

## **Photosynthetic response of pico- and nanoplanktonic algae to UVB, UVA and PAR in a high mountain lake**

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### ABSTRACT

The objective of the present study was to evaluate the influence of UV radiation on photosynthesis rate, *in situ* and in laboratory incubations, on size fractionated natural algal assemblages (picoplankton: 0.2–2µm, nanoplankton: >2µm) and whole water (total organic carbon TOC). Near surface samples from a mesotrophic high mountain lake (LCD: L. Cadagno, Swiss Alps, Switzerland, 1923 m a.s.l.) and from the oligotrophic pre-alpine L. Lucerne (LLU: Swiss Alps, Switzerland, 434 m a.s.l.) were both incubated at a depth of 30 cm (50% of surface UV at 323 nm) in L. Cadagno. At the same time, biological weighting functions for UV inhibition of photosynthesis (BWFs) were determined for the autotrophic picoplankton and whole fraction in a spectral incubator. Photosynthetic assimilation of the pico- and nanoplanktonic algal communities as well as the assimilated total organic carbon (TOC) was estimated separately by <sup>14</sup>C uptake under three irradiance conditions: PAR (photosynthetically active radiation), PAR + UVA and PAR + UVA + UVB. UV radiation reduced significantly photosynthesis rate in samples from both lakes (LLU: P = 0.0012; LCD: P = 0.0001). It appears that UVA plus UVB significantly affect the algal assemblage in both lakes; however most of the effect is due to UVA (Mann Whitney U test, two tailed: P = 0.0022). The natural assemblages from LLU transplanted to LCD were more inhibited by UV than the autochthonous assemblages of LCD. Photosynthetic rates of picoplankton from LLU and LCD under full UV exposure was reduced by 73% and 55% respectively relative to PAR only. A higher sensitivity of autotrophic picoplankton to the UV radiation, with respect to the nanoplankton, was observed in the biological weighting functions. However this difference was not statistically significant for the *in situ* incubations.

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There is disagreement among phycologists as to whether the degree of photosynthesis inhibition by ultraviolet radiation (UVR, 280–400 nm) depends on the size of the phytoplankton (Karentz et al., 1991; Helbling et al., 1992; Milot-Roy and Vincent, 1994; Halac et al., 1997; Bertoni and Callieri, 1999). These conflicting results come from a variety of approaches and methods used to assess the response of phytoplankton (mainly short and long term incubations) under natural or artificial UV exposure. For example, in a subarctic lake, Laurion and Vincent (1998) showed that cell size is not a good index of UV sensitivity and that the smallest phytoplankton cells are relatively resistant to UVR while Bertoni and Callieri (1999) found by artificially increasing the UV irradiance in Lago Maggiore, a sub-alpine oligotrophic lake, that picocyanobacteria were more affected by ultraviolet-B (UVB, 280–320 nm) than nanoplankton. More recently, Kasai et al. (in press) obtained evidence of a higher vulnerability of smaller algae to UVR and that ultraviolet-A (UVA, 320–400 nm) radiation has a higher damaging effect on phytoplankton communities.

