained by the enhancement of 18:3n-3 (α-linolenic acid, ALA). In contrast, P enrichment lowered the content of highly unsaturated fatty acids (HUFA), the HUFA:PUFA ratio and, at high P loads, seston C:P ratio. Although phytoplankton assemblages dominated by Chlorophytes were not rich in HUFA, seston in the control had substantially higher 20:4n-6 (arachidonic acid, ARA) content (79% of HUFA) than did P-enriched enclosures.
3. UVR increased the content of ω3-PUFA and TFA in seston at the two ends of the trophic gradient generated at ambient and high concentrations of P, but decreased seston C:P and HUFA at all points on this gradient. ARA was not detected in the presence of UVR.
4. The interaction between P and UVR was significant for seston HUFA and C:P ratios, indicating that the effect of UVR in reducing HUFA (decreased food quality) and C:P ratios (enhanced food quality) was most pronounced at the low

nutrient concentrations characteristic of oligotrophic conditions and disappeared as P increased. Therefore, any future increase in UVR fluxes will probably affect most strongly the food quality of algae inhabiting oligotrophic pristine waters although, at least in the Mediterranean region, these effects could be offset by greater deposition of P from the atmosphere.

INTRODUCTION

Light and nutrients are among the most important variables regulating the composition of autotrophs, which in turn affects that of herbivores via the quality of their food (Urabe & Sterner, 1996; Paul & Gwynn-Jones, 2003) and, hence, the efficiency at which energy is transferred through the food web (Sterner & Elser, 2002). Though the role of photosynthetic active radiation (PAR) on autotroph biochemical composition has received considerable attention, the indirect role of UVR is unclear. While there are reports that UVR enhances the food quality of herbivores, many data support the opposite conclusion. For example, decreased C:P ratios (enhanced nutritional quality) have been found after exposing autotrophs to UVR (Xenopoulos *et al.*, 2002; Carrillo *et al.*, 2008), but also in epilithon in the absence of ambient UVR (Watkins *et al.*, 2001). Similarly, the effect of UVR on the quality of algae in terms of fatty acid composition remains unclear. Thus, some studies have reported a decrease in certain polyunsaturated fatty acids (PUFAs) under UVR (Goes *et al.*, 1994; Skerratt *et al.*, 1998), whereas others show no influence or an increase in certain types of PUFA (Skerratt *et al.*, 1998; Leu *et al.*, 2006a).

Perhaps, the species-specific response of biochemical composition to the stress of UVR or the relatively short-term nature of most of these studies underlies these conflicting results. However, other stressors such as nutrient availability (either in shortage or in excess) need also to be considered when studying the impact of UVR on the elemental and biochemical composition of autotrophs (Davison *et al.*, 2007). This is crucial given that the effects of UVR on the growth and somatic content of organisms are related to the availability of limiting mineral nutrients (Medina-Sánchez *et al.*, 2006). In the context of global change, understanding and predicting the simultaneous effects of multiple stressors, such as increasing UVR and nutrient concentrations, on

species and food web interactions is a current challenge for ecosystem research and management. To achieve this goal, experiments that combine multiple stressors in the field are important, as they may have interdependent effects (not easily assessed from single-factor experiments) on food quality at the base of food webs.

Our aim was to examine the joint role of UV radiation and P enrichment in the cellular stoichiometry and biochemical composition (fatty acids and Chl a:C ratio) of autotrophs. Our task was thus, firstly to elucidate the single role of each factor (P or UVR) on the biochemical composition of seston and, secondly, to study their joint effect (i.e. whether their single effects on seston interact). We used relatively long term experiments (over many generations of algae) which may have enabled the beneficial photorepair and/or photoprotective UVR mechanisms that ultimately determined the biochemical composition of algae.

METHODS

Experimental set-up. The experiment was performed using field mesocosms to manipulate the supply of P and light quality (presence and absence of UVR) in La Caldera, an ultraoligotrophic fishless mountain lake in the National Park of Sierra Nevada (Southern Spain, 36º55′–37º15′N, 2º31′–3º40′W; 3050 m a.s.l.). The strong incidence of UV radiation at this latitude and altitude (Carrillo *et al.*, 2002), and the scarcity of nutrients (Villar-Argaiz *et al.*, 2001), make of La Caldera lake an ideal site at which to test the effects of nutrients and sunlight on seston. The experiment started on 1 August 2003. Unscreened lake water was pumped from 5 m depth into ten polyethylene cylindrical enclosures, closed at their lower end, and each with diameter of 1 m, depth of 7 m and volume of 2.7 m^3. Five of the enclosures, with polyethylene lid, received the

full spectrum of solar radiation (+UVR treatment) and the other five (-UVR treatment) were covered with Plexiglas UF3 (Atohaas North America) sheets that blocked direct and refractory exposure to UV radiation. The optical properties of the filters used in the light treatments were verified before the experiments using a double-beam spectrophotometer (Perkin Elmer Lambda 40, Perkin Elmer Corporation, Norwalk, USA). The polyethylene plastic used in the +UVR treatment transmitted 90% of PAR, 60% of UVB and 75% of UVA, while the long-wave-pass Plexiglass transmitted 90% of PAR but completely blocked UV radiation (<390 nm). In both series of enclosures, phosphate (NaH_2PO_4) was added at the start to create an increasing P gradient of four concentrations (20, 30, 40 and 60 μg P L^{-1}), and inorganic nitrogen (NH_4NO_3) was added to give a final N:P molar ratio of 30 according to concentrations of inorganic nutrients (NO_2^-, NO_3^-, NH_4^+ and total dissolved phosphorus, TDP) measured in the lake the day before the experiment. For each light treatment, one enclosure received no phosphate and served as a control to give a two (light) x five (P) factorial design with one replicate of each. Enclosures were sampled periodically (days 1, 3, 10 and 20 of incubations) for soluble reactive phosphorus determinations. After soluble reactive phosphorus (SRP) became depleted on day 20 of incubations, enclosures were sampled three times every two days (days 30, 32 and 34 of incubations) for phytoplankton abundance and seston (suspended particulate matter > 1.0 μm) elemental and biochemical determinations, using a plastic bucket and after gently mixing the entire length of the enclosure (total number of samples per enclosure = 3).

