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^{14}C photosynthesis and pigment pattern of phytoplankton as size related adaptation strategies in alpine lakes

Katrin Teubner[1,*], Aitziber Sarobe[2], Maria Rosaria Vadrucci[3] and Martin T. Dokulil[1]

[1] Institute of Limnology, Austrian Academy of Science, Mondseestrasse 9, A-5310 Mondsee, Austria
[2] Universidad Pais Vasco, Laboratorio de Ecologia, Barrio Sarriema SN, Apdo 644, E-48080 Bilbao, Spain
[3] Dip. di Biologia, Lab. di Ecologia, Università di Lecce, Via Prov. Lecce-Monteroni, I-73100 Lecce, Italia

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ABSTRACT

The hypothesis that both photosynthesis and pigment pattern are more reflective of size related strategies than taxonomic composition of the assemblages was tested under natural conditions in alpine lakes during fall 1999. The small size fraction (< 10 µm) in Lake Lucerne, Mondsee and Traunsee and in an additional incubator experiment contributed 55–67% to the total integral of chlorophyll-a and photosynthesis per m^2. The photosynthetic depression induced by UV-A in Lake Lucerne, measured during the 7th GAP-workshop, markedly increased to 65% with PAR up to 500 µmol m^{-2} s^{-1}. At super-saturating light intensities near the surface, UV-A inhibition further increased marginally to 69%. Effects of light acclimation and pigment adaptation on photosynthetic rates and efficiencies of phytoplankton indicated size related strategies that were more important than the taxonomic composition of the assemblage measured in Traunsee and Mondsee. Algal communities and small size fractions (< 10 µm), that had a high maximum light utilisation coefficient (α*) were adapted to low light by high ratios of chlorophyll-*a* to photo-protective β-carotene. Algae at high solar radiation and of large size (> 10 µm) were photosynthetically less efficient but high light adapted having low ratios of chlorophyll-*a* to β-carotene. In contrast to low light adapted small cells the high light adapted large-cells increased their relative proportion of photo-protective carotenoids above saturating light levels. At light limitation in deeper water layers lipophilic accessory photo-synthetic versus photo-protective pigments increased for all fractions.

* Corresponding author, e-mail: katrin.teubner@oeaw.ac.at.