During the working group on aquatic productivity (GAP) workshop, held in September 1999 in Switzerland, two experiments were conducted to measure the sensitivity of different size fractions to spectral UV radiation treatments. During the first experiment, *in situ* incubations under different screening treatments were carried out to measure photosynthesis rate ( $\text{mgC m}^{-3} \text{h}^{-1}$ ) of pico- and nanoplanktonic algae as well as whole communities. Natural algal assemblages sampled at 6:00 h, from Lake Lucerne (LLU: 434 m a.s.l., central Switzerland) and Lake Cadagno (LCD: 1923 m a.s.l., Val Piora, Swiss Alps) were incubated at 30 cm in L. Cadagno for 4 hours (11:00 h – 15:00 h CEST). Phytoplankton carbon assimilation (total and particulate) was measured in duplicates using the  $^{14}\text{C}$  ( $12.5 \mu\text{Ci NaH}^{14}\text{CO}_3$ ) method under three spectral treatments: quartz tubes (PAB: PAR+UVB+UVA), quartz tubes wrapped with Mylar D (PA: PAR+UVA), quartz tubes wrapped with Ultraphan (P: effects of PAR). Mylar D film (cut off filter <320 nm) and Ultraphan film (cut of filter <395 nm) had a transmittance of 90% above their respective cut-off wavelength. The autotrophic carbon fixation was determined by post incubation differential filtration (Fahnenstiel et al., 1994) using 2 and 0.2  $\mu\text{m}$  Nuclepore polycarbonate filters. Algae larger than 20  $\mu\text{m}$  were not removed as their biomass was negligible in both lakes as determined by a microscopical inspection, the samples were however prefiltered through a 126  $\mu\text{m}$  mesh to remove grazers. Total organic carbon assimilated (TOC) was determined using the acid bubbling technique (Gächter and Mares, 1979). Total inorganic carbon was estimated by pH and alkalinity measurements. Chlorophyll *a* was measured by HPLC on the pico- and nano fraction at the beginning of the incubation.

In a second set of experiments, laboratory measurements of the spectral sensitivity of the algal community was studied using a photoinhibitor (2500 W Xenon lamp with 72 spectral treatments, see Neale et al., 2001 b, this issue). One hour  $^{14}\text{C}$  incubation of a picoplankton and unfractionated (whole) sample were conducted under 72 spectral treatments (8 spectra  $\times$  9 intensities) to measure the total organic carbon incorporation. The picoplanktonic fraction was filtered through a 2  $\mu\text{m}$  (Nuclepore) before the incubation. Biological weighting functions (BWFs) for the inhibition of photosynthesis were estimated using the principal component analysis method (see Cullen and Neale, 1997; Neale et al., 2001 a; and Neale et al., 2001 b, this

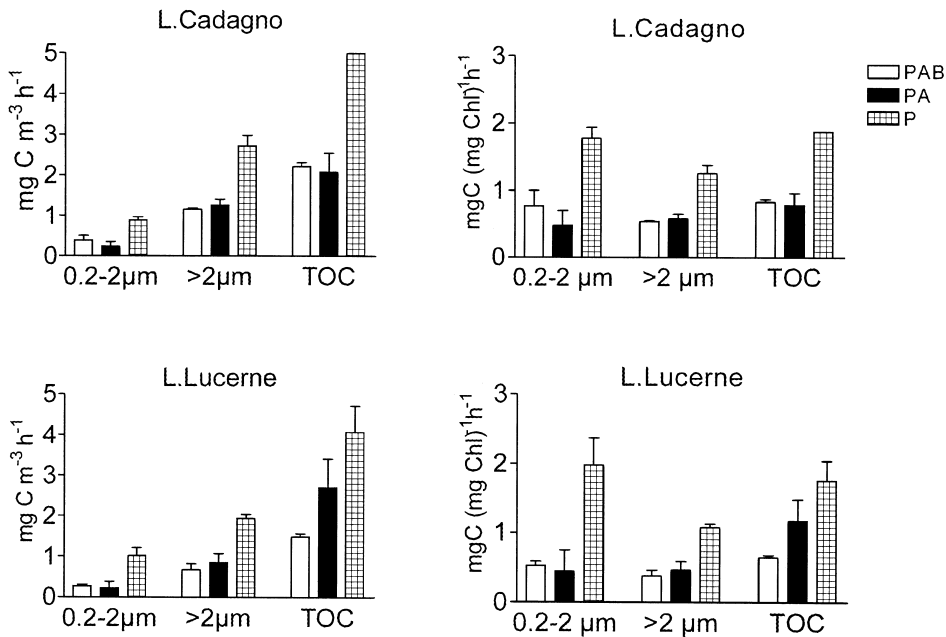
**Table 1.** Results of two-way ANOVA test on the differences of photosynthetic rate ( $\text{mgC m}^{-3} \text{h}^{-1}$ ) and chlorophyll specific photosynthetic rate ( $\text{mgC (mg Chl)}^{-1} \text{h}^{-1}$ ) between treatments (PAB, PA, P: see definition in the text), for LLU and LCD. Interaction between treatments and fractions was not significant in LLU and significant in LCD. The differences between the two fractions ( $0.2\text{--}2 \mu\text{m}$ ,  $> 2 \mu\text{m}$ ) was tested at the two most significant treatments PAB and P