Biological and biochemical analyses. Phytoplankton samples were fixed with Lugol´s solution. A 50-ml aliquot from the phytoplankton was counted at 1000X magnification using an inverted microscope (Leitz Fluovert FS, Leica, Wetzlar, Germany) to estimate cell abundance.

Seston (pre-screened through a 40 μm mesh to remove macrozooplankton) was collected onto pre-combusted (24 h at 500ºC) 1-μm glass-fibre filters (Whatman GF/B) at low pressure (100 mm of Hg). Samples were analysed for C and N using a CHN analyser (Perkin-Elmer Model 2400) or for P content by colorimetric means after persulphate oxidation (APHA, 1992). All C:P ratios were calculated on a molar basis. Filters for Chl a were extracted in acetone and concentrations determined by fluorometry (APHA, 1992). A Chl a standard (Fluka Chlorophyll a from algae) was used to transform the fluorescence data into Chl a concentrations.

Fatty acids in seston were analysed after extraction and transmethylation (Christie, 1982) using a gas chromatograph (Fisons Instruments GC 8000 Series, Thermo Electron Co., Rodano, Italy) equipped with a fused silica open tubular column (Tracer, TR-WAX, Tecknokroma, Spain) and a cold on-column injection system. We defined PUFA as polyunsaturated fatty acids with a chain length of 18 or more carbon atoms, and HUFA as a subset of PUFA molecules with 20 or more atoms of carbon. Units for fatty acids in this study are μg per mg of carbon in the seston.

Statistical analysis. The effects of P enrichment for each light treatment on seston elemental and biochemical variables were assessed by regression analysis of mean values for the three sampling dates against P-enrichment level. It was checked that data fulfilled criteria for regression analysis. When no linearity was observed, differences between each level of P enrichment and the control with no P added were assessed by dependent paired *t*-tests. For the effects of UVR, differences in biochemical and elemental composition at each P level were assessed by dependent paired *t*-tests. For the analysis of the interactive UVR x P effects on seston composition ANOVA could not be performed, due to the unreplicated design of the experiment. Alternatively, interactive UVR x P effects on food quality variables were specifically tested depending

on whether variables adjusted or not to linear trends when regressed against P-enrichment. When regressions were significant for the two light treatments, effects of UVR were tested by analysis of covariance. Statistically different slopes were indicative of an interaction between UVR and P, whereas homogeneous slopes indicated no interaction between UVR and P. In contrast, when no linearity was observed, analysis of covariance was precluded, and seston variables were relativized to the control (dividing the observation for each P level in +UVR by the respective control treatment replicate in –UVR) and regressed against P-enrichment. The existence of a linear trend between the UVR-relativized variable across the P gradient would indicate a significant UVR x P effect on the biochemical constituent. To aid in the visualization of these results, and regardless of the method used to test for interactive effects, variables were relativized to the control before being represented graphically. The statistical analyses were performed using Statistica 7.0 for Windows software (StatSoft, 1997).

RESULTS

The abundance of phytoplankton in the control enclosures without added phosphorus (Fig. 1) was within the range of those previously reported for lake La Caldera (Villar-Argaiz *et al.*, 2001). Algal growth is strongly limited by phosphorus in this lake, as reflected by the low concentration of soluble reactive phosphorus (SRP) in lake water (0.34-2.86 μgP L^{-1}) during the experiment and the dissolved inorganic nitrogen : total phosphorus ratio (DIN:TP) between 61 of 12 (Villar-Argaiz *et al.*, 2002), largely higher than the threshold of 12 proposed for P limitation by Morris & Lewis (1988). P enrichment enhanced phytoplankton abundance by two orders of magnitude (Fig. 1) and the Chlorophyta *Dyctiosphaerium chlorelloides* (Nauman;

Komárek & Perman) dominated in all experimental conditions. Variations in algal biochemical or stoichiometric content should, therefore, not be attributed to differences in algal communities among enclosures. Sampling for seston biochemical and elemental determinations was initiated after P (measured as SRP) became depleted after thirty days of incubation in the most enriched enclosures (Fig. 2) and clear differences in seston C:P ratio were detected.

Algal fatty acid content was more sensitive to P manipulation than to UVR (Fig. 3). Thus, P increased the fatty acid content linearly in terms of TFA and ω3-PUFA (Fig. 3a, c). The strength of this stimulation, however, varied between constituents, being much more pronounced for the ω3-PUFA [as shown by the over eight fold increase relative to the control (Fig. 3c) or the high slope of the regression between ω3-PUFA and P enrichment (Table 1)]. For example, the absolute content of ω3-PUFA increased from 14 to 124 μgFA mgC^{-1} in +UVR enclosures and from 7 to 81 μgFA mgC^{-1} in the – UVR enclosures (data not shown). The strongest increases in FAs as a consequence of the enrichment with P were found in the concentrations of ω3-PUFA 18:3n-3 (α-linolenic acid, ALA) and 18:2n-6 (linoleic acid, LIN) which were 249-809% and 37-123% higher in the enriched treatments, respectively (Table 2; Fig. 4). The most abundant FA, however, was the saturated fatty acid (SFA) 16:0 that accounted for >25% of TFA in all enclosures or up to 123 μgFA mgC^{-1} in the 60 μgP L^{-1}enclosure (Fig. 4). The SFA 18:0 was found in high concentrations in the control treatments, although it decreased after the addition of P and particularly in the 20 μgP L^{-1} and 30 μgP L^{-1} enclosures.

