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Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes

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SUMMARY

Photoacclimation of *Scenedesmus protuberans* Fritsch and *Microcystis aeruginosa* Kützing emend. Elenkin to high and fluctuating PPFD was studied in continuous cultures with computer-controlled variable light regimes. The aim of the work was to provide a better understanding of species-specific acclimation to high PPFD (as encountered by cyanobacteria in surface waterblooms), and of suppression of the growth of colony-forming cyanobacteria during periods of prolonged mixing in lakes. The dynamics of a set of variables was followed during the light period, including pigment composition, maximum rate, efficiency and minimum quantum requirement of photosynthesis, PS II cross-sections, and fluorescence variables. Both the green alga and the cyanobacterium displayed strong photo-inhibition of photosynthesis in the sinusoidal light regime, which simulated a natural light regime in the absence of mixing. P_{max}, α, QR and the ratio of variable to maximum fluorescence declined, and the number of inactive PS II centres and PS II_β centres increased towards midday. Introduction of oscillations in the diurnal light regime, simulating different intensities of wind-induced mixing in lakes, mitigated photo-inhibition. *Microcystis* showed a prompt non-photochemical quenching of fluorescence in all light regimes, even at low to moderate PPFD. The sustained presence of zeaxanthin in *Microcystis* possibly induced instant, thermal dissipation of excitation energy from the antenna. *Microcystis* also exhibited a more reluctant acclimation to fluctuating PPFD. Growth rate of *Scenedesmus* was higher in all light regimes. This implied that if (known) differences in loss processes were ignored, *Scenedesmus* would outcompete *Microcystis* in lakes. The results underlined the importance of buoyancy regulation in increasing the daily light dose of cyanobacteria (but at the same time preventing over-excitation), and ultimately in the success in *Microcystis* in stable lakes.

Key words: Fluctuating PPFD, cyanobacterial versus green algal photoacclimation, photosystem II, fluorescence, photo-inhibition, zeaxanthin, PS II heterogeneity.

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Abbreviations and units: A, absorbance (cm^{-1}); a^*_{ph}, chlorophyll specific optical absorption cross-section ($m^2\ mg^{-1}$ chlorophyll); DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F, fluorescence level. Subscripts o, i, d, and p represent the fluorescence level during light induction at the origin, intermediate peak, dip and plateau levels, respectively. Subscripts o, s, m and v indicate minimum, steady-state, maximum and variable levels respectively. If the symbol is followed by a prime, the measurement was made in the presence of actinic illumination. I_k, the PPFD at which photosynthesis would saturate if the photosynthesis response was linear with PPFD (μmol photons $m^{-2}\ s^{-1}$); K, rate constant for closure of PS II centres during induction of fluorescence (s^{-1}). Subscripts αi, αf and β denote constants for initial and final rate of closure of PS II_α centres and PS II_β centres respectively. LHC II, light-harvesting Chl *a*,*b*-protein complex; PAM, pulse amplitude modulation; P/I, photosynthesis versus irradiance curve; P_{max}, gross maximum oxygen production rate (mg $O_2\ h^{-1}\ mg^{-1}$ protein); PS I, photosystem I; PS II, photosystem II. Subscripts α and β denote pools with and without peripheral antenna respectively; PSU, photosynthetic size unit, the number of chlorophyll molecules participating in the evolution of one molecule of O_2; PQ, plastoquinone pool; Q_A, quinone A, first stable electron acceptor of PS II; Q_B, quinone B, second stable electron acceptor of PS II; QR, minimum quantum requirement for photosynthesis (mol photons mol^{-1} O_2); R, dark respiration rate (mg $O_2\ h^{-1}\ mg^{-1}$ protein); TDLD, total daily light dose (mol photons m^{-2}); Z_m, depth of the mixed layer in a lake (m); Z_{eu}, depth of the euphotic layer in a lake (m); α, efficiency of light-limited photosynthesis (μg $O_2\ h^{-1}\ mg^{-1}$ protein μmol photons $m^{-2}\ s^{-1}$); ϕ_p, photochemical efficiency of PS II per absorbed photon (calculated as $F'_m - F_s/F'_m$); μ, growth rate (d^{-1}).

INTRODUCTION

During waterbloom formation a population of gas-vacuolate, buoyant cyanobacteria becomes telescoped to the lake surface (Reynolds & Walsby, 1975). Stability of the water column is a prerequisite for bloom formation. Mixed conditions, however, not only prevent waterbloom formation, but also arrest the growth of colony-forming cyanobacteria (Reynolds, Wiseman & Clarke, 1983*a*, Reynolds *et al.*, 1983*b*). Observations on populations of the cyanobacteria *Microcystis* sp. and *Aphanizomenon* sp. in the relatively shallow Lake IJsselmeer, The Netherlands, showed that they mainly developed during periods of partial water-column stability (Ibelings, unpublished data). Green algae, mainly *Scenedesmus* spp., dominated during periods of more intensive mixing. Artificially induced mixing of lakes has been applied to reduce nuisance populations of cyanobacteria (see Paerl, 1988). One of the causes of reduced growth under mixed conditions may be the reluctant acclimation of cyanobacteria to changes in PPFD as found by Ward & Wetzel (1980) and Collins & Boylen (1982). These studies examined acclimation to a single shift in PPFD, whereas the light field in lakes is highly variable. Naturally available PPFD for phytoplankton fluctuates as a complex function of the daily passage of the sun, meteorological conditions, wave action and mixing over the underwater light gradient (Marra, 1978; Shin, Rhee & Chen, 1987; Grobbelaar, 1989; Stramski, Rosenberg & Legendre, 1993).

In this study we made a direct comparison between the cyanobacterium *Microcystis aeruginosa* Kützing emend. Elenkin and the eukaryotic green alga *Scenedesmus protuberans* Fritsch with respect to acclimation to fluctuations in PPFD, simulating those induced by wind-induced mixing over the underwater light gradient in lakes. Microstratification in the near-surface layers largely determines the depth to which phytoplankton is circulated (Reynolds, Walsby & Oliver, 1987; Ibelings, Mur & Walsby, 1991), and will predominantly control the light conditions for photosynthesis. Vincent, Neale & Richerson (1984) found that shifts in algal photochemistry closely followed the diel cycle of microstratification and mixing in Lake Titicaca.

Cells acclimate to a change in PPFD by adjusting their pigment content and composition, the number and size of photosynthetic size units (PSU), the ratio of PS II to PSI and, on shorter time scales, by mechanisms affecting fluorescence yield and redox carriers (e.g. Falkowski, 1984; Shin *et al.*, 1987). The measurement of fluorescence is a fast and non-invasive technique. It permits changes in photochemistry to be followed at time scales equal to the PPFD fluctuations of the cells *in situ* (Kroon, 1992). At room temperature most fluorescence is emitted by Chl *a* of PS II. Fluorescence accounts for a small proportion of the light absorbed, it competes weakly with photochemistry, thermal dissipation of energy and excitation transfer to non-fluorescent pigments (see Krause & Weis, 1991 for a review).

In the absence of wind, cyanobacteria are able to rise to the lake surface to form surface waterblooms, and are, on cloudless days, exposed to high PPFD, the pattern of which follows a sine curve. This type of light regime was incorporated in the study in order to compare the acclimation of the two species to high PPFD. Abeliovich & Shilo (1972) and Eloff, Steinitz & Shilo (1976) found photo-oxidation of cyanobacteria in surface waterblooms. Photo-oxidation results from prolonged exposure to conditions that cause photo-inhibition. Photo-inhibition results in a decrease of photosynthesis, and usually follows exposure of cells to a PPFD level above that to which the cells are acclimated (Powles, 1984). Under photo-inhibitory conditions excessive energy may be dissipated as heat by PS II, either in the antenna or in the reaction centre itself (Krause & Weis, 1991). Increased thermal energy dissipation in PS II manifests itself as an increase in non-photochemical quenching of fluorescence (see Krause, 1988). Demmig-Adams *et al.* (1990*a*, *b*) proposed a relationship between non-photochemical quenching of fluorescence and the xanthophyll zeaxanthin, which is formed by de-epoxidation of violaxanthin in the xanthophyll cycle. The xanthophyll cycle is absent in prokaryotes, although most are able to form zeaxanthin slowly (over days), presumably from β-carotene. When the energy-dissipating mechanisms are saturated, net photo-inhibition will occur. This leads initially to inhibition of electron transport through PS II and, subsequently, to degradation of the D_1-reaction centre protein (see Aro, Virgin & Andersson, 1993). A repair cycle for damaged PS II centres has been suggested in which the damaged D_1-protein is being replaced (Melis, 1991). The initial step in this repair is removal of the peripheral antenna, resulting in PS II centres with a smaller effective PS II cross-section, classified as PS II_β centres, as opposed to PS II_α centres, which have the peripheral antenna in place. In addition to the above-mentioned heterogeneity with respect to the antenna size, a heterogeneity with respect to the capacity to reduce the PQ pool (active, Q_B-reducing and inactive, Q_B-non-reducing centres) has been suggested.

All of the above-mentioned processes were studied in their role in photoacclimation of *Microcystis aeruginosa* and *Scenedesmus protuberans* to high and fluctuating PPFD. Our aim was to use the results of this study to interpret shifts between cyanobacterial and green algal dominance in lakes, induced by changes in the underwater light climate as a result of varying mixing intensities (see Reynolds, 1987).

MATERIALS AND METHODS

Organisms and growth conditions

The cyanobacterium *Microcystis aeruginosa* CCAP 1450/11 and the green alga *Scenedesmus protuberans* (culture collection, Laboratory for Microbiology, University of Amsterdam) were grown in continuous cultures on a 10:14 hour light:dark cycle. The mineral medium that was used has been described by van Liere & Mur (1978). Cultures were flushed with air at a rate of 150 l h^{-1} to ensure CO_2 supply and mixing; 200 mg l^{-1} HCO_3^- was added as supplementary carbon. All cultures were kept within 10 % of an absorbance (A) of 0·15 cm^{-1}; measurements were made at the start of the light period on a Pharmacia-LKB Novaspec II spectrophotometer at 750 nm. The gas vesicles of *Microcystis* were collapsed before measuring A. The flat culture vessel that was used had the dimensions 237 × 30 × 450 mm. The temperature was kept at 20 °C by a water jacket, connected to a Colora thermocryostat, and placed between the light source and the vessel; illumination was provided by a high-intensity light source (Osram Metallogen HMI 1200 W/GS), the spectral composition of which resembled the solar spectrum. PPFD was measured as PAR on the surface of the vessel with a Licor LI-190SB quantum sensor connected to a chart recorder. PPFD impinging on the vessel was varied by the angular slat displacement of a Venetian blind, connected to a stepping motor. Specially designed softwater controlled the diurnal light regime. When dark conditions were required a pneumatically operated door was closed (a detailed description of the culture unit can be found in Kroon, van Hes & Mur, 1992*a*).