$\text{mgC m}^{-3} \text{h}^{-1}$	Lake Lucerne	Lake Cadagno
PAB, PA, P	** P = 0.0012	*** P < 0.0001
PAB, PA	n.s.	n.s.
PA, P	* P = 0.0189	*** P = 0.0001
$\text{mgC (mg Chl)}^{-1} \text{h}^{-1}$		
PAB, PA, P	*** P = 0.0004	*** P < 0.0001
PAB, PA	n.s.	n.s.
PA, P	** P = 0.0061	*** P = 0.0001
$\text{mgC m}^{-3} \text{h}^{-1}$		
$0.2\text{--}2 \mu\text{m}$ , $> 2 \mu\text{m}$	** P = 0.0075	*** P = 0.0009
$\text{mgC (mg Chl)}^{-1} \text{h}^{-1}$		
$0.2\text{--}2 \mu\text{m}$ , $> 2 \mu\text{m}$	n.s. P = 0.0599	n.s. P = 0.0697

issue). Profiles of photosynthetically active radiation (PAR, 400–700 nm) were measured by an underwater sensor LI-192 SA and LI-COR 250 datalogger and underwater UV penetration by Satlantic Inc. multichannel radiometer (STOR-DAT with 6 channels OCI-200 head 323, 338, 380, 443, 490 and 555 nm). Incident, above surface, UV and PAR irradiance were monitored during the incubation using MACAM broadband sensors. For more information on the assessment of radiation and limnological conditions in the Lakes Lucerne and Cadagno during the GAP workshop, see Bossard et al. (2001) and Neale et al. (2001c), this issue.

Statistical analysis was performed using a two-way ANOVA test (treatments against fractions) for each lake.

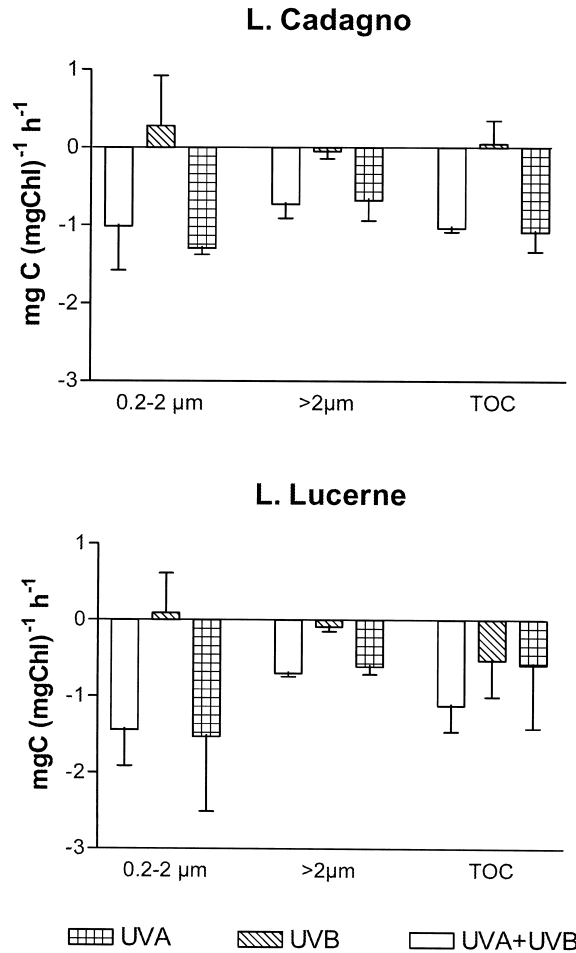
The results of the *in situ* experiments show UVR inhibition of photosynthesis in the PAB and PA treatments relative to the PAR only tubes (P) for all the fractions in both lakes (Fig.1). The photosynthetic and chlorophyll *a* specific photosynthetic rates were both significantly lower in respect to the tubes with PAR only. Differences in absolute photosynthetic rate were significant (two-way ANOVA, Table 1) for both LLU and LCD if tested for all the treatments together, similar results were obtained using data for the chlorophyll specific rates (Table 1). Nevertheless PAB and PA were not significantly different in both lakes, while PA and P were different. Differences between the fractions are also shown in Table 1. Differences between the PAB and P treatments are statistically significant for absolute photosynthetic rates but not significant for chlorophyll *a* specific rates. As it is common in most lakes (Stockner et al. 2000), picoplankton are less productive than nanoplankton in both lakes Lucerne and Cadagno. However photosynthetic efficiency, estimated as chlorophyll *a* specific photosynthetic rate, is similar or even higher (though not sta-