Most seston FAs (16:0, 16:4, 18:1n-9, 18:2n-6 and 18:3n-3) were also significantly enhanced by UVR although to a lesser extent than by P, particularly at the ends of the P gradient, i.e. in the controls with no P added and the most enriched

enclosures (Fig. 4; Table 3). The strongest differences between UVR treatments were found in the concentrations of 18:1n-9 and 18:2n-6, which were over 200% higher in +UVR relative to –UVR in the 60 μgP L^{-1} enclosures (Table 3; Fig. 4). In contrast, HUFA was negatively affected by UVR and the addition of P. Thus, arachidonic acid (ARA [20:4n6]) reached 79% of HUFA and >5% of TFA only in the control in –UVR, but was not detected in the presence of UVR or after the addition of P (Fig. 4). Such a decline, together with the general increase in ω3-PUFA (Fig. 3c), was responsible for the strong decline in the HUFA:PUFA ratios with P (all *t*-test, $P < 0.01$; Table 2; Fig. 3e). Significant UVR x P effects were found on HUFA content (slope of the regression line: $F_{1, 6} = 7.87$, $P = 0.031$; Table 4). These effects were antagonistic, with a diminished effect of UVR as the enrichment with P increased (Fig. 3b).

Phosphorus enrichment did not cause a decrease in seston C:P at a linear rate in +UVR enclosures (Table 1; Fig. 5a). The relatively low C:P of 273 in the control and the post-bloom depletion of P in the least P-enriched enclosures were responsible for this result. Thus, seston C:P ratio was 360 in the 20 μgP L^{-1} (~30% higher relative to the control), decreased progressively with increasing P concentration, and achieved values below the control of 180 exclusively in the 60 μgP L^{-1} enclosure (Table 2; Fig. 5; note that only relativized response variables are shown). In contrast, UVR reduced mean seston C:P in all treatments and, particularly, in enclosures receiving ≤ 30 μgP L^{-1}, where seston C:P in the control was 34% lower in the +UVR enclosure than in the –UVR enclosure (Table 3; Fig. 5b). In addition, UVR and P showed an interactive effect on seston C:P (Table 4) which resulted in a reduced effect of UVR decreasing seston C:P at high P concentrations (Fig. 5b). Phosphorus and UVR effects on P cell quota differed. While P enrichment strongly decreased P cell quota (Table 3; Fig. 5c), the effect of UVR on P cell quota was significant and positive only in the control with no P

added (Table 3; Fig. 5d). As a consequence, a significant UVR x P effect was found on P cell quota indicating a stimulatory effect of UVR on P uptake that attenuated at high P enrichment levels (Table 4).

Seston C:N ratios generally increased due to enrichment with P, and only remained unaltered relative to the control in the 60 μgP L^{-1} (Fig. 5e; Table 2). Also, seston C:N was not affected by UVR, except for the 20 μgP L^{-1} enclosure where this ratio was lower under UVR (from 9.9 ± 0.1 to 8.7 ± 0.3 in –UVR vs. +UVR enclosures; Table 3; Fig. 5f).

Chl a:C ratios strongly increased across the P gradient (Fig. 5g; Table 1), and was generally higher in the enclosures receiving UVR (Fig. 5h), and particularly in the control where UVR increased mean Chl a:C by >25% (Table 3).

DISCUSSION

In this study we examined how a prolonged exposure to UVR affected phytoplankton biochemical and elemental composition with increasing pulses of phosphorus, the latter resembling natural inputs to this lake carried by winds from the Sahara (Morales-Baquero *et al.*, 2006). Our results contribute to our knowledge of the simultaneous effects of multiple stressors on the biochemical composition of primary producers. We found that P and UVR are important determinants of seston elemental and biochemical composition. While P was the main driver for most seston biochemical parameters, however, the precise effect of UVR depended strongly on the availability of P. Thus, consistently with an interactive UVR x P effect, the role of UVR in reducing HUFA and C:P ratios was most pronounced under oligotrophic conditions but vanished as P concentration increased.

Single and interactive P x UVR effects on fatty acid composition

A seston fatty acid profile dominated by palmitic acid (16:0), as well as by the monounsaturated oleic acid (18:1n-9) and α-linolenic acid (18:3n-3), agrees well with expectations for a Chlorophyte dominated phytoplankton community (Brett *et al.*, 2006). However, seston fatty acid composition varied as a consequence of experimental manipulation. Thus, we found strong effects of P enrichment on algal fatty acid composition, whereas the effects of UVR *per se* and its interaction with P were generally less pronounced. Notably, ω3-PUFA increased eight-fold in response to P enrichment which corroborates observations for high arctic systems (Elser *et al.*, 2001; Leu *et al.*, 2006b). In particular, the higher concentration of ω3-PUFA was largely explained by the synthesis of C18 PUFAs and, particularly, of ALA. As discussed by Leu *et al.* 2006a, the pathway for the biosynthesis of C18 PUFAs starts at the SFA 18:0. Probably the enrichment with P caused the activation of this biosynthetic pathway towards the formation of 18C PUFAs (after the insertion of double bonds) at the expenses of the precursor 18:0.

Interestingly, the content of ω3-PUFA was also enhanced by UVR, although only at the ends of the experimental P gradient. Similar increments in ω3-PUFA due to UVR have been reported previously, but observations have not always being consistent among studies (Goes *et al.*, 1994; Skerratt *et al.*, 1998). Although the proximate reasons for the increase in PUFA synthesis due to UVR are not obvious, it has been suggested that UVR could induce gene expression of desaturase enzymes responsible of the biosynthesis of C18 PUFAs (Leu *et al.*, 2006a). Ultimately, the increase in structural lipids could indicate enhanced cell growth and metabolism, benefiting the acclimation of UVR-stressed cells.

