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Photosystem II electron transport rates and oxygen production in natural waterblooms of freshwater cyanobacteria during a diel cycle

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[6]TO WHOM CORRESPONDENCE SHOULD BE ADDRESSED

The relationship between electron transport rate through PSII and photosynthetic oxygen evolution in cyanobacterial surface waterblooms was followed over a diel cycle. Chlorophyll fluorescence and photosynthetic oxygen evolution (PSOE) measurements were performed in a small-volume incubation chamber on samples taken from a fish pond. Measurement of light-response curves showed a close to linear relationship between electron transport rates (ETR) and PSOE up to irradiancies of 800 µmol quanta m^{-2} s^{-1}, except during mid-morning conditions. At higher irradiances, the relationship was non-linear. The regression coefficient κ (= PSOE/ETR) exhibited wide variation during the day (3.8–9.2), indicating that the use of ETR as a measure of PSOE in cyanobacterial waterblooms should be approached with caution. The involvement of alternate oxygen-consuming electron transfer pathways is discussed as a possible explanation for this discrepancy.

INTRODUCTION

In nature, cyanobacteria are often subjected to large irradiance gradients, especially in turbid waters. Such variations determine the structure, physiology and species composition of the phytoplankton population. The worsening of the underwater light climate due to the massive development of microalgal biomass represents one of the most important consequences of pronounced eutrophication of Czech fish ponds (Pechar, 1995, 2000).

In the absence of wind, gas-vacuolated, buoyant cyanobacteria float to the water surface to form waterblooms (Reynolds and Walsby, 1975; Walsby *et al*., 1992). Stability of the water column is a prerequisite for such bloom formation. Cyanobacteria may benefit from being close to the air–water interface because of a resupply of inorganic carbon, CO_2 diffusing in from air (Paerl and Ustach, 1982). This potential advantage may be counteracted by the risk of prolonged exposure to high irradiance (Ibelings and Maberly, 1998). Therefore, waterbloom-forming species need effective photoadaptation to avoid injury to their photosynthetic apparatus.

The activity and adaptive mechanisms of phytoplankton are traditionally measured by oxygen evolution measurements or radiolabelled CO_2 fixation. As measurement of the quantum yield of O_2 evolution (Φ_{O2}) and CO_2 fixation (Φ_{CO2}) is laborious and time-consuming, much attention has recently been focused on the use of variable chlorophyll fluorescence to provide rapid, real-time information on both photosynthesis and overall acclimation status of the photosynthetic apparatus (Krause and Weis, 1991).

PAM (pulse amplitude modulation) fluorometers can measure fluorescence yield with high selectivity against the background of ambient light (Schreiber *et al*., 1986). This equipment has allowed the application of saturating light pulses for transient saturation (closure) of PSII reaction centres. In this way, photochemical and non-photochemical quenching coefficients can be differentiated (Schreiber *et al*., 1986). Chlorophyll fluorescence quenching analyses

have been applied with considerable success for fast, non-invasive assessment of photosynthetic performance of terrestrial plants [for reviews see (Krause and Weis, 1991)], algae (Ting and Owens, 1992; Kroon, 1994; Hofstraat *et al.*, 1994) and cyanobacteria [e.g. (Hofstraat *et al.*, 1994; Campbell *et al.*, 1998, Hartig *et al.*, 1998)].

Weis and Berry were the first to derive a semi-empirical equation according to which the rate of CO_2 fixation could be predicted from fluorescence measurements (Weis and Berry, 1987). Later, the advance of quenching analysis made it possible to calculate the steady-state photosynthetic activity quantitatively, similar to conventional gas exchange methods in higher plants (Genty *et al.*, 1989). In higher plants, it was shown that under a variety of conditions, a linear relationship generally exists between the quantum yield of CO_2 fixation (Φ_{CO2}) and the effective PSII quantum yield, Φ_P, measured by chlorophyll fluorescence [e.g. (Genty *et al.*, 1989; Krall and Edwards, 1991)]. Similarly, by means of a pump-and-probe fluorescence technique, Kolber and Falkowski also constructed a semi-empirical model to estimate photosynthetic rates from light-stimulated changes in the quantum yield of chlorophyll fluorescence (Kolber and Falkowski, 1993). In algae, experimental comparison of quantum yields of PSII photochemistry (Φ_P), O_2 production (Φ_{O2}) and CO_2 fixation (Φ_{CO2}) has given contradictory results. Some authors observed a linear relationship between Φ_P and Φ_{O2} in the green alga *Scenedesmus* [e.g. (Heinze *et al.*, 1996)]. Similarly, photochemical efficiency of PSII, Φ_P, appeared to correlate well with growth rates in laboratory cultures of *Dunaliella tertiolecta* (Hofstraat *et al.*, 1994). On the contrary, in several algal classes, varying Φ_P/Φ_{O2} ratios were observed (Schreiber *et al.*, 1995; Gilbert *et al.*, 2000). Flameling and Kromkamp also showed considerable light-dependent variability in the Φ_P/Φ_{O2} ratio in four aquatic microalgae representing different taxonomic groups (Flameling and Kromkamp, 1998). In diatoms, a linear relationship between the rate of photosynthetic oxygen evolution and the rate of PSII electron transport was found only at limiting light intensities; approaching light saturation, the relation became curvilinear (Geel *et al.*, 1997).