Light regimes

Three different light regimes were investigated. The first regime simulated the absence of mixing in a situation where the algae remain at the lake surface during the day, i.e. the mixing depth, Z_m, equals zero ($Z_m = 0$). The diurnal variation in PPFD followed a sine curve. The second light regime simulated the presence of density microstratification in the water column, in which mixing was restricted to the near-surface layer. The algae were assumed to be circulated to a depth equal to 70 % of the euphotic zone ($Z_m = 0{\cdot}7\ Z_{eu}$). The third regime simulated more intensive mixing in which the algae were assumed to be mixed below the euphotic zone; they spent 30 min in the light followed by a dark period of 30 min ($Z_m = 2\ Z_{eu}$). In the simulation mixing rates were assumed to remain constant, although this is in fact a simplification (MacIntyre, 1993). The maximal PPFD of 1100 μmol m^{-2} s^{-1} was similar for all three regimes. Figure 1 shows the variation in PPFD for the three regimes. The 10 h light period started at 08.00 h local time, and lasted till 18.00 h. Values of the total daily light dose (TDLD) were calculated as described in Kroon *et al.* (1992*a*), and can be found in Table 1. Samples were taken from the continuous cultures at regular intervals between 07.00 and 19.00 h. A number of variables were measured in order to follow the dynamics of photosynthesis in the fluctuating light field. All time series were studied in duplicate, and the data represent the average values of the two series. Daily averaged values were calculated for all suitable variables. The estimation

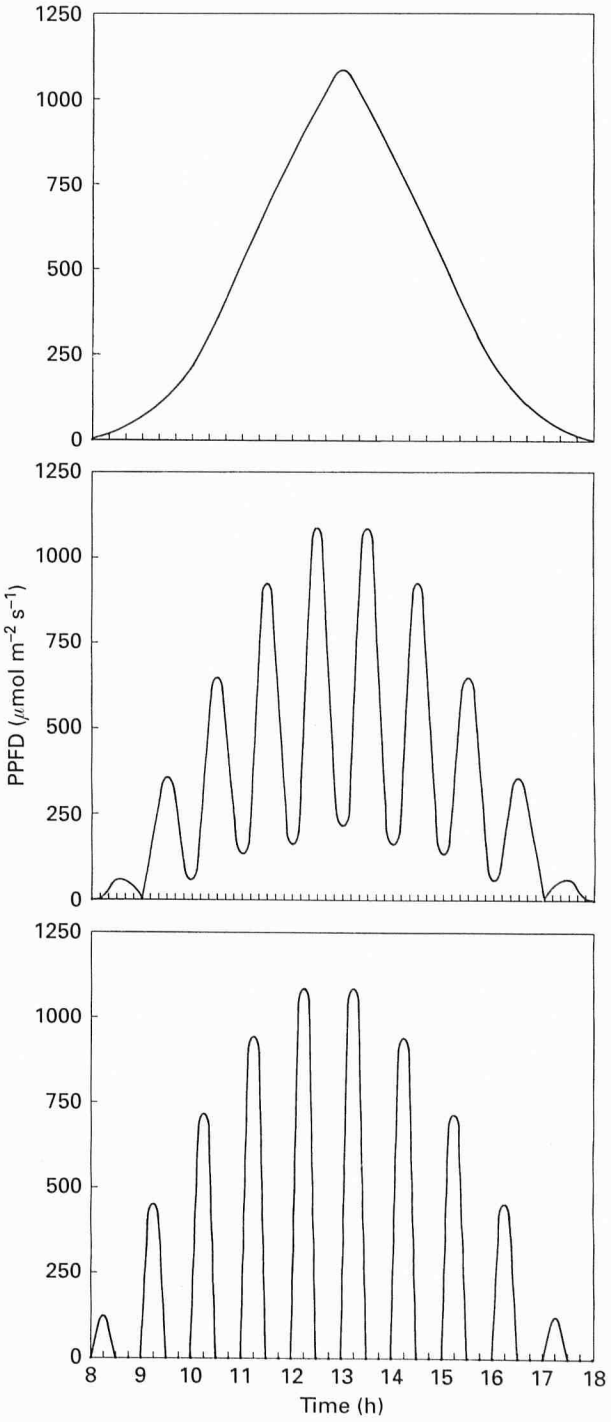

Figure 1. Variation in PPFD (μmol m^{-2} s^{-1}) during the light period (08.00–18.00 h) of three dynamic light regimes: $Z_m = 0$ (top); $Z_m = 0{\cdot}7\ Z_{eu}$ (middle), $Z_m = 2\ Z_{eu}$ (bottom).

Table 1. *Growth rates* (d^{-1}) *of* Scenedesmus protuberans *and* Microcystis aeruginosa *in the three light regimes of this study as a function of the total daily light dose* [*TDLD* (*mol photons* m^{-2})]

	$Z_m = 0$	$Z_m = 0{\cdot}7\ Z_{eu}$	$Z_m = 2\ Z_{eu}$
TDLD (mol photons m^{-2})	22·9	16·3	7·7
Average PPFD (μmol m^{-2} s^{-1})	636	453	214
μ *Scenedesmus* (d^{-1})	0·34	0·29	0·19
μ *Microcystis* (d^{-1})	0·17	0·15	0·13

of significant differences was based on the 95 % confidence levels; the number of degrees of freedom was six for $Z_n = 0$, and 12 for $Z_m = 0{\cdot}7\ Z_{eu}$ and $Z_m = 2\ Z_{eu}$.

Biomass, carbohydrate, buoyancy and pigments

The protein content of a sample was taken as a measure of biomass. Protein was determined in triplicate after hydrolysis in 0·1 M NaOH according to the Folin method, with bovine serum albumin as the standard (Herbert, Phipps & Strange, 1971). Carbohydrate was determined in triplicate after acid hydrolysis according to the anthrone method with D-glucose as standard (Herbert *et al.*, 1971). Standard errors were less than 5 % of the mean. Pigments (chlorophylls and carotenoids) were determined by HPLC (Isco model 2350) using reversed-phase ion-pair chromatography. The wave-length for detection of pigments was 455 nm. Pigments of *Microcystis* were extracted in the dark, in 90 % acetone at room temperature for 30 min, after 4 × 30 s sonication. Pigments of *Scenedesmus* were extracted in the dark, in hot methanol for 2 × 2 min. Duplicate determinations were made.

The buoyancy state of *Microcystis* cells was determined in Microslides (Camlab) in triplicate after centrifugation at 200 ***g***, according to the method of Oliver & Walsby (1988). Standard errors were less than 5 % of the mean.

Photosynthesis/irradiance

The photosynthesis/irradiance relationship (P/I curve) was determined from the rates of oxygen production in the system described by Dubinsky *et al.* (1987). The samples were flushed with nitrogen for 20 s before the start. Dark respiration was measured during the first 15 min. Subsequent photosynthesis measurements were made at 15 PPFDs between 4 and 2000 μmol photons m^{-2} s^{-1}, of which seven were below the I_k value. The set of measurements was completed within 30 min. The short incubation times used ensured that photo-inhibition induced during determination of the P/I relationship was minimal (see Henley, 1993). P_{max}, the maximum gross rate of oxygen production, was calculated as the average of the three highest values. α, the efficiency of photosynthesis, was determined via linear regression of the light-limiting part of the P/I relationship.

Cross-sections and quantum requirement

The chlorophyll specific optical-absorption cross-section (a^*_{ph}), was determined as described by Kroon *et al.* (1992*b*) from *in vivo* absorption spectra determined on an Aminco (DW 2000 spectrophotometer, with the cuvette placed in front of the photomultiplier, and assuming a light source with a constant spectral composition. The minimum quantum requirement for oxygen production (QR) was calculated as the ratio of a^*_{ph} and α (Tilzer, 1984).

Fluorescence measurements

Fluorescence measurements were made using a PAM-Walz 101 fluorometer (Schreiber, Schliwa & Bilger, 1986). All measurements were performed at room temperature. A light-emitting diode delivered a low-intensity, non-actinic, pulsed measuring beam of peak wavelength 650 nm, half-bandwidth 20 nm. This beam, and additional actinic illumination, was guided to the sample through a bundle of fibre optics that also collected fluorescence from the sample. The very weak measuring beam alone did not induce variable fluorescence and was used to determine F_0, the minimal fluorescence yield of previously dark-adapted cells (all PS II reaction centres open), and F_s, steady-state fluorescence yield in the presence of actinic light. In the *in situ* saturation pulse analysis the fibre-optic holder was placed directly on the surface of the culture vessel. A Schott KL-1500 light source gave saturation pulses of 0·9 s duration. This pulse of 12000 μmol photons m^{-2} s^{-1} closed all PS II reaction centres, which induced maximal fluorescence, F_m (after dark adaptation) or F'_m (in the light; see van Kooten & Snel, 1990). The saturating pulse was administered at 90 s intervals.

Fluorescence induction curves were made in triplicate in the presence or absence of 10 μM DCMU. Samples were allowed 10 min relaxation in the dark and were subsequently concentrated on Gelman 0·45 μm membrane filters. Analysis of the DCMU induction curves followed procedures outlined by Owens (1986). The measured value for F_m was not corrected, even though it is often difficult to determine the true F_m value correctly (see Appendix of Owens, 1986). The kinetics of fluorescence induction represent the closure of PS II reaction centres (see Krause & Weis, 1991). Fluorescence induction curves were measured for culture conditions $Z_m = 0{\cdot}7\ Z_{eu}$ and $Z_m = 2\ Z_{eu}$ only.

RESULTS

Light dose and growth rate

TDLD and the average PPFD during the 10 h light period varied almost three-fold between the three regimes. Efficient acclimation of cells to the lower TDLD can explain why growth rate did not increase in proportion to the light dose (see Falkowski, 1980). It must be realized, however, that an average PPFD of 214 μmol m^{-2} s^{-1} when $Z_m = 2\ Z_{eu}$, still significantly exceeds the average PPFD used in most studies on photoacclimation in phytoplankton. Alternatively the high TDLD when $Z_m = 0$ may have induced photo-inhibition of photosynthesis, resulting in a lowered growth rate, although photo-inhibition may also reflect a regulatory mechanism that allows growth under dynamic PPFD (see discussion). Growth rates of *Scenedesmus* were higher than the corresponding growth rates of *Microcystis*. Even the growth rate of *Scenedesmus* when $Z_m = 2\ Z_{eu}$ still exceeded the growth rate of *Microcystis* when $Z_m = 0$.

Biomass and pigments

Protein. Protein content of the cells showed an increase during the light period (data not shown). In *Scenedesmus*, when $Z_m = 0$, protein increased both at the start and the end, but not in the middle of the light period, whilst in *Microcystis* the increase was restricted to the morning. The rate of increase was less pronounced in the other two light regimes. Daily averaged protein concentrations were not significantly different for the three light regimes, but protein contents of *Microcystis* were significantly higher than those for *Scenedesmus*.