The fact that the HUFA ARA was found in substantial amounts in the control enclosure with no UVR, but disappeared when enclosures were P enriched, agrees with previous findings reporting an active accumulation of carbon in the form of ARA deposited in triacylglycerols under nutrient deprivation in unicellular chlorophytes (Merzlyak *et al.*, 2007). Further, it is generally accepted that algae acclimate to stressful environments by alteration of their lipid composition and some species actively synthesize triacylglycerols (TGA) as an efficient carbon sink (Guschina & Harwood, 2006). The inhibition of ARA by UVR in the control enclosure can be explained by the detrimental effect of UVR in peroxidating HUFA rather than the more resistant short-chain PUFAs (Girotti, 2001). This view is supported by the finding of a higher content of 16:0, 16:4 or 18:1n-9 in the control in +UVR relative to –UVR enclosures.

As expected, the finding of an interactive antagonistic effect of UVR and P on the content of HUFA indicated that the detrimental effect of UVR in reducing HUFA and HUFA:PUFA was more intense at low P concentration. This is characteristic of oligotrophic conditions, under which UVR can penetrate deeper before extinction and less algae-shading effect is expected.

Single and interactive P x UVR effects on stoichiometric composition

The finding of higher seston C:P ratios in the low P enrichment enclosures relative to the control after thirty days of incubations was not surprising given: (1) the intensive depletion of P in P-enriched enclosures after the algal bloom (as reflected by the mark reduction in P cell quota; Fig. 5c), and (2) the low seston C:P ratio in the unenriched +UVR treatment, which resembles those of lake La Caldera under natural conditions (181 and 115 are average seston C:P ratios for 30 samples dates during 1996 and 1997; data from (Villar-Argaiz *et al.*, 2002). The results of Xenopoulos *et al.* (2002)

and, more recently, Carrillo *et al.* (2008) and Hessen *et al.* (2008) suggest that UVR has a key role in reducing seston C:P ratios. The finding in this study of a higher P cell quota in the control in +UVR relative to –UVR is consistent with previous work that relates the decrease in seston C:P in the presence of UVR with the increased P acquisition by algae (Leu *et al.*, 2006a; Carrillo *et al.*, 2008, Hessen *et al.*, 2008), although enhanced photosynthetic carbon release by algae can also have an important effect (Carrillo *et al.* 2008). The extra demands for P could be due to the high energetic cost (and consequently demand for ATP) associated with the bioconversion of ALA and other PUFAs to HUFA through the elongation and desaturation processes (Kanawaza *et al.*, 1979; Brett & Müller-Navarra, 1997), or with other beneficial photo-mediated mechanisms such as nucleotide repair or enhanced protein biosynthesis (Hessen *et al.*, 2008). Together these observations are in line with previous work reporting that phytoplankton sensitivity to UVR is modulated by their P nutritional deficiency (Aubriot *et al.*, 2004).

As for HUFA, the fact that the effect of UVR in decreasing seston C:P was manifest at low P concentration and vanished as P load increased implied an interactive UVR x P effect, a result previously shown in three of five bioassays performed in two lakes at the Experimental Lake Area in Canada (Xenopoulos *et al.*, 2002). Therefore, P and UVR affected phytoplankton elemental composition by simultaneously decreasing C:P ratios (at least at the high P loads in this study). Thus, UVR might be of prime importance for phytoplankton elemental and biochemical composition in pristine waters where UVR penetrates more deeply (Fleischmann, 1989), and could help to explain deviations from the Redfield ratio towards lower C:P (Medina-Sanchez *et al.*, 2006).

Single and interactive P x UVR effects on Chl a:C ratio

Our observation that Chl a:C ratio strongly increased across the P gradient is supported by previous findings for green algae cultures (Hessen *et al*., 2002). Further, UVR enhanced Chl a:C ratios, particularly in the controls, a result consistent with observations of strong C:Chl a disruptions caused by UVR (Xenopoulos *et al.*, 2002). Previous work has clearly shown that chlorophyll and carbon content depends strongly on the growth of the autotroph concerned (Healey & Hendzel, 1979), for which solar radiation and nutrients are essential factors. Therefore, responses to UVR and P could lead to multiple effects on photosynthetic C fixation/release (Carrillo *et al.*, 2008) and pigment synthesis/photolysis processes, by operating at different rates, could lead to variable Chl a:C ratios. It is logical to assume that a high Chl a per unit C is characteristic of healthy algae and makes then a better food quality for consumers. Although chlorophyll is chiefly used as an indicator of food quantity, the pronounced changes in Chl a:C observed in this study suggest that this factor may have pronounced consequences for zooplankton growth.

Implications for food quality

It is widely accepted that food quantity places the largest constraint on the growth of zooplankters in oligotrophic systems, and that food quality is more important as systems become more productive (Sterner, 1997; Persson *et al.*, 2007), but see Becker & Boersma (2003). This indeed might be the case in the present experiment, where food was well below the incipient limiting level (ILL) for most zooplankters in control enclosures, but well above these thresholds (Villar-Argaiz *et al.*, 2002) in the enriched enclosures.

With respect to the biochemical composition of seston, the strong synthesis of ALA due to P enrichment may have implications for food quality, due to the known

ability of herbivores to convert this fatty acid to long-chain polyunsaturated fatty acids (HUFAs) through elongation and desaturation processes (Brett & Müller-Navarra, 1997). Further, the relevance of ALA content in seston for zooplankton growth has been suggested based on field correlations between seston and zooplankton FA content (Sushchik *et al.*, 2003) or bioassays using natural seston or ALA-enriched algae as a food source for zooplankton growth (Wacker & Von Elert, 2001; Park *et al.*, 2003; Von Elert, 2002). However, simply because P and also UVR stimulated the content of ω3-PUFA, it may not be safe to conclude that these factors enhance food quality for zooplankton (Leu *et al.*, 2006a). In fact, our results indicate that the addition of P was detrimental to the content of HUFA, a well known essential group of biochemicals in animals (Müller-Navarra *et al.*, 2000). This is consistent with observations by Müller-Navarra *et al.* (2004) of more nutritious algae for zooplankton at low P concentration, due to their higher HUFA content per unit carbon.