**Figure 1.** Photosynthetic rate (left panels) and chlorophyll specific photosynthetic rate (right panels) of the pico- (0.2 – 2 μm) and nanoplankton (>2 μm) and total carbon (TOC), under the three different treatments (see explanation in the text), of samples from L. Cadagno and L. Lucerne

tistically significant) than that of nanoplankton comparing the PAB and P treatments (Fig.1).

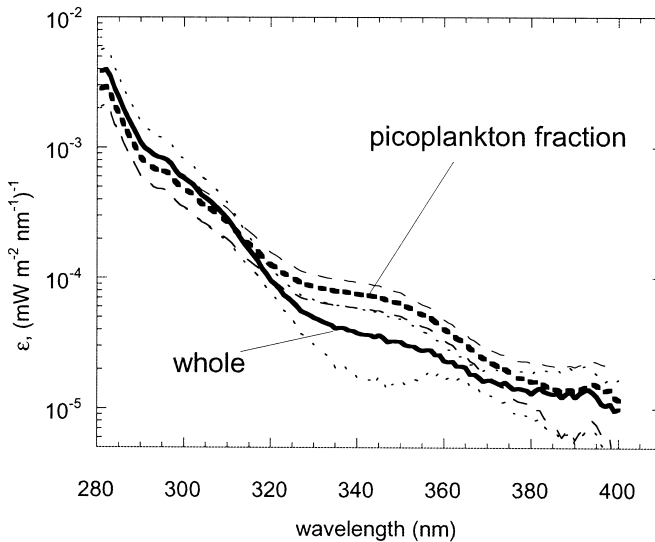
To compare the inhibition of photosynthesis by UV on pico- and nanoplankton we calculated the successive differences between treatments, i.e. UVA effect is P-PA, UVB effect is PA-PAB, and UVA + UVB effect is P-PAB (Fig.2). It appears that UVA plus UVB significantly affect the algal assemblage in both lakes; however most of the effect is due to UVA (Mann Whitney U test, two tailed: P=0.0022). In both lakes picoplankton were inhibited primarily by UVA. We calculated the ratios PAB:P and PA:P of pico- and nanoplankton to evaluate hypothetical difference of photosynthesis inhibition. From bio-optical theory smaller cells should be more vulnerable to UV than larger cells as accumulation of sunscreens is not an effective protection against UV radiation, due to short cell radii (Garcia-Pichel, 1994). The Mann-Whitney U test (non parametric test) revealed no significant differences between pico and nanoplankton photoinhibition when all data from LCD and LLU were pooled together. Nevertheless, in the laboratory, under controlled conditions, the results of the biological weighting functions show a greater sensitivity of the picoplanktonic assemblage (Fig. 3). Although for LCD the BWFs are identical at <320 nm and >380 nm they show that the autotrophic picoplankton photosynthesis is more sensitive to UV than photosynthesis in the whole water portion between 330 to 360 nm (Fig. 3). Using the approach of Neale et al. (2001 b, this