Pigments of Scenedesmus. The daily averaged value of the ratio of Chl($a+b$)/protein of *Scenedesmus* did not differ significantly between regimes (Fig. 2*a*). This is seemingly in contradiction with the ability of green algae to adjust their chlorophyll content to irradiance level (e.g. Falkowski, 1980; Richardson, Beardall & Raven, 1983; Humbeck, Hoffmann & Senger, 1988). The chlorophyll content of the cells in all three regimes increased during the first 2–3 h of the light period. When $Z_m = 0$ the Chl/protein ratio decreased slightly after 13.00 h, mainly due to a lower Chl *b* content. The daily averaged Chl *a*/*b* ratio when $Z_m = 2\ Z_{eu}$ was significantly higher than for the other two regimes (Fig. 2*b*). Almost all of the Chl *b* is contained within the light-harvesting antenna of PS II (e.g. Busheva *et al.*, 1991). A high Chl *a*/*b* ratio when $Z_m = 2\ Z_{eu}$ can be interpreted as a decrease in the number of light-harvesting complexes of PS II (LHC II), despite the fact that conditions when $Z_m = 2\ Z_{eu}$ provided the lowest TDLD. A decrease in Chl *b*, compared to only a limited decrease in Chl *a*, led to a sharp rise in the *a*/*b* ratio during the light period when $Z_m = 0$. Increase in Chl *a*/*b* ratios usually follows a transition from low to high PPFD (Richardson *et al.*, 1983; Osborne & Raven, 1986), although Humbeck *et al.* (1988) found little variation in Chl *a*/*b* between low and high PPFD-acclimated *Scenedesmus*. The Chl *a*/*b* ratio varied between two and four; comparable ratios were found by Kroon *et al.* (1992*b*), when the green alga *Chlorella pyrenoidosa* was grown under fluctuating PPFD.

The pigment composition of *Scenedesmus* in all three light regimes resembled what is generally found in a green alga (Hager & Stransky, 1970): chlorophyll *a* and *b*, α- and β-carotene, and lutein as the dominant xanthophyll. Besides lutein, the xanthophylls violaxanthin, neoxanthin and antheraxanthin were found. Unfortunately zeaxanthin could not be quantified since, during HPLC, it co-eluted with the larger lutein peak. The separation of neo- and violaxanthin was also inadequate. Thus information on the xanthophyll cycle remained incomplete. A decrease in the lutein content during the light period when $Z_m = 0$, both on a per-protein and Chl ($a+b$) basis, contributed to the decrease in light-harvesting capacity during the light period when $Z_m = 0$ (Fig. 2*c*). Lutein is almost exclusively found in LHC II, improving light harvesting at low PPFD (Anderson & Osmond, 1987), as does β-carotene, although this pigment is more closely associated with the reaction centre of PS I (Sieferman-Harms, 1985; Oilazola & Duerr, 1990). In our study the lutein and β-carotene (Fig. 2*d*) contents of *Scenedesmus* when $Z_m = 2\ Z_{eu}$ were significantly higher than when $Z_m = 0$, and $Z_m = 0{\cdot}7\ Z_{eu}$ showed intermediate values. The ratio of total carotenoid/Chl ($a+b$) closely resembled the dominating lutein patterns, i.e. the lower TDLD had a higher ratio.

Pigments of Microcystis. In *Microcystis* the Chl/protein ratio displayed little variation during the light period of all three regimes (Fig. 2*a*). Cells in the $Z_m = 2\ Z_{eu}$ regime contained twice the amount of Chl/protein of cells when $Z_m = 0$ or $Z_m = 0{\cdot}7\ Z_{eu}$. Gibson (1985) found a sharp decline in Chl/protein of cyanobacteria with an increasing length of the photoperiod. Our study did not include quantification of the major cyanobacterial light-harvesting complexes, the phycobilisomes. Several authors (e.g. Raps *et al.*, 1983; Zevenboom & Mur, 1984; Nultsch & Agel, 1986), however, showed that the ratio of Chl/phycobilisomes remained constant over a wide range of TDLD. The carotenoids zeaxanthin, myxoxanthophyll and β-carotene were present in varying quantities. Of these myxoxanthophyll is typical for photosynthetic prokaryotes (Stransky & Hager, 1970). A similar carotenoid composition in *Microcystis* was found by Paerl, Tucker & Bland (1983). Raps *et al.* (1983) found echinenone in addition, and detected myxoxanthophyll only in cultures grown at

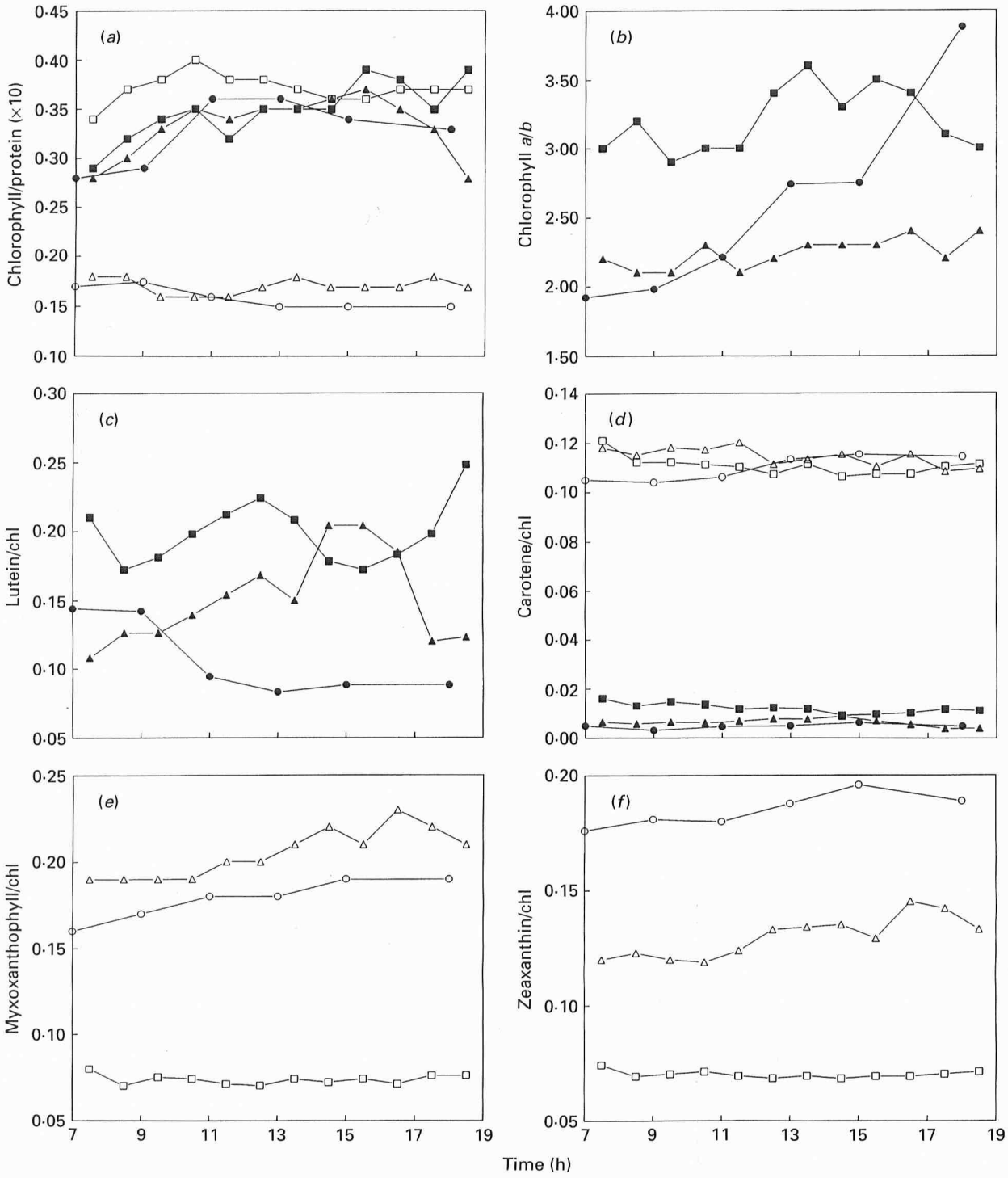

Figure 2. Diel variation in pigment contents of cells of *Scenedesmus protuberans* (solid symbols) and *Microcystis aeruginosa* (open symbols) during the light period (08.00–18.00 h) of three dynamic light regimes: $Z_m = 0$ (●, ○); $Z_m = 0{\cdot}7\ Z_{eu}$ (▲, △); $Z_m = 2\ Z_{eu}$ (■, □). (*a*) Chlorophyll $(a+b)$/protein of *Scenedesmus* and *Microcystis*; (*b*) chlorophyll a/b of *Scenedesmus*; (*c*) lutein/chlorophyll $(a+b)$ of *Scenedesmus*; (*d*) carotene/chlorophyll $(a+b)$ of *Scenedesmus* and *Microcystis*; (*e*) myxoxanthophyll/chlorophyll of *Microcystis*; (*f*) zeaxanthin/chlorophyll of *Microcystis*.

high PPFD. The values of β-carotene/Chl were not significantly different for the three light regimes (Fig. 2*d*). Myxoxanthophyll was high when $Z_m = 0$ and $Z_m = 0{\cdot}7\ Z_{eu}$, but significantly lower when $Z_m = 2\ Z_{eu}$ (Fig. 2*e*). The zeaxanthin content increased with an increase in TDLD (Fig. 2*f*). Zeaxanthin has been identified as a major carotenoid involved in photoprotection (Demmig-Adams *et al.*, 1990*a*, *b*). Increased myxoxanthophyll and zeaxanthin contents led to higher carotenoid/chlorophyll ratios at higher TDLD. This form of acclimation to higher light doses is generally found in cyanobacteria (Collins & Boylen, 1982; Nultsch & Angel, 1986; Garcia-Pichel & Castenholz, 1991). Individual caro-