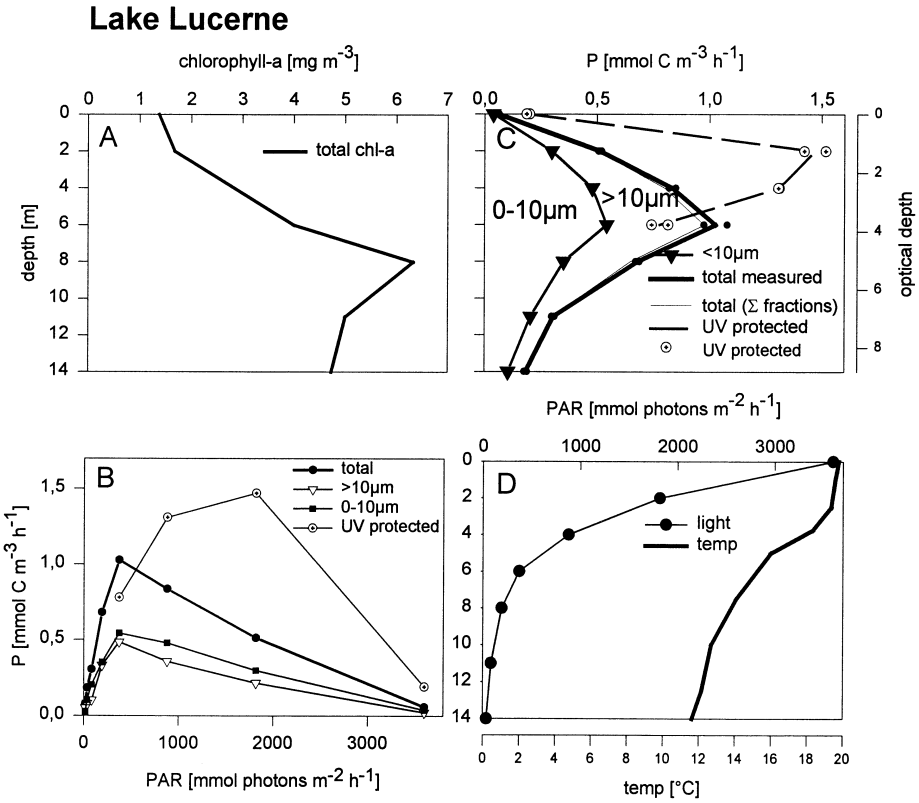

Figure 2. Lake Lucerne: A: Vertical profiles of concentrations of chlorophyll-*a*, B: Photosynthetic rate (P) vs. light as PAR. C: photosynthetic rates (P) of the total sample, fractions 0–10 µm and >10 µm, the sum of both fractions and UV-protected bottles, D: photosynthetic available radiation (PAR) and temperature (temp)

ferred to vials and covered by 1 ml 1N HCL (HCl-liquid in Fig. 1A) or put into petri-dishes on paper soaked with HCL and left over night (HCl-paper in Fig. 1A). In the latter case, filters were transferred to vials the next morning. Tissue solubilizer (SOLUEN) was added for both filter treatments. Results for both filter treatments were comparable (Fig. 1A). Ten ml scintillation cocktail (Packard, Ultima Gold) was added to all samples and mixed with the liquid phase. As Figure 1A and Figure 2–3 indicates, the sum of the fractions (filtrate plus filter) closely resembled the total values measured. Photosynthetic rates were calculated from total activity of added ^{14}C, the ^{14}C-uptake values and DIC-concentrations of lake water (Gächter et al., 1984). For each depth and fraction the value for the dark bottle was subtracted from the corresponding light bottle (Markager, 1998).

For the incubator experiment an integrated sample from 0–15 m was used. Subsamples, 10 ml each were filled either directly or pre-fractionated through 10 µm Nylon netting into 20 ml glass bottles, ^{14}C added and the bottles placed in a water-cooled incubator (CULLEN-type) in the laboratory at Kastanienbaum. Illumina-