**Figure 2.** Inhibition of chlorophyll specific photosynthetic rate by UVA, UVB and UVA + UVB on the autotrophic picoplankton (0.2–2  $\mu\text{m}$ ), nanoplankton (>2  $\mu\text{m}$ ) and total carbon (TOC) in L. Cadagno and L. Lucerne, on 14 september 1999

issue), the BWFs for picoplankton and whole were applied to *in situ* spectral irradiance at 30 cm (results not shown). The percent decrease in photosynthesis is predicted to be 50% in the picoplankton versus 35% in the whole sample. This difference could have been hidden during the *in situ* incubation due to the limited numbers of replicates and large variability between replicates. Alternatively, the disagreement could be more methodological, since incubation results were obtained with post-incubation and the BWF was conducted on filtrate obtained with pre-incubation. If the picoplankton were damaged during filtration, they may become more sensitive to subsequent UV exposure.

Conflicting results have been obtained in previous studies of differences in UV vulnerability between picoplankton and nanoplankton algae. Although we obtain-



**Figure 3.** Biological weighting functions ( $\text{mW m}^{-2} \text{nm}^{-1}$ ) for inhibition of photosynthesis for *L. Cadagno* picoplankton (blue line) and whole assemblage (red line) on September 13, 1999. Broken lines show estimated 95% confidence interval for individual coefficient estimates

ed somewhat different results in the incubations and *in situ* experiments, the contrast between fractions is in any case not large, ranging between 2–9% *in situ* and 15% based on the BWF (relative to PAR only). In LCD the percent decrease of chlorophyll specific photosynthetic rate is 55% and 57% and in LLU 73% and 65% in pico and nano fractions respectively. Although small cells receive greater UV exposure, the potentially greater damage may be counteracted through such mechanisms as greater concentrations of antioxidants and enhanced repair capability.

The results from the spectral treatments are however clear: UVA was by far the most inhibiting radiation under the conditions tested. The higher effect of UVA on algae with respect to UVB has been found both in marine (Helbling et al., 1994) and freshwater environments (Bühlmann et al., 1987; Kim and Watanabe, 1993; Villafañe et al., 1999). In Lake Titicaca (Villafañe et al., 1999) photosynthetic inhibition by UVA accounted for 60% and UVB for 20%. Indirect evidence for a strong effect of UVA on the activity of natural picoplanktonic assemblages has been also shown in another subalpine Italian lake (Bertoni and Callieri, 1999).

Nevertheless we know from literature (reviewed by Karentz et al., 1994) and from the laboratory experiments where the UVR radiant exposure at different spectral irradiance can be regulated, that the UVB radiation usually has the most deleterious effects on the organisms (Cullen et al., 1992; Cullen and Neale, 1994) on a per photon basis. The BWFs for *L. Cadagno* and *L. Lucerne* also show very high sensitivity to UVB (Fig. 3, and Neale et al., 2001b, this issue). The negligible impact of UVB on LCD algae *in situ* could be due to the lower weighted irradiance brought about by the high  $K_d$  at short wavelength (Table 2) and low incident flux, whereas in the UVA the weighted irradiance is higher due to a greater incident flux and

**Table 2.** Attenuation coefficients ( $K_d$ ) for UV and visible for Lake Lucerne and Lake Cadagno; depths with 1% of surface radiation at different wavelengths; percentage of radiation at the depth of incubation in Lake Cadagno

Wavelength nm	Lake Lucerne		Lake Cadagno		Lake Cadagno Transparency at 30 cm %	Instrument used
	$K_d$ m <sup>-1</sup>	1% depth m	$K_d$ m <sup>-1</sup>	1% depth m		
305	1.65		3.19		40	PUV
323	1.16	4.0	2.31	2.0	47	Satlantic
338	0.90	5.1	1.98	2.3	56	Satlantic
380	0.49	9.4	1.21	3.8	70	Satlantic
PAR	0.35	15	0.47	10	87	LI-COR

lower  $K_d$ . This conclusion is supported by the application of the measured BWFs to *in situ* irradiance spectra: UV in the 290–315 nm region accounts for only 15% of total weighted irradiance (Neale et al., 2001 b, this issue).

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