In cyanobacteria, the measurement and interpretation of chlorophyll fluorescence data presents several serious problems (Büchel and Wilhelm, 1993; Campbell *et al.*, 1998). The first problem is the significant contribution of other fluorescing pigments (chlorophylls of PSI and phycobilins) to the total signal. This complicates the use of some parameters developed for higher plants whose use is based on the assumption that fluorescence signals originate predominantly from the PSII complex (F_V/F_M, $\Delta F/F_M'$, qN, NPQ). The second problem is the existence of state transitions (Fujita *et al.*, 1994; van Thor *et al.*, 1998; Koblížek *et al.*, 1998). State transitions are the most apparent, rapid (tens of seconds) light adaptation processes in cyanobacteria, considered to be a mechanism for redistributing excitation energy between the photosystems to avoid imbalance in photosynthetic electron transport. It is manifested by an efficient quenching of fluorescence that is present even in the dark. This feature hampers calculations of the extent of non-photochemical quenching (qN), as it usually needs comparison with the non-quenched state (which is, in higher plants and most algae, usually assured by dark adaptation).

To date, available information on the Φ_P/Φ_{O2} relationship in cyanobacteria is limited. As an example, in the cyanobacterium *Nostoc*, the rate of photosynthetic oxygen evolution was progressively slowed down compared with the electron transport rate at irradiances above the growth light level, allegedly reflecting increased electron flow to oxygen under excess irradiance (Sundberg *et al.*, 1997).

The aim of this investigation was to study the Φ_P/Φ_{O2} relationship in natural cyanobacterial surface waterblooms over a diel cycle. From the information gathered we aimed to understand the photosynthetic condition of the algae and possible stress, since they are important for development of phytoplankton in the fish ponds of South Bohemia.

METHOD

Organisms

Samples of a natural waterbloom were collected as a 0.5 cm surface layer from the Opatovický fish pond, close to the Institute of Microbiology, at various times during a calm, windless day. Fish ponds in South Bohemia are mostly eutrophic reservoirs which support summer average phytoplankton maxima up to 200 µg chlorophyll (Chl) l^{-1}. The phytoplankton mixture was dominated by the cyanobacterial species *Anabaena spiroides*, *Aphanizomenon gracile*, *Anabaena lemmermannii* and *Microcystis flos-aquae*, which formed 94% of the phytoplankton biomass (based on biovolume). Such a species composition is representative of late-summer waterblooms in these hypertrophic fish ponds of the Třeboň Basin (Pechar, 1995).

After collecting, the samples were transferred within minutes to a measuring chamber. The average chlorophyll concentration in samples was about 3 mg l^{-1}. Chlorophyll content was determined spectrophotometrically in 100% methanol (Lichtenthaler and Wellburn, 1983).

Set-up of equipment

Photosynthetic oxygen evolution and chlorophyll fluorescence quenching were measured simultaneously in a temperature-controlled (22°C), cylindrical chamber (5 ml

total volume) described by Bartoš *et al.* (Bartoš *et al.*, 1975), with the light path being about 15 mm. An oxygen electrode (Clark type), a magnetic stirrer and a multifurcated fibre optic cable (for fluorescence measurements) were mounted in the wall of the chamber, perpendicularly to the path of actinic light. Chlorophyll fluorescence parameters were determined by a low-intensity, modulated (1.6 kHz) beam from light-emitting diodes, with the excitation wavelength at 655 nm, and detected above 710 nm using the modulated fluorometer PAM 101–103 [H. Walz, Germany (Schreiber *et al.*, 1986)]. Oxygen concentrations in the solution were kept at a level corresponding maximally to 200% air saturation in order to avoid the promotion of photoinhibition at higher oxygen concentration, as noted by van Wijk and Krause (van Wijk and Krause, 1991). When oxygen concentration in the measuring chamber had increased twofold above normal saturation, it was depressed by flushing with nitrogen + 1% CO_2.