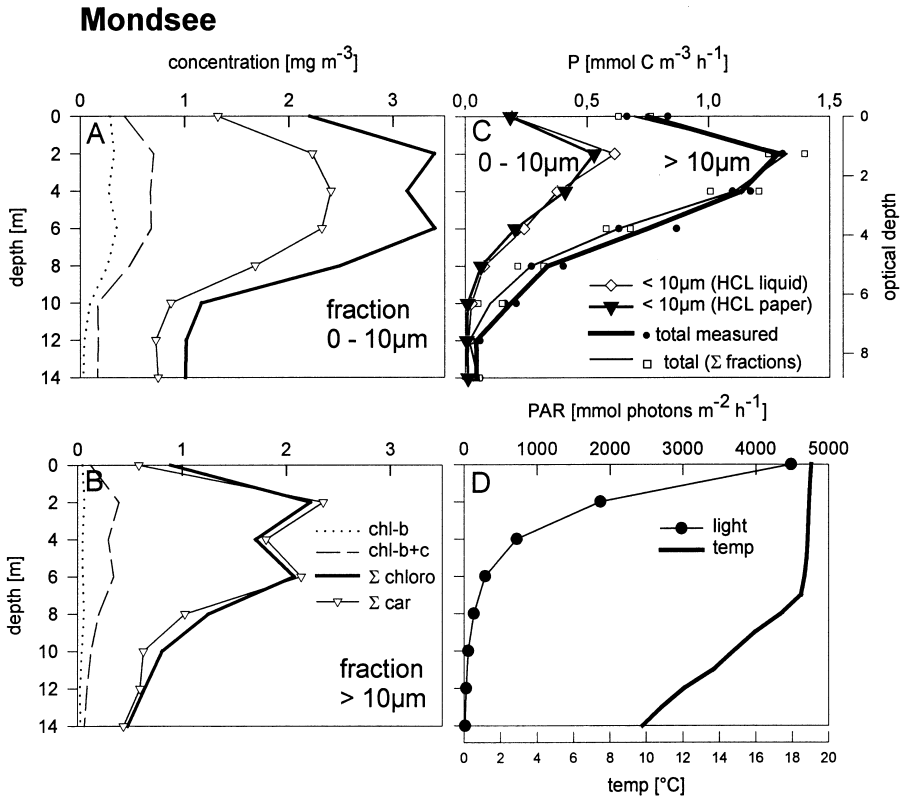

Figure 3. Mondsee, vertical profiles: A–B pigments: concentrations of chlorophyll-*b*, chlorophyll-*b* plus -*c*, the sum of chlorophyll-*a*, -*b*, and -*c* and the sum of carotenoids in single fractions (0–10 µm in A, >10 µm in B); C: Photosynthetic rates (P) vs. depth for the total and fractions, for different filter treatment refer to method; D: photosynthetic available radiation (PAR) and temperature (temp) vs. depth

tion of the incubator was from two HQI/D, OSRAM lamps (PAR_{max} = 800 µmol m^{-2} s^{-1}). Spectral measurements indicated significant emissions of ultra-violet.

Chlorophyll measurements for Lake Lucerne were done by high performance liquid chromatography (HPLC) in laboratory at the Kastanienbaum Institute. Pigments for Traunsee and Mondsee were analysed according to Wright et al. (1991) using HPLC at the Mondsee Institute. Two litres of lake water were filtered (Whatman GF/F) for total pigment concentrations, frozen and extracted by 90% acetone. To maintain the calibration settings a mixed pigment standard used containing carotenoids in the quantitative proportion to chlorophyll-*a* as described in Wilhelm et al. (1991) and measured in algal cultures respectively. For size fractionation additionally 2 l water were filtered in cascades through 11 µm Nylon-filter (47 mm diameter) and GF/F. Analysis of variance indicated close agreement between the sum of the fractions and the total chlorophylls and carotenoids respectively (Fig. 1B). Therefore for both photosynthesis and cell pigments the fraction < 10 µm was mea-