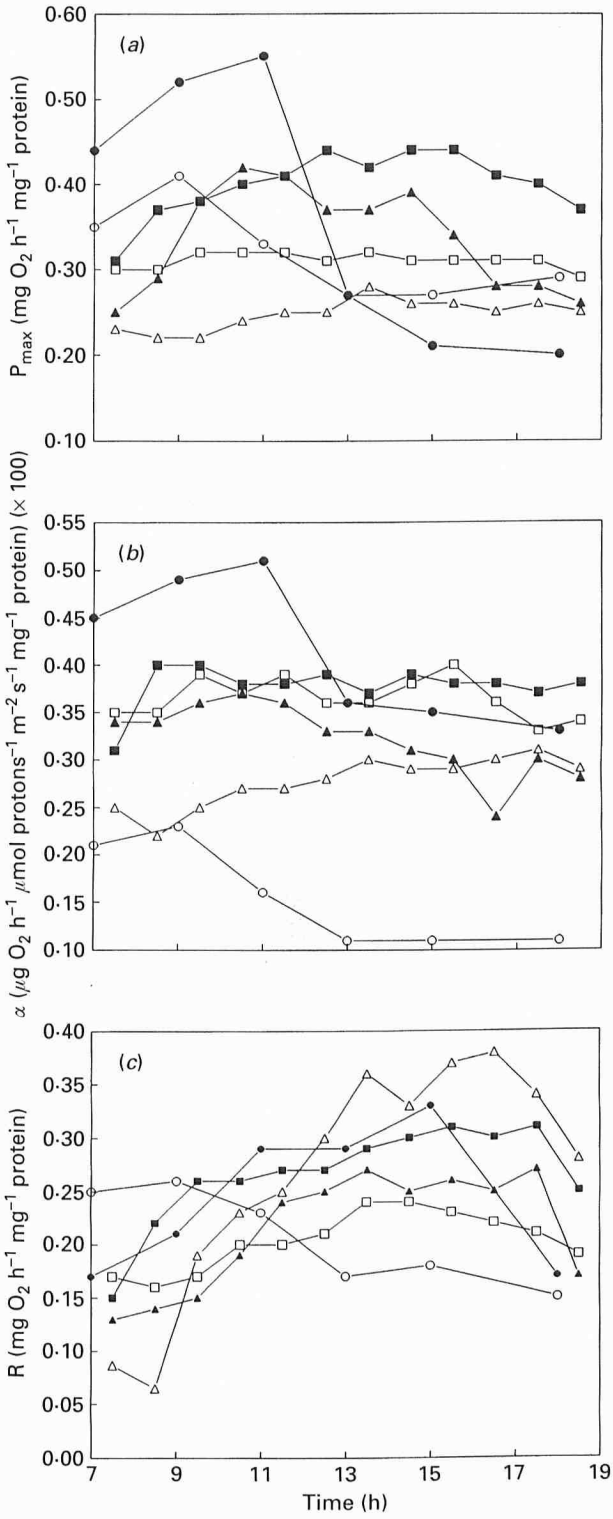

Figure 3. Diel variation in photosynthesis–irradiance variables of *Scenedesmus protuberans* (solid symbols) and *Microcystis aeruginosa* (open symbols) cells during the light period (08.00–18.00 h) of three dynamic light regimes: $Z_m = 0$ (●, ○); $Z_m = 0{\cdot}7\ Z_{eu}$ (▲, △); $Z_m = 2\ Z_{eu}$ (■, □). (*a*) Maximum oxygen production rate, P_{max}/protein; (*b*) efficiency of photosynthesis, α/protein; (*c*) respiration rate, R/protein.

tenoids showed little variation, and acclimation to the diurnal variation in PPFD was not observed, in contrast to the patterns of lutein in *Scenedesmus*.

Photosynthesis and respiration

P_{max} and α for Scenedesmus. All P/I variables were expressed on a per-protein basis. Characteristic of the patterns in P_{max} and α for *Scenedesmus* was the decline during the $Z_m = 0$ light regime (Fig. 3*a*, *b*). P_{max} and α initially increased, as new Chl was synthesized in the early hours of the light period, but decreased from 11.00 h onwards. A combined drop in both P_{max} and α is a strong indication of photoinhibition of photosynthesis (Vincent *et al.*, 1984; Neale, 1987). The depression of P_{max} and α did not revert completely towards the end of the photoperiod, as was observed in the green alga *Chlorella pyrenoidosa* during a sinusoidal light regime (Grobbelaar *et al.*, 1992). The patterns of P_{max} and α when $Z_m = 0{\cdot}7\ Z_{eu}$ resembled the pattern discussed when $Z_m = 0$. The decline, however, was less pronounced. P_{max} increased when $Z_m = 2\ Z_{eu}$, whereas α remained stable. The daily averaged values of α in *Scenedesmus* were not significantly different for the three light regimes. Cells grown at low TDLD did not use light energy more efficiently than cells grown at high TDLD. Normally α, on a per-cell or per-biomass basis, increases with acclimation to low irradiance (Herzig & Dubinsky, 1992; Wilhelm, 1993).

P_{max} and α for Microcystis. When $Z_m = 0$, the patterns of P_{max} and α resembled those described for *Scenedesmus*. In *Microcystis* P_{max} and α increased slightly when $Z_m = 0{\cdot}7\ Z_{eu}$. When $Z_m = 2\ Z_{eu}$ the values of P_{max} and α were relatively stable. With a decrease in TDLD α/protein increased significantly. This type of acclimation has been described before for *Microcystis* (Zevenboom & Mur, 1984), and is more in line with general findings (Wilhelm, 1993).

Dark respiration. Respiration in *Scenedesmus* increased gradually during the light period of all three regimes (Fig. 3*c*). It is well established that light enhances subsequent mitochondrial dark respiration (Stone & Ganf, 1981; Falkowski, Dubinsky & Santostefano, 1985). Photorespiration itself is also stimulated by a history of high PPFD. It probably reduced net C-fixation in the second half of the light period, especially in the regimes with a high TDLD (see results on carbohydrate accumulation). Respiration in *Microcystis* declined during the light period when $Z_m = 0$, though it showed the more common light stimulation in the other two light regimes. Shyman, Raghavendra & Sane (1993) made the observation that dark respiration in the cyanobacterium *Anacystis nidulans* declined after extended exposure to high PPFD.

Carbohydrate storage in Scenedesmus. Excess photosynthate accumulates in the carbohydrate pool of cells (Post *et al.*, 1985), and can be used as energy

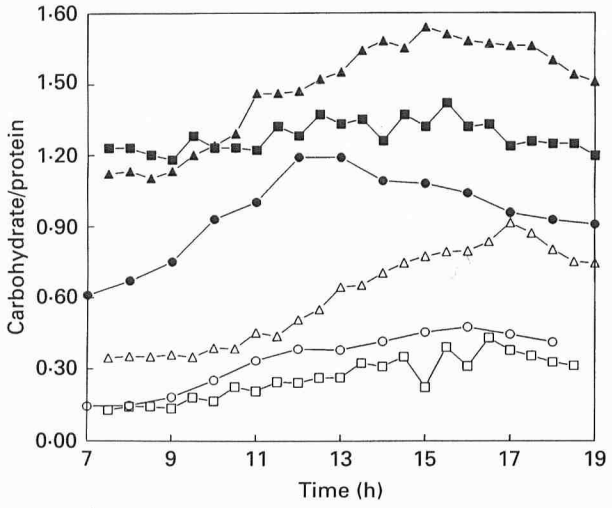

Figure 4. Diel variation in carbohydrate storage by cells of *Scenedesmus protuberans* (solid symbols) and *Microcystis aeruginosa* (open symbols) during the light period (08.00–18.00 h) of three dynamic light regimes: $Z_m = 0$ (●, ○); $Z_m = 0{\cdot}7\ Z_{eu}$ (▲, △); $Z_m = 2\ Z_{eu}$ (■, □).

supply for biosynthesis during the dark period. In *Scenedesmus* the amount of carbohydrate that accumulated during the light period increased with TDLD (Fig. 4). Nevertheless, the smallest total carbohydrate content was found when $Z_m = 0$, where carbohydrate accumulated until 12.00 h only, and decreased in the second part of the light period. Similar observations were made (but to a lesser extent) for the other two regimes where carbohydrate contents of the cells also started to decrease before the end of the light period. Stimulated photorespiration probably restricted net C-fixation during this part of the light period. In addition, there was an increased energy flow towards protein synthesis, the rate of increase of which rose towards the end of the light period (data not shown). Accumulation of carbohydrate was restricted when $Z_m = 2\ Z_{eu}$. Carbohydrate that was stored during the 30 min light periods was immediately respired during the following 30 min dark period, especially in the second half of the diurnal light period, emphasizing once more that light enhances the subsequent level of dark respiration.

Carbohydrate storage in Microcystis. In *Microcystis* cessation of carbohydrate accumulation was found when $Z_m = 0$ during the 3 h with the highest PPFD, a further increase being noted between 14.00 and 16.00 h. Thus the cyanobacterium did not show the drop in carbohydrate storage early in the photoperiod that was seen in the green alga. When $Z_m = 0{\cdot}7\ Z_{eu}$ the accumulation of carbohydrate increased sharply between 11.00 and 17.00 h. In the second part of the diurnal light period when $Z_m = 2\ Z_{eu}$, carbohydrate stored in the 30 min light periods was largely respired during the alternating dark periods, as was found for *Scenedesmus*.

Changes in buoyancy of *Microcystis* cells closely followed the patterns in carbohydrate storage (data not shown). This is in agreement with current understanding that diel changes in buoyancy in *Microcystis* are mediated through variation in ballast content only (Kromkamp & Mur, 1984). Loss of buoyancy was inhibited during the period of high PPFD when $Z_m = 0$. A complete loss of buoyancy was found between 15.00 and 17.00 h when $Z_m = 0{\cdot}7\ Z_{eu}$, whereas buoyancy fluctuated in the alternating light–dark periods of the $Z_m = 2\ Z_{eu}$ light regime.

Minimum quantum requirement. The minimum quantum requirement for photosynthesis, QR, was consistently higher than its theoretical value of 8 mol photons $(\text{mol } O_2)^{-1}$, although an unrealistically low value close to the theoretical minimum was measured for *Microcystis* at the start of the $Z_m = 0$ light regime (Fig. 5). The actual limit is closer to 10 mol photons $(\text{mol } O_2)^{-1}$ (see Schofield *et al.*, 1993). QR when $Z_m = 0$ increased sharply during the light period for *Scenedesmus*, due to a decline in α. When $Z_m = 2\ Z_{eu}$ the value fluctuated around a relatively high level throughout the photoperiod, whilst when $Z_m = 0{\cdot}7\ Z_{eu}$, QR increased between 12 and 14 h. The diurnal patterns of QR in *Microcystis* were clearly correlated to variations in α. When $Z_m = 0$, QR increased during the photoperiod; when $Z_m = 0{\cdot}7\ Z_{eu}$ the opposite was observed, whilst when $Z_m = 2\ Z_{eu}$ QR showed fluctuations around a value that was significantly higher than for the other two regimes.