The question then rises as to which factor exerts the most relevant role determining food quality for herbivore consumers? Whether changes in the potential food quality of autotrophs, due to the simultaneous effect of P and UVR, add or offset each other is not a trivial question. For example, the beneficial effect of UVR or P in increasing ω3-PUFA and decreasing C:P could be offset by the detrimental effect of UVR peroxidating HUFA. Further, the diversity of potentially limiting substances to zooplankton is still highly debated (Sterner & Schulz, 1998; Ferrao *et al.*, 2007), and particularly since different zooplankton taxa might be limited by different characteristics of the food (Boersma & Stelzer, 2000). The strength of this food limitation may also vary greatly with zooplankton ontogeny (DeMott *et al.*, 2001) and lake trophic state (Persson *et al.*, 2007). Finally, there is covariation among seston food quality variables, making discrimination among the key food traits responsible for

zooplankton nutritional limitation in nature far from straightforward. For example, a tight correlation between Chl a:C and ω3-PUFA ($r^2 = 0.82$, $P < 0.001$, $df = 1, 8$) was found in this study.

In summary, P addition had a positive effect on the nutritional quality of algae in terms of TFA, ω3-PUFA and Chl a:C ratios, negative effects on HUFA, HUFA:PUFA and C:N ratios, and effects on seston C:P that were specific to the concentration of P. Considering the magnitude of the responses and the ability of zooplankton to convert ω3-PUFA to HUFA, we suggest that the strong stimulation of ω3-PUFA may be the most relevant enrichment effect improving the nutritional quality of seston as food source for consumers in this study. The response to UVR depended, however, on the degree of P enrichment. Overall, UVR had minor effects enhancing food quality in terms of PUFA at both ends of the P gradient applied, but adversely affected HUFA content by lowering, as a consequence, the HUFA:PUFA ratio. Nonetheless, this potentially detrimental effect of UVR could be counteracted by the simultaneously beneficial effect of decreasing seston C:P.

Global change has strong effects, not only on the amount of UV reaching the Earth (Shindell *et al.*, 1998; McKenzie *et al.*, 2007) including mid-latitudes (Seckmeyer & McKenzie, 1992; KeilJakson & Hort, 2007), but also on the frequency and intensity of P inputs from the atmosphere reaching the Mediterranean region (Morales-Baquero *et al.*, 2006). Our results show interdependent and contradictory effects of UVR and P on the quality of algae for herbivore consumers. UVR has a predominant role in lowering seston C:P ratios (enhanced food quality) and HUFA content (decreased food quality) in low nutrient waters, but this effect vanishes as P load increases. This, in addition to food quantitative effects, could have consequences for the efficiency with which mass is transferred up the food web, particularly in highly oligotrophic systems.

Acknowledgments

The authors would like to thank Dag Hessen and an anonymous reviewer for their valuable comments on the manuscript. We would also like to thank Alan Hildrew for editing the manuscript. This research was supported by the Spanish Ministries of Environment (PN2003 / 25), Education and Science (GL2005 / 01564 / BOS) and Foreign Affairs and Cooperation (AECI 0562), and two Spanish government FPU grants to FJB and JADM. We are particularly grateful to the staff of Sierra Nevada National Park for their help in the field and permission to work in the National Park.

References

APHA (1992) *Standard Methods for the Examination of Water and Wastewater.,* 18th edn American Public Health Association, Washington, DC.

Aubriot L., Conde D., Bonilla S. & Sommaruga R. (2004) Phosphate uptake behavior of natural phytoplankton during exposure to solar ultraviolet radiation in a shallow coastal lagoon. *Marine Biology*, **144,** 623–631.

Becker C. & Boersma M. (2003) Resource quality effects on life histories of *Daphnia*. *Limnology and Oceanography*, **48,** 700–706.

Boersma M. & Stelzer C.P. (2000) Response of a zooplankton community to the addition of unsaturated fatty acids: an enclosure study. *Freshwater Biology*, **45,** 179–188.

Brett M.T. & Müller-Navarra D.C. (1997) The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biology*, **38,** 483–499.

Brett M.T., Müller-Navarra D.C., Ballantyne A.P., Ravet J.L. & Goldman, C.R. (2006) *Daphnia* fatty acid composition reflects that of their diet. *Limnology and Oceanography*, **51,** 2428–2437.

Carrillo P., Delgado-Molina J.A., Medina-Sánchez J.M., Bullejos F.J. & Villar-Argaiz M. (2008) Phosphorus inputs unmask negative effects of ultraviolet radiation on algae in a high mountain lake. *Global Change Biology*, **14,** 423–439.

Carrillo P., Medina-Sánchez J.M. & Villar-Argaiz M. (2002) The interaction of phytoplankton and bacteria in a high mountain lake: Importance of the spectral composition of solar radiation. *Limnology and Oceanography*, **47,** 1294–1306.

Christie W.W. (1982) A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. *Journal of Lipid Reseach*, **23**, 1072–1075.

Davison I.R., Jordan T.L., Fegley J.C. & Grobe C.W. (2007) Response of *Laminaria saccharina* (phaeophyta) growth and photosynthesis to simultaneous ultraviolet radiation and nitrogen limitation. *Journal of Phycology*, **43,** 636–646.

DeMott W.R., Gulati R.D. & Van Donk E. (2001) *Daphnia* food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. *Limnology and Oceanography*, **46,** 2054–2060.