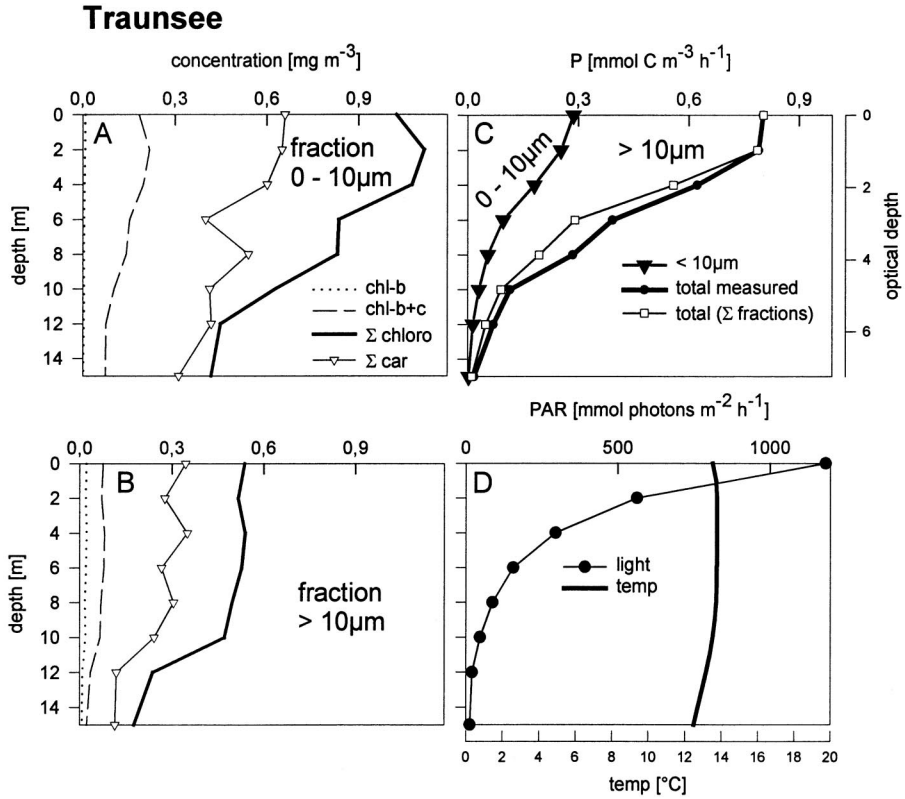

Figure 4. Traunsee, vertical profiles: A–D same as Figure 3, but no different filter treatment in C

sured and not estimated by difference. The sum of all fractions was used as a control.

Recommended units in mol for carbon fixation, the same time unit for photosynthetic rates and light in P vs E curves and the nomenclature follows the suggestions by Sakshaug et al. (1997). Carotenoids in Figure 7 were categorised according to their distinct function as 'photo-synthetic' (involved in light harvesting such as fucoxanthin and fucoxanthin-like pigments, peridinin, prasinoxanthin and α-carotene) and photo-protective pigments (protecting the cells against photo-oxidation, the remaining carotenoids; Rowan, 1989; Bricaud et al., 1995; Stuart et al., 1998).

According to long-term phytoplankton analyses the contribution of size fractions larger and smaller than 10 µm were 44% and 56% respectively in Traunsee and Mondsee. Accordingly filters of 11 µm pore size were chosen to discriminate the respective size fractions of nearly the same biovolume contribution, similar to techniques used elsewhere (e.g. Koschel and Scheffler, 1985; Happey-Wood, 1993; Lafond et al., 1990).

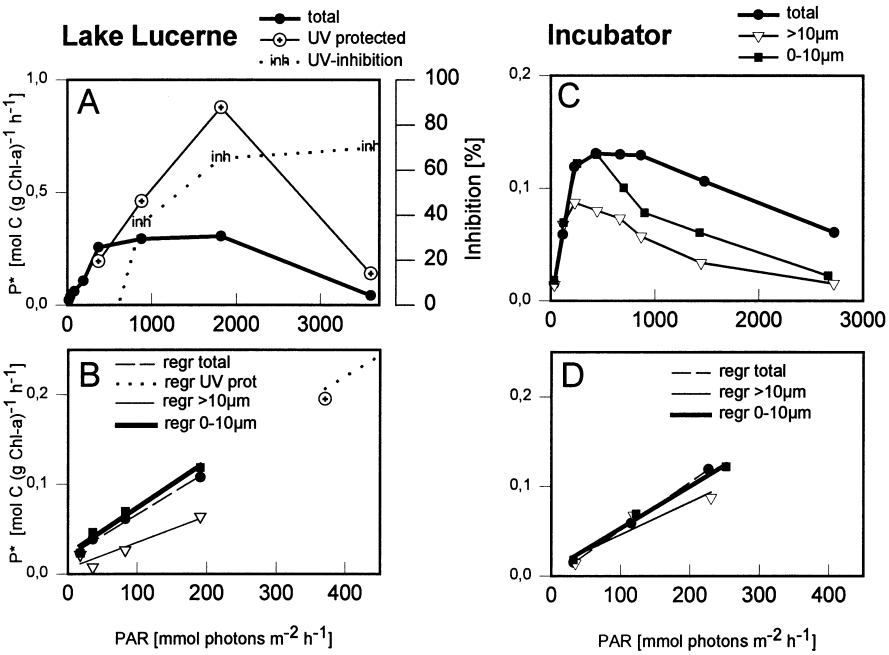

Figure 5. Lake Lucerne: photosynthetic activity (P*) measured in situ (A, B) and in incubator (C, D) vs. light as PAR. Linear part of P* in B and D respectively. Inhibition by UV calculated as difference between covered and uncovered incubation bottle related to covered bottle (100%, Bühlmann et al. 1987) vs. PAR is shown in A