Fluorescence

In situ *saturation pulse analysis in* Scenedesmus. In

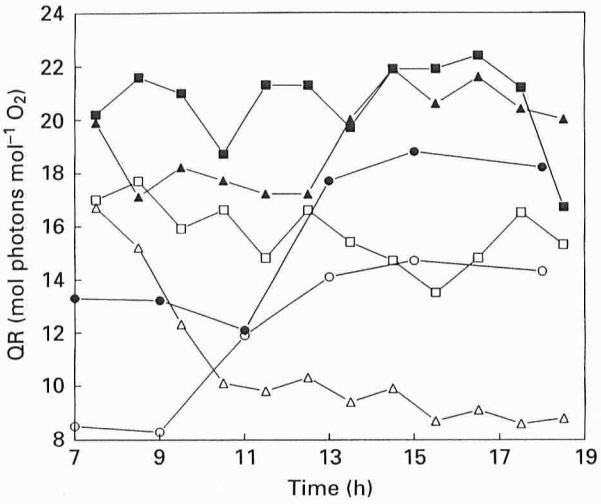

Figure 5. Diel variation in minimum quantum requirement for photosynthesis of cells of *Scenedesmus protuberans* (solid symbols) and *Microcystis aeruginosa* (open symbols) during the light period (08.00–18.00 h) of three dynamic light regimes: $Z_m = 0$ (●, ○); $Z_m = 0{\cdot}7\ Z_{eu}$ (▲, △); $Z_m = 2\ Z_{eu}$ (■, □).

the saturation pulse analysis, the steady-state fluorescence yield, F_s, and the maximum fluorescence yield, F'_m, were monitored during the photoperiod *in situ* (see van Kooten & Snel, 1990). In *Scenedesmus* cultures F_s increased, when PPFD rose to moderate values (at the beginning and the end of the photoperiods), or when exposure to high PPFD was short-lived (around noon when $Z_m = 2\ Z_{eu}$). This was presumably caused by a decrease in photochemical quenching. In contrast, quenching of F_s occurred during exposure to high PPFD (between 11.00 and 16.00 h when $Z_m = 0$ or $Z_m = 0{\cdot}7\ Z_{eu}$). Figures 6 and 7 show the fluorescence traces during the $Z_m = 0$ and $Z_m = 2\ Z_{eu}$ light regimes. The pattern of F'_m was inversely related to the pattern of PPFD. The value of F'_m is independent of photochemical quenching because all reaction centres are closed. Non-photochemical quenching increased when PPFD rose, probably initially as a result of energization of the thylakoid membrane, later in the light period when $Z_m = 0$ or $Z_m = 0{\cdot}7\ Z_{eu}$ also as a result of photo-inhibition (see Horton & Ruban, 1992). F'_m was usually higher than F_s; an exception was found during the period of high PPFD when $Z_m = 0$ or $Z_m = 0{\cdot}7\ Z_{eu}$, when the difference between F'_m and F_s was close to zero. Apparently all reaction centres were already closed by the actinic illumination. A similar observation was made by Kroon (1992) and van Wijk *et al.* (1993).

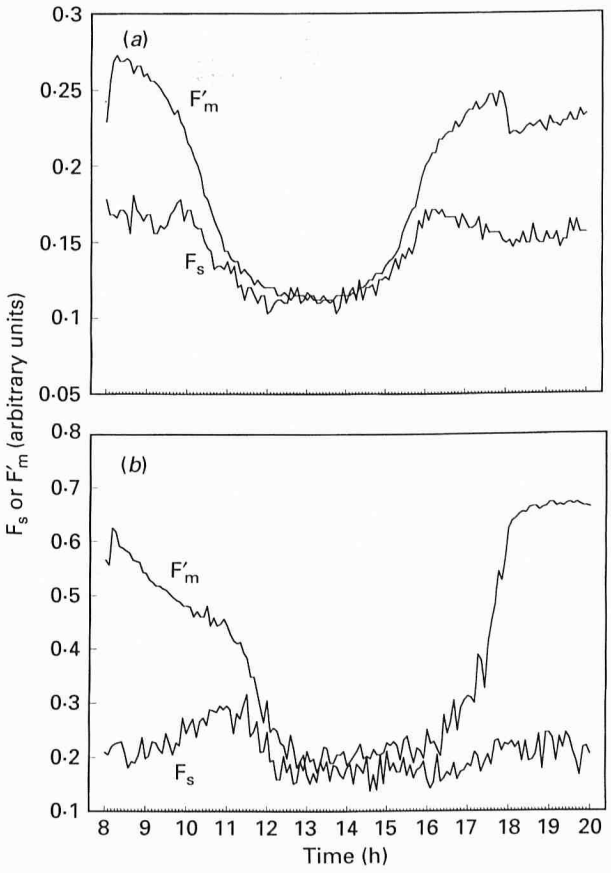

Figure 6. Diel patterns of steady-state fluorescence F_s and maximum fluorescence F'_m of (*a*) *Microcystis aeruginosa* and (*b*) *Scenedesmus protuberans* during *in situ* saturation pulse analysis in the light period when $Z_m = 0$ (08.00–18.00 h).

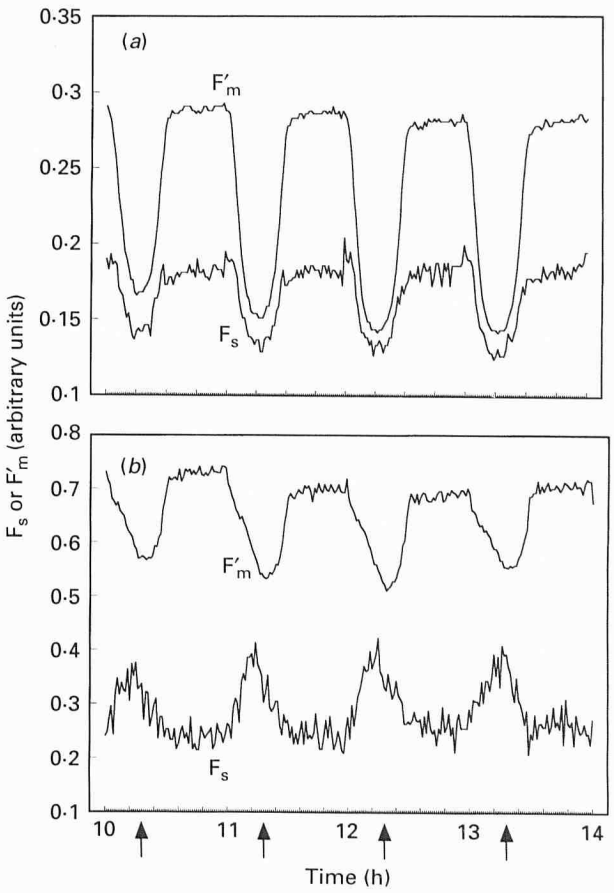

Figure 7. Diel patterns of steady-state fluorescence F_s (lower trace) and maximum fluorescence F'_m (upper trace) of (*a*) *Microcystis aeruginosa* and (*b*) *Scenedesmus protuberans* during *in situ* saturation pulse analysis between 10.00 and 14.00 h in the light period when $Z_m = 2\ Z_{eu}$. The arrows mark the time of maximum PPFD during the 30 min light periods, which were alternated with 30 min dark periods in a 10 h diurnal light regime.

In situ *saturation pulse analysis in* Microcystis. In *Microcystis* F_s was quenched more readily than in *Scenedesmus*; with quenching occurring even at low to moderate PPFD. This was found for all three light regimes, including $Z_m = 2\ Z_{eu}$, where F_s was quenched throughout the 30 min light periods, except for the first and last few minutes (Figs 6 and 7). Thus the initial rise in F_s that was observed for *Scenedesmus* when $Z_m = 0$ was absent. F'_m was related to PPFD in a manner similar to that seen in *Scenedesmus*, but F'_m decreased upon termination of the light period, and displayed a sudden increase immediately upon the start of the light period. Romero, Lara & Sivak (1992) made similar observations for the cyanobacterium *Anacystis nidulans*.

Photon yield of PS II. The photochemical efficiency of PS II per absorbed photon, or photon yield, ϕ_p, as formulated by Genty, Briantais & Baker (1989), was

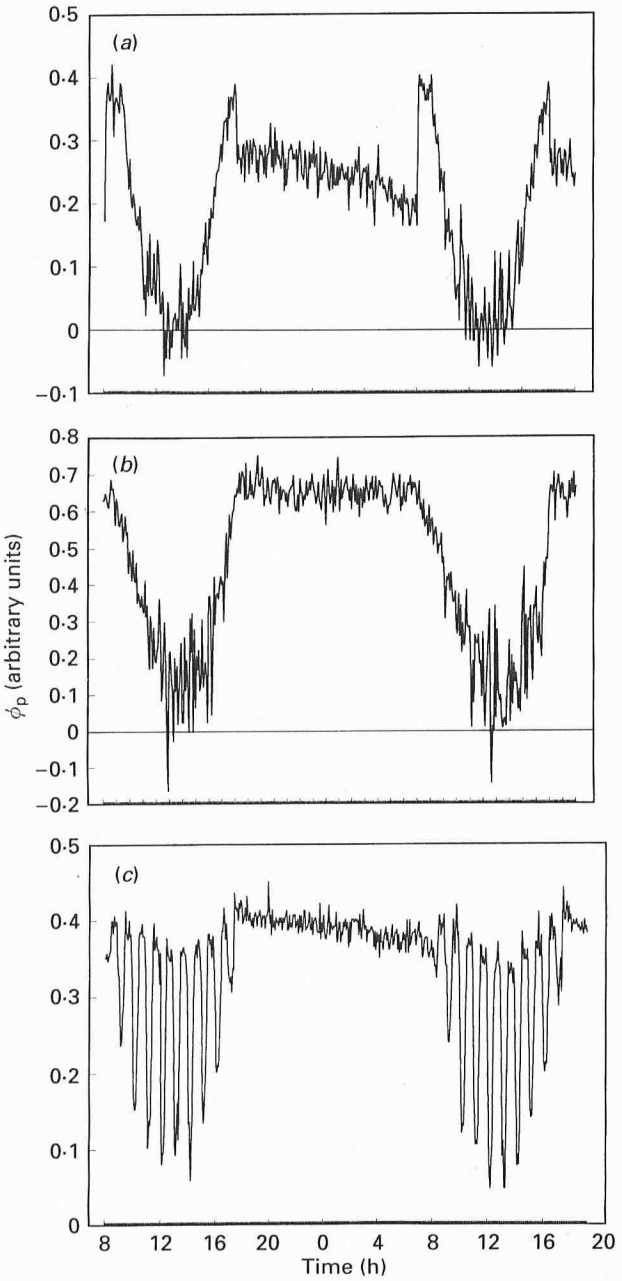

Figure 8. Diel patterns of ϕ_p, the photon yield of PS II, calculated as $(F'_m - F/F'_m)$, during the light period (08.00–18.00 h) when $Z_m = 0$ for (*a*) *Microcystis aeruginosa*, and (*b*) *Scenedesmus protuberans*, and (*c*) during the light period when $Z_m = 2\ Z_{eu}$ for *Microcystis aeruginosa*. Note that the time period covers 1·5 diel cycles.

calculated as $(F'_m - F_s)/F'_m$. It gives a good estimate for the efficiency of linear electron transport through the photosystem (Hofstraat *et al.*, 1994). The yield was inversely related to PPFD in all light regimes (Fig. 8). This is in agreement with the results of Kroon (1992). The noisiness of the data is caused by bubbling the cultures with air. During the dark period ϕ_p remained at a stable, high level for *Scenedesmus* cultures. In *Microcystis*, a steady decline in ϕ_p was observed during the dark period, when $Z_m = 0$. The decrease measured up to 50% of the maximal decrease in the preceding light period. The value of ϕ_p decreased with duration of the dark conditions, whilst the decrease in the dark period of $Z_m = 2\ Z_{eu}$ was only small (Fig. 8). It has been suggested that cyanobacteria are in state 2 after dark acclimation (Allen, Sanders & Holmes, 1985). This would result in a distribution of excitation energy away from PS II, in favour of PS I, and can perhaps explain the lowered ϕ_p in darkness (see Discussion).