Elser J.J., Hayakawa K. & Urabe J. (2001) Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. *Ecology*, **82,** 898–903.

Ferrao A.D., Tessier A.J. & DeMott W.R. (2007) Sensitivity of herbivorous zooplankton to phosphorus-deficient diets: Testing stoichiometric theory and the growth rate hypothesis. *Limnology and Oceanography*, **52,** 407–415.

Fleischmann E.M. (1989) The measurement and penetration of ultraviolet radiation into tropical marine water. *Limnology and Oceanography*, **34,** 1623–1162.

Girotti A.W. (2001) Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. *Journal of Photochemistry and Photobiology B-Biology*, **63,** 103–113.

Goes J.I., Handa N., Taguchi S. & Hama T. (1994) Effect of UV-B radiation on the fatty acid composition of the marine phytoplankton *Tetraselmis* sp. - relationship to cellular pigments. *Marine Ecology Progress Series*, **114,** 259–274.

Guschina I.A. & Harwood J.L. (2006) Lipids and lipid metabolism in eukaryotic algae. *Progress in Lipid Research*, **45,** 160–186.

Gutseit K., Berglund O. & Graneli W. (2007) Food quality for *Daphnia* in humic and clear water lakes. *Freshwater Biology*, **52,** 344–356.

Healey F.P. & Hendzel L.L. (1979) Indicators of phosphorus and nitrogen deficiency in five algae in culture. *Canadian Journal of Fisheries and Aquatic Sciences*, **36,** 1364–1369.

Hessen D.O., Faerovig P.J. & Andersen T. (2002) Light, nutrients, and P : C ratios in algae: Grazer performance related to food quality and quantity. *Ecology*, **83,** 1886–1898.

Hessen D.O., Leu E., Faerovig P.J. & Petersen S.F. (2008) Light and spectral properties as determinants of C:N:P-ratios in phytoplankton. *Deep-Sea Research II,* doi:10.1016/j.dsr2.2008.05.013.

Kanawaza A., Teshima S. & Ono K. (1979) Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. *Comparative Biochemistry and Physiology B-Physiology*, **63,** 295–298.

Keil M., Jakson D.R. & Hort M.C. (2007) The January 2006 low ozone event over the UK 2006. *Atmospheric Chemistry and Physics*, **7,** 961–971.

Leu E., Faerovig P.J. & Hessen D.O. (2006a) UV effects on stoichiometry and PUFAs of *Selenastrum capricornutum* and their consequences for the grazer *Daphnia magna*. *Freshwater Biology*, **51,** 2296–2308.

Leu E., Falk-Petersen S., Kwasniewski S., Wulff A., Edvardsen K. & Hessen D.O. (2006b) Fatty acid dynamics during the spring bloom in a High Arctic fjord: importance of abiotic factors versus community changes. *Canadian Journal of Fisheries and Aquatic Sciences*, **63,** 2760–2779.

Mckenzie R.L., Aucamp P.J., Bais A.F., Björn L.O. & Ilyas M. (2007) Changes in biologically-active ultraviolet radiation reaching the Earth's surface. *Photochemical and Photobiological Sciences*, **6,** 218–231.

Medina-Sánchez J.M., Villar-Argaiz M. & Carrillo P. (2006) Solar radiation-nutrient interaction enhances the resource and predation algal control on bacterioplankton: A short-term experimental study. *Limnology and Oceanography*, **51,** 913–924.

Merzlyak M.N., Chivkunova O.B., Gorelova O.A., Reshetnikova I.V., Solovchenko A.E., Khozin-Goldberg I. & Cohen Z. (2007) Effect of nitrogen starvation on optical properties, pigments, and arachidonic acid content of the unicellular green alga *Parietochloris incisa* (Trebouxiophyceae, Chlorophyta). *Journal of Phycology*, **43,** 833–843.

Morales-Baquero R., Pulido-Villena E. & Reche I. (2006) Atmospheric inputs of phosphorus and nitrogen to the southwest Mediterranean region: biochemical response of high mountain lakes. *Limnology and Oceanography*, **51**, 830–837.

Morris D.P. & Lewis W.M. (1988) Phytoplankton nutrient limitation in Colorado mountain lakes. *Freshwater Biology*, **20**, 315–327.

Müller-Navarra D.C., Brett M.T., Liston A.M. & Goldman C.R. (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, **403,** 74–77.

Müller-Navarra D.C., Brett M.T., Park S., Chandra S., Ballantyne A.P., Zorita E. & Goldman C.R. (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature*, **427**, 69–72.

Park S., Müller-Navarra D.C. & Goldman C.R. (2003) Seston essential fatty acids and carbon to phosphorus ratios as predictors for *Daphnia pulex* dynamics in a large reservoir, Lake Berryessa. *Hydrobiologia*, **505,** 171–178.

Paul N.D. & Gwynn-Jones D. (2003) Ecological roles of solar UV radiation: towards an integrated approach. *Trends in Ecology and Evolution*, **18,** 48–55.

Persson J., Brett M.T., Vrede T. & Ravet J.L. (2007) Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (*Daphnia*) in pelagic freshwater food webs. *Oikos*, **116,** 1152–1163.

Seckmeyer G. & Mckenzie R.L. (1992) Increased ultraviolet radiation in New Zealand (45º S) relative to Germany (48º N). *Nature*, **359,** 135–137.

Shindell D.T., Rind D. & Lonergan P. (1998) Increased polar stratospheric ozone losses and delayed eventual recovery owing to increasing greenhouse-gas concentrations. *Nature*, **392,** 589–592.

Skerratt J.H., Davidson A.D., Nichols P.D. & McMeekin T.A. (1998) Effect of UV-B on lipid content of three Antarctic marine phytoplankton. *Phytochemistry*, **49,** 999–1007.