Results

Vertical profiles of photosynthetic rates, light and temperature for the three alpine lakes are shown in Figures 2–4. As indicated by temperature profiles, both Lake Lucerne and Mondsee were still stratified in autumn while Traunsee was mixed throughout the euphotic zone. Chlorophyll concentrations reached a single maximum at 8 m in Lake Lucerne and had two peaks at 2 and 6 m in Mondsee. In contrast, concentrations of chlorophyll in Traunsee were much lower in general, peaking at 2 m and gradually declining to very low levels in deeper layers. Profiles of photosynthetic rates closely resembled the vertical distribution of chlorophylls in all three lakes.

The fraction smaller than 10 µm contributed on average about 63% to the total integral chlorophyll-a in Mondsee and Traunsee. The contribution to the integral photosynthetic rate, however, was about 2–4% higher than the share of chlorophyll-a. The contribution of the fraction <10 µm to the total photosynthetic rate in Lake Lucerne (55%) was almost the same as in the other two lakes.

The PAR of 3600–4320 mmol m^{-2} h^{-1} (= 1000–1200 µmol m^{-2} s^{-1}) at the lake surface in both Lake Lucerne and Mondsee was considerably higher than that measured in Traunsee (1188 mmol m^{-2} h^{-1} = 330 µmol m^{-2} s^{-1}) for the days of observa-

tion (Fig. 2D, 3D, 4D). Photoinhibition at the surface was 94% of maximum photosynthesis (P_m) in Lake Lucerne, about 42% in Mondsee and 53% in the incubator. If the phytoplankton assemblage was shielded from the UV-A-part of the spectrum, photosynthetic rates shifted nearer to the surface (6 m to 2 m, Fig. 2C). For Lake Lucerne an UV-A inhibition of 69% was calculated for the surface sample compared with the non-protected surface-sample (Fig. 5 A, surface: 3600 mmol photons m^{-2} h^{-1} = 1000 µmol m^{-2} s^{-1}, compare. to the 40% UV exposed/UV excluded estimated by Neale et al. (2001, this issue). UV-A inhibitions declined rapidly in layers deeper than 2 m (Fig. 2C), which was equal to PAR lower than 505 µmol m^{-2} s^{-1} (1818 mmol m^{-2} h^{-1} in Fig. 5 A). The inhibition at surface calculated from the maximum photosynthetic rate (P^*_m) for the non-covered bottles (83%, P^*_m at 6 m) was, however, the same as for UV-protected bottles (84%, P^*_m at 2 m). In contrast, no photoinhibition was observed in Traunsee due to overcast and hence low light intensities.

Photosynthetic rates and activities versus light (P vs. E) are shown in Figure 2B, 5 and 6, the summary of derived parameters in Table 2. Photosynthetic activity per unit chlorophyll-*a* of the total assemblage was almost identical to the activities of both fractions for Traunsee and Mondsee (Fig. 6B and F). A much higher activity was estimated for the UV-protected samples in Lake Lucerne (Fig. 5A).

Photosynthetic efficiency (α^*) for algae smaller than 10 µm was consistently higher than for the >10 P^*_m m fraction for all experiments in the field and in the laboratory (Fig. 5 D and 6 D, H, Table 2). Photosynthesis in the field saturated (E_K) at lower light levels in the <10 µm fraction when compared with larger sized algae (Table 2).