Kautsky effect. Fluorescence induction of dark-acclimated cells follows a characteristic pattern, known as the Kautsky effect. Fluorescence rises from a minimal level F_0 to an intermediate maximum F_i, followed by a plateau or dip, F_d, and finally to its peak level F_p (Q_A fully reduced, all PS II reaction centres closed). Romero *et al.* (1992) reported that the cyanobacterium *Anacystis* did not show the Kautsky effect, F_v of dark acclimated cells was minimal, and state transitions interfered with the Kautsky effect upon actinic illumination. *Microcystis* did show a typical Kautsky effect; although caution is needed to interpret the data. The 10 min dark relaxation before each measurement did slightly reduce F_p. However, the brief light pulse used to induce fluorescence was insufficient to cause a state transition; no actinic light was present, so there was no state transition simultaneous with the Kautsky effect. For both species, when $Z_m = 0{\cdot}7\ Z_{eu}$, F_p was preferentially quenched. The quenching of F_i and F_0 was less pronounced, with F_0 remaining constant in *Scenedesmus* (Fig. 9). F_d was equal to F_i in both species (not shown). An increase in F_0 may reflect damage to the photosystem caused by exposure to high PPFD (Demmig-Adams & Adams, 1992), and has been found in cyanobacteria (Sivak & Vonshak, 1988) and red algae (Hanelt, Huppert & Nultsch, 1992). When $Z_m = 2\ Z_{eu}$, quenching of F_0, F_i and F_p was minimal (*Microcystis*) or absent (*Scenedesmus*).

Quenching of variable fluorescence. Minimum and maximum fluorescence as determined during fluorescence induction in the presence of DCMU were used to calculate variable fluorescence ($F_v = F_m - F_0$), and the ratio of variable to maximum fluorescence (F_v/F_m). In *Scenedesmus* F_v and F_v/F_m were quenched during the middle part of the light period of $Z_m = 0{\cdot}7\ Z_{eu}$ only, whilst F_v and F_v/F_m were quenched from the start of the light period onwards in *Microcystis* (data not shown). F_v/F_m returned to its initial value towards the end of the light period of $Z_m = 0{\cdot}7\ Z_{eu}$ in both organisms. The main component of non-photochemical quenching of variable fluorescence has been related to energization of the thylakoid membrane (q_e). This type of quenching, however, is promptly reversed after a brief period of dark relaxation (see Krause & Weis, 1991), which was not observed in these studies. Quenching through state transitions (q_t) could also be excluded, since 10 min illumination with light of 714 nm,

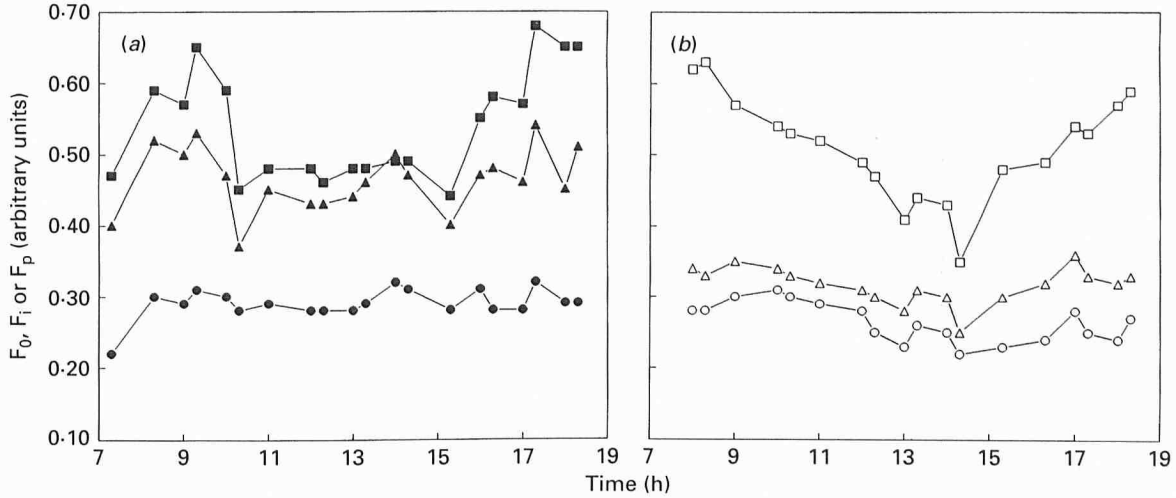

Figure 9. Changes in fluorescence levels characteristic of Kautsky induction curves for dark-acclimated cells of (*a*) *Scenedesmus protuberans* (solid symbols) and (*b*) *Microcystis aeruginosa* (open symbols), during the light period when $Z_m = 0{\cdot}7\ Z_{eu}$. Fluorescence near origin of induction curve F_o (●, ○); at the intermediate peak F_i (▲, △); and at the plateau F_p (■, □).

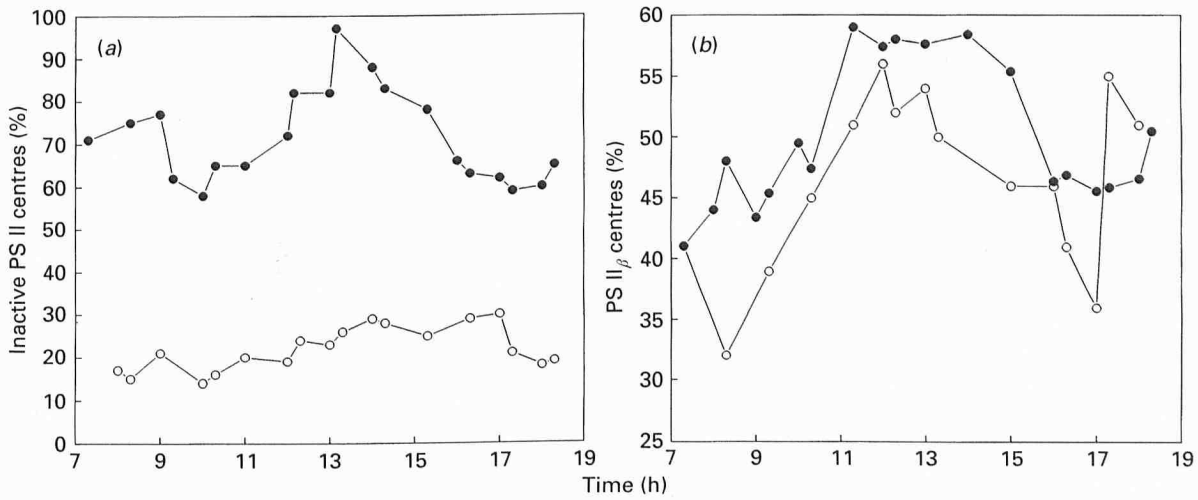

Figure 10. Diel variation in the amount of (*a*) inactive, PS II Q_B-non-reducing centres, calculated as $(F_i - F_o)/(F_p - F_o)$, and (*b*) PS II$_\beta$ centres, during the light period (08.00–18.00 h) when $Z_m = 0{\cdot}7\ Z_{eu}$, in *Scenedesmus protuberans* (solid symbols), and *Microcystis aeruginosa* (open symbols).

administered at the peak in PPFD in the diurnal light regime, did not reverse quenching of F_v. State transitions may have occurred earlier in the photoperiod at lower PPFD, when according to Walters & Horton (1993) they are more likely to occur. It was therefore concluded that the observed quenching of F_v at the peak in PPFD occurred through photoinhibition (q_i). Quenching of the ratio F_v/F_m has been used as a quantitative indicator of photoinhibition, with restoration of its value indicating recovery from photo-inhibition (see for example Demmig-Adams & Adams, 1992; Oquist, Chow & Anderson, 1992).

PS II Q_B-non reducing centres. The initial rapid rise from F_0 to F_i is believed to result from the presence of a pool of inactive PS II reaction centres (Cao & Govindjee, 1990; Ort & Whitmarsh, 1990). These centres are unable to reduce PQ, and are named Q_B-non-reducing (or inactive) centres. In *Scenedesmus* the amount of Q_B-non-reducing centres when $Z_m = 0{\cdot}7\ Z_{eu}$, calculated as $(F_i - F_0)/(F_p - F_0)$, increased towards a maximum of 0·98 at the peak in PPFD and decreased from this time onwards (Fig. 10*a*). A similar high value was reported for the green alga *Chlamydomonas reinhardtii* by Falk *et al.*, 1992. The pattern for *Scenedesmus* when $Z_m = 2\ Z_{eu}$ was similar to that seen when $Z_m = 0{\cdot}7\ Z_{eu}$, but with a much decreased amplitude. It is possible that the rise in inactive centres when $Z_m = 0{\cdot}7\ Z_{eu}$ was partly responsible for the quenching of F_v at high PPFD, since they are thought to convert excitation energy to heat (Krause, 1988). In *Microcystis* the fraction of inactive centres did not exceed 0·3, and varied little during the photoperiods when $Z_m = 0{\cdot}7\ Z_{eu}$ or $Z_m = 2\ Z_{eu}$.

PS II_α and PS II_β centres. From a semilogarithmic transformation of the increase in area above the fluorescence induction curve of DCMU-poisoned cells (see Owens, 1986), a biphasic behaviour emerged for *Scenedesmus*, indicating the presence of two types of PS II reaction centres. The PS II_α and PS II_β centres reside in different areas of the thylakoid membranes (Melis, 1991). The cross-section of PS II_α (responsible for the fast phase of the induction curve) is usually two to three times larger than the cross-section of PS II_β (Krause & Weis, 1991). PS II_β centres do not have a peripheral LHC II complex. It has been proposed that these centres are an intermediate in a PS II repair cycle (Melis, 1991). Kinetic analysis of the DCMU curves of *Microcystis* also showed biphasic behaviour, which by analogy to green algae, would indicate two pools of PS II reaction centres with different cross-sections. Assuming all PS II centres showed identical fluorescence yields (van Wijk *et al.*, 1993), the fraction of PS II_β when $Z_m = 0{\cdot}7\ Z_{eu}$ increased from 44 % (*Scenedesmus*) or 32 % (*Microcystis*) at the start of the light period to a maximum of about 60 % at the peak in PPFD (Fig. 10*b*). When $Z_m = 2\ Z_{eu}$ the amplitude of the variation was smaller. A decrease in number of PS II_α centres can result from photoinhibition (Neale & Melis, 1991). Falk *et al.* (1992) found a 20 % decrease in PS II_α centres after exposure to high PPFD.