Statsoft I. (1997) *Statistica for windows,* Release 7.1, Tulsa, OK, U.S.A.

Sterner R.W. (1997) Modelling interactions of food quality and quantity in homeostatic consumers. *Freshwater Biology*, **38,** 473–481.

Sterner R.W. & Elser J.J. (2002) *Ecological stoichiometry: The biology of elements from molecules to the biosphere,* Princeton University Press, Princeton, NJ, U.S.A.

Sterner R.W. & Schulz K.L. (1998) Zooplankton nutrition: recent progress and a reality check. *Aquatic Ecology*, **32,** 261–279.

Sushchik N.N., Gladyshev M.I., Kalachova G.S., Kravchuk E.S., Dubovskaya O.P. & Ivanova E.A. (2003) Particulate fatty acids in two small Siberian reservoirs dominated by different groups of phytoplankton. *Freshwater Biology*, **48,** 394–403.

Urabe J. & Sterner R.W. (1996) Regulation of herbivore growth by the balance of light and nutrients. *Proceedings of the National Academy of Sciences of the United States of America*, **93,** 8465–8469.

Villar-Argaiz M., Medina-Sánchez J.M. & Carrillo P. (2002) Interannual changes in the C:N:P ratios of seston and zooplankton of a high mountain lake in Sierra Nevada, Spain. *Water, Air and Soil Pollution Focus*, **2,** 359–378.

Villar-Argaiz M., Medina-Sánchez J.M., Cruz-Pizarro L. & Carrillo P. (2001) Inter- and intra-annual variability in the phytoplankton community of a high mountain lake: the influence of external (atmospheric) and internal (recycled) sources of phosphorus. *Freshwater Biology*, **46,** 1017–1034.

Von Elert E. (2002) Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. *Limnology & Oceanography*, **47,** 1764–1773.

Wacker A. & Von Elert E. (2001) Polyunsaturated fatty acids: Evidence for non-substitutable biochemical resources in *Daphnia galeata*. *Ecology*, **82,** 2507–2520.

Watkins E.M., Schindler D.W., Turner M.A. & Findlay D. (2001) Effects of solar ultraviolet radiation on epilithic metabolism, and nutrient and community composition in a clear-water boreal lake. *Canadian Journal of Fisheries and Aquatic Sciences*, **58,** 2059–2070.

Xenopoulos M.A., Frost P.C. & Elser J.J. (2002) Joint effects of UV radiation and phosphorus supply on algal growth rate and elemental composition. *Ecology*, **83,** 423–435.

Table 1. Effects of P enrichment, tested via regression analysis, on the biochemical and elemental composition of seston for each light treatment. Significant predictors are shown in bold. TFA, total fatty acids; SFA, saturated fatty acids; ω3-PUFA, ω3-polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids.

		Linear regression			
		Slope	Y-intercept	r^2	*P*
TFA (μgFA mgC^{-1})	+UVR	**3.63**	**204.21**	**0.775**	**0.046**
	-UVR	1.29	196.91	0.335	0.306
SFA (μgFA mgC^{-1})	+UVR	0.743	112.71	0.212	0.435
	-UVR	-0.30	110.31	0.269	0.370
*ω*3-PUFA (μgFA mgC^{-1})	+UVR	**2.00**	**13.81**	**0.932**	**0.008**
	-UVR	**1.351**	**16.120**	**0.791**	**0.044**
HUFA (μgFA mgC^{-1})	+UVR	**-0.05**	**6.847**	**0.792**	**0.043**
	-UVR	**-0.15**	**11.26**	**0.888**	**0.016**
HUFA : PUFA	+UVR	-0.318	17.476	0.719	0.069
	-UVR	-0.754	37.543	0.653	0.098
C : P	+UVR	-1.822	338.03	0.363	0.283
	-UVR	**-3.929**	**452.18**	**0.886**	**0.017**
pgP per cell	+UVR	-0.003	0.166	0.499	0.182
	-UVR	-0.002	0.158	0.489	0.189
C : N	+UVR	0.007	7.551	0.023	0.809
	-UVR	0.012	7.752	0.033	0.769
Chl a : C (μgChl a mgC^{-1})	+UVR	**0.679**	**32.130**	**0.956**	**0.004**
	-UVR	**0.767**	**28.684**	**0.863**	**0.022**

Table 2. Effects of the enrichment with P on seston biochemical and elemental composition for each light treatment. Numbers give the magnitude (%) and sign (-, inhibition; +, stimulation) of each P concentration relative to the control (no P added). Only food quality variables that did not show a linear response to the enrichment with P (see Table 1) are shown here. Asterisks indicate the results of paired dependent *t*-test between the enriched treatments and the controls with no P added (n.s., not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; n.a., not applicable). Abbreviations as in Table 1.

	Factor (μgP L^{-1})	20		30		40		60	
	Light	+UVR	-UVR	+UVR	-UVR	+UVR	-UVR	+UVR	-UVR
SFA (μgFA mg C^{-1})		-37 (n.s.)	-22 (n.s.)	-31 (n.s.)	-19*	7 (n.s.)	-4 (n.s.)	17 (n.s.)	-23*
HUFA:PUFA		-74**	-82**	-74**	-88**	-86**	-93**	-90**	-94**
16 : 0		-23 (n.s.)	14 (n.s.)	-10 (n.s.)	17 (n.s.)	17 (n.s.)	43 (n.s.)	48*	6 (n.s.)
16 : 4		234**	(n.a.)	322***	(n.a.)	253**	(n.a.)	624**	(n.a.)
18 : 0		-52*	-36 (n.s.)	-53*	-50*	-21 (n.s.)	-41*	-25*	-52*
18 : 1n-9		1 (n.s.)	219***	-0.2 (n.s.)	168**	-5 (n.s.)	135*	22 (n.s.)	17 (n.s.)
18 : 2n-6		30 (n.s.)	156***	37*	162**	126*	143*	123**	17 (n.s.)