When photosynthesis was scaled to unit carotenoids (P_{car}), clearer differences in activity between size fractions became evident (Fig. 6C, G) compared to photosyn-

Table 2. Summary of P vs. E characteristics for different treatments (size fractions, UV-protection) in three alpine lakes and in an incubator. P^*_m = maximum photosynthetic rate (mol C (g Chl-*a*)$^{-1}$ h^{-1}); E_K = light saturation index (mmol photons m^{-2} h^{-1}); α^* = maximum light utilization coefficient (initial slope of P versus E curve) [mol C (g Chl-*a*)$^{-1}$ (mmol photons)$^{-1}$$m^2$]; r^2 = variance, P-value (paired t-test for >10 µm vs. 0–10 µm, n.s. = non-significant $P > 0.005$), *integral P* [mmol C m^{-2} d^{-1}]

	Treatment	P^*_m	E_K	α^* [10^{-3}] (r^2)	P	integral P
Lake Lucerne	total	0.306	651	0.47 (0.99)		90
(Switzerland)	UV protected	0.877	1867	0.47 (0.99)		
	> 10 µm	0.281	779	0.36 (0.99)		
	0–10 µm	0.351	676	0.52 (0.98)	< 0.05	
Mondsee	total	0.294	653	0.45 (0.80)		82
(Austria)	> 10 µm	0.299	763	0.38 (0.93)		
	0–10 µm	0.293	687	0.43 (0.80	< 0.05	
Traunsee	total	0.578	286	2.02 (0.91)		43
(Austria)	> 10 µm	0.620	365	1.70 (0.95)		
	0–10 µm	0.613	270	2.27 (0.93)	< 0.05	
Incubator	total	0.131	247	0.53 (0.99)		
(Cullen-type)	> 10 µm	0.087	242	0.36 (0.89)		
	0–10 µm	0.130	277	0.47 (0.99)	n.s.	

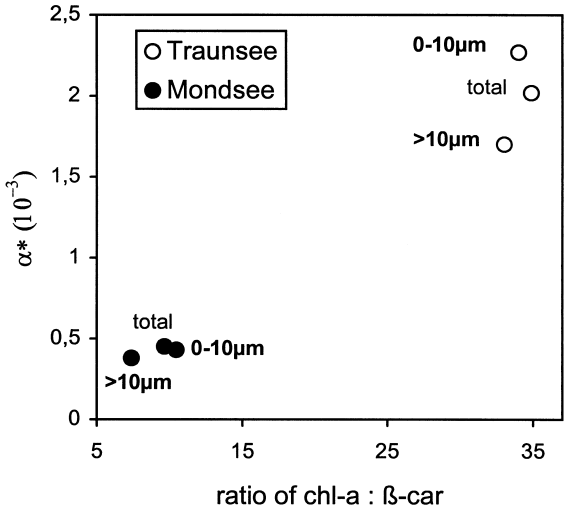

Figure 8. Relation between the ratio of chlorophyll-*a* to *β*-carotene (mg m^{-3} : mg m^{-3}) and the photosynthetic efficiency [α^*, mol C (g Chl-*a*)$^{-1}$ (mmol photons)$^{-1}$m^2, refer to Table 2] for the fractions < 10 µm (0–10 µm), > 10 µm and the total for Mondsee and Traunsee

lengths from 300 nm upwards and favour photoinhibition by UV-A (320–400 nm; Bühlmann et al., 1987; Kim and Watanabe, 1993, 1994; Villafañe et al., 1999; Bertoni and Callieri, 1999). The inhibition due to UV-A in Lake Lucerne measured during the GAP-workshop was about 15% higher than those expected from experiments by Bühlmann et al. (1987). The degree of inhibition markedly increased with PAR up to 500 µmol m^{-2} s^{-1}, but levelled off at super-saturating light intensities. These results are in accordance with Bühlmann et al. (1987) which showed that there is a more sensitive photosynthetic reaction to UV-A at low light intensities than at high radiation. Recent studies have shown that photoinhibition is a net result of photodamage, mainly due to shorter UV (e.g. DNA damage by UV-B) and recovery processes by longer waves (e.g. DNA damage repair by UV-A and visible light) and therefore the proportion between different radiation is biologically relevant (e.g. Kim and Watanabe, 1994; Cullen and Neale, 1994; Quesada et al., 1995; Buma et al., 1996; Gieskes and Buma, 1997, Villafañe et al., 1999).