Rate constants for closure of PS II. The rate constants for the closure of PS II reaction centres during induction of fluorescence are indicative of the effective absorption cross-sections of the PS II centres. The initial rate of closure of PS II_α centres ($K_{\alpha i}$) was slower than the final rate of closure ($K_{\alpha f}$). A possible explanation is the transfer of excitons from closed PS II_α reaction centres to open PS II_α centres. This phenomenon, called connectivity, has been described for eukaryotes and assumes a statistical bed of antenna complexes, where excitons can move freely from one reaction centre to another (Owens, 1986). Theoretically it should improve quantum yield at low PPFD (Baker & Webber, 1987). No significant difference in connectivity was observed between the green alga and the cyanobacterium in this study (data not shown). In *Scenedesmus*, both $K_{\alpha i}$ and K_{af} dropped during the first hour of the light period when $Z_m = 0{\cdot}7\ Z_{eu}$. $K_{\alpha i}$ remained at a low level afterwards, but $K_{\alpha f}$ recovered partly after 13.00 h (Fig. 11), similar to the pattern described for *Chlorella* by Kroon (1992). When $Z_m = 2\ Z_{eu}$, the rate constants stayed at a constant, relatively low level throughout the light period. K_β, the rate constant for closure of PS II_β centres in *Scenedesmus*, was about one-sixth of $K_{\alpha i}$, indicating that the difference in effective cross-sections between PS II_α and PS II_β was larger than that found in previous studies (Krause & Weis, 1991). Initially there was a decrease in K_β when $Z_m = 0{\cdot}7\ Z_{eu}$, but a full recovery was observed towards the end of the light period. When $Z_m = 2\ Z_{eu}$, K_β remained constant during the light period. The rate constants for closure of PS II reaction centres in *Microcystis* showed only a limited response to variation in PPFD. $K_{\alpha i}$ and K_β remained at stable, relatively high values throughout the light period, whereas $K_{\alpha f}$ showed an irregular, but generally decreasing pattern (data not shown).

DISCUSSION

Comparison with other studies

In this study diurnal variation in photosynthetic variables has been investigated in light regimes simulating different degrees of mixing through the underwater light gradient. Several papers (e.g.

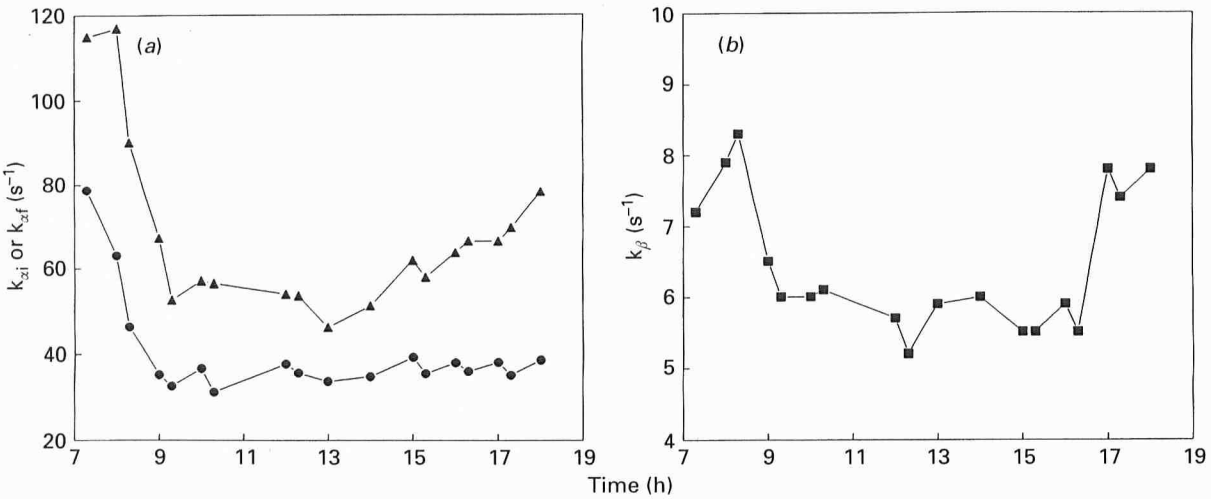

Figure 11. Changes in *Scenedesmus protuberans*, in rate constants for closure of PS II_α and PS II_β centres during the light period (08.00–18.00 h) when $Z_m = 0{\cdot}7\ Z_{eu}$. (*a*) $K_{\alpha i}$, initial rate constant for closure of PS II_α centres (●); $K_{\alpha f}$, final rate constant for closure of PS II_α centres (▲); (*b*) K_β, rate constant for closure of PS II_β centres (■).

Marra, 1978; Kroon *et al.*, 1992*b*, *c*; Kromkamp & Limbeek, 1993) have reported on the effect of fluctuations in the light regime while keeping TDLD constant. Others have discussed the effect of varying TDLD while keeping PPFD at a constant level during the light period (e.g. Foy, Gibson & Smith, 1976; Raps *et al.*, 1983; Zevenboom & Mur, 1984). We chose to let a decrease in TDLD be a consequence of an increase in light fluctuations. This parallels the situation in lakes, where wind-induced mixing may not only increase the fluctuations in PPFD but also reduce the average light dose received by the alga. The different approaches followed by other authors, however, made it difficult to compare the various studies on the effect of dynamic light regimes on photosynthesis. As well as investigating different organisms, both the period of oscillation and the maximum PPFD often differed from our study (Marra, 1978; Cosper, 1982; Shin *et al.*, 1987; Kroon *et al.*, 1992*b*, *c*). Our study offered the advantage that direct comparison between two different taxonomic groups in the phytoplankton could be made. This should enhance interpretation of the data with respect to our understanding of the role of photoacclimation in natural populations.

Growth rates

Growth rates of the organisms were not proportional to TDLD (Table 1). Similar results have been obtained before. Post, Loogman & Mur (1986) concluded that the daily light dose does not always account for the resultant light-limited growth. Acclimation to short light periods was less efficient than acclimation to low PPFD. Zevenboom & Mur (1984) found that growth rate of *Microcystis* was not proportional to the product of PPFD and length of the photoperiod. Photo-inhibition of growth of cyanobacteria has been reported by Eloff *et al.* (1976), and it may be an explanation for depression of growth when $Z_m = 0$ (and to a lesser extent when $Z_m = 0{\cdot}7\ Z_{eu}$).

Pigments

In the green alga *Scenedesmus* the chlorophyll content of the cells was independent of the light regime (Fig. 2*a*), whereas in the cyanobacterium *Microcystis* chlorophyll/protein in the $Z_m = 2\ Z_{eu}$ light regime showed the more common response of an increase with lower TDLD (e.g. Zevenboom & Mur, 1984). Osborne & Raven (1986) have discussed how an increase in pigment content could decrease the specific absorption coefficient through a rise in the package effect. Inefficient investment in pigments can be a severe energy constraint at low PPFD. This could partly explain the lack of variation in intracellular chlorophyll in the green alga. The light-harvesting phycobilisomes in *Microcystis* have a more peripheral organization, which should allow for a greater amount of pigment per unit area of thylakoid without increasing the package effect (Osborne & Raven, 1986). Moreover, there might be a difference in the way cyanobacteria and green algae acclimate to fluctuations in PPFD. Green algae may adjust their chlorophyll content in response to the maximum PPFD, whereas cyanobacteria may respond mainly to TDLD. Most studies have looked at light–dark acclimation, using a non-fluctuating light regime (see, for example, Richardson *et al.*, 1983). In these studies not only TDLD, but also the maximal PPFD varied between the cultures (in contrast to our study where the maximum PPFD was constant). It is therefore not possible from these experiments to draw conclusions about the nature of the regulating factor.

Minimum quantum requirements

Minimum quantum requirements for cultures grown under light-limiting conditions are expected to remain constant, at values close to the theoretical minimum (Tilzer, 1984). Senger & Fleischhacker (1978) found that QRs for *Scenedesmus* grown at high and low PPFD were identical, though Shin *et al.* (1987) reported an unexplained higher QR for *Scenedesmus* grown under fast-fluctuating PPFD, as was found by Kroon *et al.* (1992*c*) for *Chlorella*. In *Scenedesmus*, it may be that the relatively high carotenoid content in the $Z_m = 2\ Z_{eu}$ light regime was responsible for the high QR observed in this study. Several carotenoids, e.g. lutein, do transfer excitons to chlorophyll, but with a relatively low efficiency (Siefermann Harms, 1985). However, since the carotenoid content in the $Z_m = 2\ Z_{eu}$ light regime of *Microcystis* was significantly lower than that found during the other two regimes, this is not enough to explain the observed high value of QR in this cyanobacterium.

Acclimation to high PPFD

Photo-inhibition. Photo-inhibition affected the diurnal patterns of protein formation, carbohydrate accumulation, pigment content and composition, P/I variables and fluorescence variables, especially when $Z_m = 0$. The onset of the changes in the various variables coincided well with each other, although the onset of recovery showed less correspondence. In a study by Havaux (1988) it was found that thermal energy dissipation started well before the decrease in photosynthetic oxygen evolution, whilst during recovery normal heat dissipation was associated with only partly restored oxygen evolution. These results suggest that photoprotection started before damage became apparent. Falk & Samuelsson (1992) and Falk *et al.* (1992) found that F_v/F_m was the most sensitive indicator of

photo-inhibition; the onset of changes in α was slower, and the decrease in P_{max} occurred even later in the photoperiod. Henley (1993) concluded from these observations that PS II electron transport capacity initially exceeds the capacity for carboxylation. Once a critical number of PS II centres have been inactivated, electron transport limits photosynthetic rates at saturating PPFD as well. It is likely that the time resolution of our measurements, when $Z_m = 0$, was insufficient to pick up differences in the onset of decrease in α and P_{max}. In *Scenedesmus*, during exposure to high PPFD, Chl *b* and lutein decreased, resulting in decreased light-harvesting capacity (Fig. 2). Both P_{max} and α declined (Fig. 3), carbohydrate accumulation and protein synthesis were discontinued (Fig. 4) and QR rose sharply (Fig. 5). Steady-state fluorescence yield, F_s, in the *in situ* pulse analysis (Fig. 6), and F_v, were quenched in a manner indicative of photo-inhibition. The number of PS II Q_B-non-reducing centres and PS II_β centres increased when PPFD intensified in the $Z_m = 0{\cdot}7\ Z_{eu}$ light regime (Fig. 10). Unfortunately some of these data are missing for the $Z_m = 0$ light regime. The response of *Microcystis* to high PPFD was similar to that of the green alga. The pigment composition of the cyanobacterium changed so that the ratios of zeaxanthin and myxoxanthophyll to Chl increased (Fig. 2*d*, *e*).