18 : 3n-3	249**	537**	378***	671**	487**	1302**	809*	1050**
20 : 4n-6	(n.a.)	(n.a.)	(n.a.)	(n.a.)	(n.a.)	(n.a.)	(n.a.)	-94*
C : P	51*	18 (n.s.)	13 (n.s.)	-10 (n.s.)	4 (n.s.)	-26 (n.s.)	-31**	-49***
pgP per cell	-91**	-89***	-92***	-90***	-91**	-89***	-87***	-84***
C : N	38*	63***	38*	47**	28*	33**	11 (n.s.)	24*

Table 3. Effect of UVR on seston biochemical and elemental composition. Numbers give the magnitude (%) and sign (-, inhibition; +, stimulation) of the UVR effects. Asterisks indicate the results of paired dependent *t*-test between +UVR and –UVR treatments for each P level (n.s., not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$). Abbreviations as in Table 1.

Factor (μgP L^{-1})	Control	20	30	40	60
TFA (μgFA mgC^{-1})	50*	3 (n.s.)	11 (n.s.)	25 (n.s.)	90**
SFA (μgFA mgC^{-1})	26*	8 (n.s.)	8 (n.s.)	41*	93**
ω3-PUFA (μgFA mgC^{-1})	96*	9 (n.s.)	19*	22*	54*
HUFA (μgFA mgC^{-1})	-42*	-35**	-15 (n.s.)	-11 (n.s.)	12 (n.s.)
HUFA:PUFA	-57**	-36**	-8 (n.s.)	-20 (n.s.)	-28*
16: 0	48*	3 (n.s.)	14 (n.s.)	22 (n.s.)	108**
16 : 4	(n.a.)	4 (n.s.)	23*	-37 (n.s.)	67**
18 : 0	13 (n.s.)	-15 (n.s.)	6 (n.s.)	53 (n.s.)	77 (n.s.)
18 : 1n-9	196*	-6 (n.s.)	10 (n.s.)	19 (n.s.)	208*
18 : 2n-6	94*	-2 (n.s.)	2 (n.s.)	81 (n.s.)	269**

18 : 3n-3	97**	8 (n.s.)	22**	-18 (n.s.)	55 (n.s.)
20 : 4n-6	(n.a.)	(n.a.)	(n.a.)	(n.a.)	(n.a.)
C : P	-34*	-12 (n.s.)	-7 (n.s.)	0.4 (n.s.)	-8 (n.s.)
pgP per cell	27*	4 (n.s.)	3 (n.s.)	-2 (n.s.)	0.4 (n.s.)
C : N	4 (n.s.)	-12*	-2 (n.s.)	0.3 (n.s.)	-7 (n.s.)
Chl a : C	27**	4*	-1 (n.s.)	-10*	5 (n.s.)

Table 4. Interactive UVR x P effects on seston biochemical and elemental composition. Interactive effects in variables showing linearity with P-enrichment for each light treatment were assessed by analysis of covariance, while variables not showing linearity were tested via regression analysis between UVR-relativized variables and P-enrichment (see Methods for further explanation). Significant predictors are shown in bold.

	Interactive UVR x P effects					
	Analysis of covariance		Linear regression (UVR-relativized variables)			
	$F_{1, 6}$	P	Slope	Y-Intercept	r^2	P
TFA (μgFA mgC^{-1})	–	–	0.007	1.130	0.202	0.447
SFA (μgFA mgC^{-1})	–	–	0.012	0.980	0.541	0.157
ω3-PUFA (μgFA mgC^{-1})	1.633	0.249	–	–	–	–
HUFA (μgFA mgC^{-1})	**7.867**	**0.031**	–	–	–	–
HUFA : PUFA	–	–	0.005	0.552	0.360	0.285
C : P	–	–	**0.005**	**0.732**	**0.870**	**0.050**
pgP per cell	–	–	**-0.004**	**1.173**	**0.678**	**0.045**

C : N	–	–	-0.001	0.992	0.103	0.600
Chl a : C (μgChl a mgC^{-1})	0.202	0.669	–	–	–	–

Figure legends

Figure 1. Taxonomic phytoplankton abundance in unenriched (control) and P-enriched enclosures under –UVR and +UVR after 30 days of incubation.

Figure 2. Changes in Soluble Reactive Phosphorus as a function of the P-enrichment in the two UVR treatments. Solid symbols represent –UVR enclosures and open symbols represent +UVR enclosures. Data show means of three laboratory measurements.

Figure 3. Effects of P (left) and UVR x P (right) manipulations on seston in terms of (a, b) total, saturated and highly unsaturated fatty acids, (c, d) ω3- PUFA, and (e, f) HUFA:PUFA ratio in experimental enclosures. Response variables were specifically relativized to show P and UVR x P effects. P-relativized variables were calculated by dividing the replicate for each P treatment by the control replica (no P added) in +UVR enclosures. For the UVR x P effect, variables were first UVR-relativized by dividing the observation for each P level in +UVR by the respective control treatment replicate in –UVR and then regressed against P-enrichment (see Table 4 for linear regression parameters and Methods for further detail). A positive response was interpreted as stimulating the constituent production or ratio and a negative response as inhibiting the constituent production or ratio. Values represent the mean and standard deviation of three sampling dates.

Figure 4. Effects of UV radiation and P manipulations on seston fatty acid composition. Values represent means from the three sampling dates. Fatty acids accounting for less than 5% of TFA in all treatments were not included.

Figure 5. Effects of P (left) and UVR x P (right) manipulations on seston in terms of (a, b) seston C:P ratio, (c, d) P cell quota, (e, f) seston C:N ratio, and (g, h) Chl a:C in experimental enclosures. See Fig. 3 text for further details.