Moreover, the susceptibility of different phytoplankton groups to radiation varies as became obvious analysing marker pigments in Mondsee and Traunsee. While Cryptophyceae and Dinophyceae avoided radiation higher than 500 µmol m^{-2} s^{-1} in Mondsee, the Bacillariophyceae and Chrysophyceae tolerated high radiation near the water surface as found elsewhere (Arvola et al., 1991; Richardson et al., 1993; UV-resistance of Bacillariophyceae ref. Bertoni and Callieri, 1999; Halac et al., 1997). Therefore, the stronger effect of UV-A at low PAR (< 500 µmol m^{-2} s^{-1}) can be enhanced by the increase of light-sensitive algae in subsurface layers.

Photo-inhibition remained at 83–84% of P^*_m regardless if bottles are UV-protected or not indicating that artefacts or factors other than UV must interfere with photosynthetic rates. Under natural conditions, algal species tolerate higher light

intensities because of circulating through the epilimnion. Conventional static in situ incubations, however, can lead to an overestimation of photo-inhibition near the surface because of prolonged exposure to over-saturating light intensities (e.g. Jewson and Wood, 1975; Dokulil et al., 1978; Marra, 1978; Nixdorf and Behrendt, 1990; Nixdorf et al., 1990; Cullen and Neale, 1994; Köhler et al., 2001, this issue).

Conclusions

Effects of light acclimation and pigment adaptation on photosynthetic rates and efficiencies of phytoplankton indicated size related strategies that were more important than the taxonomic composition of the assemblage. Analysis of the impact by UV-A in Lake Lucerne substantiated the general believe that photoinhibition is largely affected by short wave radiation. The extent of the inhibition was consistent with earlier measurements in Lake Lucerne and with observations in fresh-waters in general.

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REFERENCES

Arvola, L., A. Ojala, F. Barbosa and S.I. Heaney, 1991. Migration behaviour of three cryptophytes in relation to environmental gradients: an experimental approach. B. Phycol. J. 26: 361–373.

Bertoni, R. and C. Callieri, 1999. Effects of UV-B radiation on freshwater autotrophic and heterotrophic picoplankton in a subalpine lake. J. Plankton Res. 21 (7): 1373–1388.

Bricaud, A., M. Babin, A. Morel and H. Claustre, 1995. Variability in the chlorophyll-specific absorption coefficients of natural phytoplankton: analysis and parameterization. J. Geophys. Res. 100: 13321–13332.

Bossard, P., S. Gammeter, C. Lehmann, F. Schanz, R. Bachofen, H.R. Bürgi, D. Steiner and U. Zimmermann, 2001. Limnological description of the Lakes Zürich, Lucerne, and Cadagno. Aquat. Sci. 63/3: 225–249.

Bühlmann, B., P. Bossard and U. Uhlinger, 1987. The influence of longwave radiation (UV-A) on the photosynthetic activity (^{14}C-assimilation) of phytoplankton. J. Plankton Res. 9 (5): 935–943.

Buma, A.G.J., H.J. Zemmelink, K. Sjollema and W.W.C. Gieskes, 1996. UV-B radiation modifies protein and photosynthetic pigment content, volume and ultrastructure of marine diatoms. Mar. Ecol. Prog. Ser. 142: 47–54.

Cullen, J.J. and P.J. Neale, 1994. Ultraviolet radiation, ozone depletion, and marine photosynthesis. Photosynthesis Res. 39: 303–320.