A striking effect of the introduction of an oscillation in the sinusoidal light regime was the partial relief from photo-inhibition, which was most prominently seen as the absence of a sharp fall in P_{max} and α (Fig. 3). Quenching of F_s in *Microcystis*, however, indicated that presumably a certain degree of photo-inhibition still occurred in the oscillating light regimes (see Fig. 7). Photo-inhibition will be suppressed in oscillating light regimes if the length of exposure to high PPFD is shorter than the time course for inhibition of photosynthesis (Marra, 1978; Yoder & Bisshop, 1985). In lakes, microstratification of the near-surface layer and wind-induced mixing largely determine the time during which phytoplankton is exposed to high PPFD (Vincent *et al.*, 1984).

Photoprotection and repair. High PPFD does not necessarily lead to net photo-inhibition or photodamage. Irradiance stress results from absorption of light in excess to that used for photosynthesis. Additional mechanisms are needed to protect the photosystem, and remove excess energy, predominantly in the form of thermal energy dissipation, in which zeaxanthin plays a role (Demmig-Adams & Adams, 1992). Exposure of PS II to excess PPFD leads to a stepwise inhibition of electron transport, resulting in degradation of the D_1-protein (see Aro *et al.*, 1993 for a review). A repair cycle has been proposed for eukaryotes, generating a continuous cycle of damage and repair in the light (Melis, 1991). Both $PSII_\beta$ and PSII Q_B-non-reducing centres are intermediates in the cycle. The rate of the repair cycle increases with PPFD. It has been suggested that photo-inhibition becomes apparent only when the rate of damage at high PPFD exceeds the rate of repair (Greer, Berry & Björkman, 1986). Krause & Weis (1991), however, have questioned the general validity of this concept. Damaged centres lose their peripheral antenna (converting them to PS II_β/QB-non-reducing centres). The antenna is re-bound in the grana after repair and activation of the damaged centre in the non-appressed thylakoid membranes (Melis, 1991). Re-binding of the antenna may not occur under high PPFD. This would result in an expansion of the pool Q_B-reducing centres of the PS II_β type, which are less sensitive to photo-inhibition than PS II_α, due to their smaller antenna size (Melis, 1991; Neale & Melis, 1991), and could be a mechanism through which the cells (partly) maintain their rate of photosynthesis at high PPFD. Our results did not confirm these findings, but are in agreement with Briantais, Cornic & Hodges (1988) and Oquist *et al.* (1992), who also found a decrease in active PS II Q_B-reducing centres after high PPFD exposure (see Fig. 10), which they held responsible for the observed inhibition of photosynthesis. Inactive centres still act as traps for excitation energy but convert it to heat. It has been proposed that this is one of the mechanisms responsible for photoinhibitory quenching of fluorescence (Giersch & Krause, 1991), and should therefore be conceived of as a protective mechanism. At 13.00 h in *Scenedesmus* almost all the centres were of the PS II Q_B-non-reducing type, but there was still a 40% population of PS II_α centres. Thus a strict relationship between PS II_β and Q_B-non-reducing centres did not seem to hold. Falk *et al.* (1992) emphasized that the methods used to study the PS II heterogeneity probably underestimate the amount of PS II Q_B-reducing centres, and are qualitative rather than quantitative. This may also (partly) explain why photosynthesis continued when fluorescence measurements indicated a population of inactive centres of nearly 100%. Similar observations were made by Falk & Samuelsson (1992).

Comparison of photoinhibition in the two species. There were noticeable differences in the quenching of F_s between the two organisms. In *Scenedesmus* F_s was quenched only during more extended exposure to high PPFD. In *Microcystis* F_s was quenched at most PPFDs (Figs 6, 7). This may indicate that *Microcystis* increased the rate constant of thermal energy dissipation over the rate constant of photochemistry, indicating that excitation energy was removed from the antenna, even during relatively short exposure to high PPFD or exposure to moderate PPFD. It is possible that the different quenching patterns in the cyanobacterium and the green alga result from the

continuous presence of zeaxanthin in *Microcystis*, whereas the xanthophyll cycle in *Scenedesmus* presumably restricted the presence of zeaxanthin to periods with an excess excitation energy. Demmig-Adams *et al.* (1990*a*), however, suggested that zeaxanthin in cyanobacteria in some lichens needed activation by excessive PPFD. Our results would suggest that zeaxanthin in *Microcystis* was activated at relatively low PPFD.

It seems that *Scenedesmus* had a more adaptable response to exposure to high PPFD. *Scenedesmus* decreased light harvesting when $Z_m = 0$ by reducing the amount of Chl *b* and lutein, whereas pigment contents of *Microcystis* showed little diurnal variation (Fig. 2). *Scenedesmus* also decreased the effective cross-section of PS II_α at the start of the light period when $Z_m = 0{\cdot}7\ Z_{eu}$, which would provide protection against over-excitation (Fig. 11). The PS II_α cross-section of *Microcystis* remained constant. The xanthophyll cycle in green algae would offer larger flexibility in readjusting the level of zeaxanthin in accordance with a temporary demand for photoprotection.

Acclimation to fluctuating PPFD

Respiration. Is *Scenedesmus* better adapted to a fluctuating light environment than the cyanobacterium? Stone & Ganf (1981) concluded that *Microcystis* was not well adapted to mixing conditions in a lake because of strong photorespiration and the enhancement of dark respiration after light exposure, decreasing net C-fixation. In this study, no significant difference between *Microcystis* and *Scenedesmus* for the ratio of P_{max}/respiration was found. In our opinion it is not so much enhancement of respiration in fluctuating PPFD that is detrimental, but a low efficiency of conversion of carbohydrate to protein, as was found for *Microcystis* by Kromkamp (1987).

The role of zeaxanthin. The absence of a xanthophyll cycle, and continuous presence of zeaxanthin, is another disadvantage in fluctuating PPFD. The proposed activation of zeaxanthin in cyanobacteria by high PPFD only (Demmig-Adams, 1990) would decrease the detrimental loss of excitation energy at low PPFD, although the results of our study suggest that the threshold for activation is low. Deactivation of zeaxanthin upon return to low PPFD, following exposure to high PPFD, however, is likely to be a relatively slow process (Demmig-Adams, 1990). This would lead to losses of excitation energy under fluctuating light conditions.

State transitions. In cyanobacteria, PQ-cytbf is an intermediate in the transport of electrons, in both photosynthesis and respiration (Matthijs & Lubberding, 1988). In *Microcystis* decreased values of ϕ_p in the dark probably indicated that the redox state of the PQ-cytbf complexes changed. This could induce a state 1–2 transition, resulting in a redistribution of excitation energy between PS I and PS II, inducing a low fluorescent state, since less excitation energy reaches PS II (Allen *et al.*, 1985; Romero *et al.*, 1992). During deep mixing phytoplankton spends alternating periods in the light and the dark, as in our $Z_m = 2\ Z_{eu}$ light regime. The distribution of PPFD over PS I and PS II could be unfavourable for photochemistry at low PPFD, which *Microcystis* would receive when it returned to the illuminated part of the water column. Other authors found that green algae, like cyanobacteria, are in state 2 after dark acclimation (see Williams & Allen, 1987). *In situ* saturation pulse analysis showed that, in contrast to *Microcystis*, F_m and ϕ_p in *Scenedesmus* remained at a high level during the dark period (Fig. 8). This would indicate that redistribution of excitation energy in favour of PS I occurred only in the cyanobacterium.

Extrapolation of the results of this study to phytoplankton competition in lakes

What is the relevance of this study for our understanding of phytoplankton ecology in lakes? Paerl *et al.* (1983, 1985), concluded that susceptibility to high PPFD is an artefact found only in isolates of *Microcystis*. In contrast Ibelings & Mur (1992) and Ibelings & de Winder (1994) found photo-inhibition in natural populations of *Microcystis*, which manifested itself as inhibition of rates of gross photosynthesis and quenching of F_v/F_m in surface blooms. Buoyancy of *Microcystis* keeps the cyanobacterium near the lake surface in lakes with stable water columns (Ibelings *et al.*, 1991). The low threshold for thermal removal of excess excitation energy in *Microcystis*, seen in Figures 6 and 7, induced prompt photo-inhibition, but this would offer protection against more severe photodamage in the natural environment. Photo-inhibition can be envisaged as a mechanism for the long-term protection of photosystem II (Oquist *et al.*, 1992); it is part of the normal physiology of cells (Ögren & Rosenqvist, 1992). Most studies on photo-inhibition focus on the response of cells, acclimated at low PPFD, to sudden exposure to high PPFD (e.g. Falk *et al.* 1992; Hanelt *et al.*, 1992). Cells acclimated to high PPFD are not expected to suffer from net D_1 degradation in the absence of additional stress factors, like high or low temperature (Horton & Ruban, 1992). Cells in the $Z_m = 0$ light regime, were acclimated to a high TDLD, but still showed a diel pattern of inhibition and recovery (although recovery of the P/I variables was limited during the light period). Photo-oxidation of a *Microcystis* population could occur during surface bloom formation following a prolonged period of deep mixing. Under these circumstances

cells, acclimated to a low average light climate, are suddenly exposed to full sunlight, and will not be able to invoke photoprotective mechanisms on a sufficiently short timescale (see Ibelings and de Winder, 1994).

Growth rates of *Scenedesmus* were higher in all regimes. This need not cause surprise, since growth rate vs. irradiance curves of cyanobacteria and green algae are expected to cross at much lower average PPFD than the average PPFDs (Table 1) used in this study (Mur, Gons & van Liere, 1977). The study of Mur *et al.* (1977) was, however, conducted under continuous light. The effect of a dynamic light regime was not taken into consideration. The higher growth rates imply that *Scenedesmus* would outcompete *Microcystis* in all three regimes. The green alga showed a more flexible response in its acclimation to high, as well as to fluctuating PPFD. On the other hand, under relatively stable conditions in a lake, the TDLD for *Microcystis* would be higher than for *Scenedesmus*, because its buoyancy would keep the colonies near the surface, at the same time decreasing the fluctuations in PPFD (Ibelings *et al.*, 1991). However, there is a demand for the regulation of buoyancy to protect *Microcystis* against excessive irradiance doses in Z_{eu}. Under stable conditions *Scenedesmus* would suffer from increased sedimentation losses, decreasing the net gain in biomass, even when growth rates are relatively high. This all might serve to demonstrate once more the prominent importance of buoyancy and the regulation of buoyancy in the success of *Microcystis* in stable lakes.

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