Measured parameters

The maximum F_M and minimum F_0 fluorescence yield were determined at set time intervals after 10–15 min of dark adaptation. F_M was reached by exposing cells to 200 µmol quanta m^{-2} s^{-1} of white light in the presence of 3-(3, 4-dichlorphenyl)-1, 1-dimethylurea to avoid the influence of state transitions (Campbell *et al.*, 1998). The maximum PSII quantum yield was calculated as $F_V/F_M = (F_M–F_0)/F_M$. ETR, called the relative electron transport rate, is the product of the effective photochemical yield of PSII, $\Phi_P = \Delta F/F_M' = (F_M'\text{-}F)/F_M'$ and photosynthetic photon flux density (PPFD) (Genty *et al.*, 1989; Geel *et al.*, 1997; Kromkamp *et al.*, 1998). F and F_M' represent the steady-state and maximum fluorescence measured in the light; the F_M' value was taken after saturating light pulses when all PSII reaction centres were closed. Non-photochemical quenching NPQ was calculated as $(F_M – F_M')/F_M'$ (Bilger and Björkman, 1990).

Rapid light-response curves of fluorescence parameters and photosynthetic oxygen production were measured for the phytoplankton samples taken from the fish pond at different times during a day. A reflector with a projector tungsten filament bulb (1000 W, Tungsram, Germany) served as the actinic light source and a carousel of eight neutral density filters was used to adjust the irradiances in the range from 0 to 2500 µmol quanta m^{-2} s^{-1}. The irradiances were measured using a cosine-corrected quantum sensor (Li-185B, Li-Cor, USA) placed horizontally.

Fluorescence and oxygen evolution were recorded simultaneously on a dual-pen chart recorder. Each actinic light exposure intensity lasted for about 90 s to obtain the steady-state fluorescence level F. Then, a saturating pulse (Schott KL1500 halogen light source, 6000 µmol quanta m^{-2} s^{-1}, 500 ms duration) was triggered to reach F_M'. The nomenclature of fluorescence parameters used was that according to van Kooten and Snel (van Kooten and Snel, 1990).

The correlation coefficient κ between PSOE and ETR was determined from linear regression of the first five data points on the light response curves measured in the light-limited region.

RESULTS

Two examples (at 08:30 and 12:00 h) of light-response fluorescence records (measured at 0, 7, 16, 28, 90, 150, 320, 780 and 1750 µmol quanta m^{-2} s^{-1}) of cyanobacterial waterblooms are shown in Figure 1. Significant

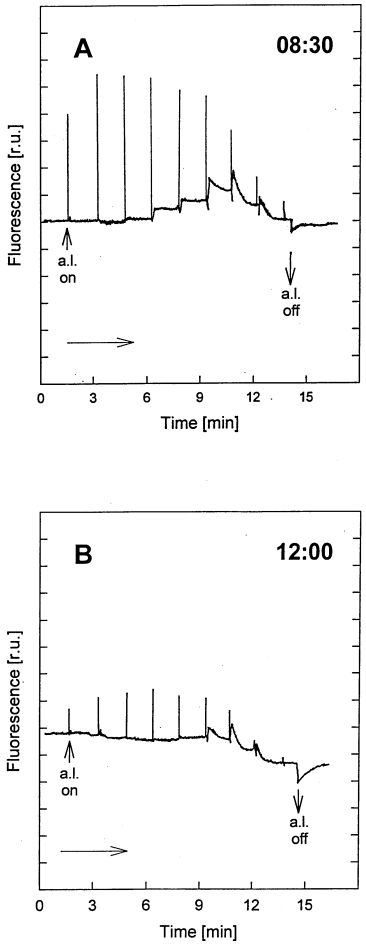

Fig. 1. Records of rapid light-response measurements of chlorophyll fluorescence using various actinic light intensities (0, 7, 16, 28, 90, 150, 320, 780 and 1750 µmol quanta m^{-2} s^{-1}). Large differences were found between samples in the morning (panel **A**) and at midday (panel **B**) with respect to the non-photochemical quenching pattern and the PSII photochemical yield $\Delta F/F_M'$ measured after saturating flashes of light. The left arrow (a.l. on) shows when the first level of actinic light (7 µmol quanta m^{-2} s^{-1}) was switched on, the arrow on the right (a.l. off) when it was switched off.

differences were seen between samples taken in the morning (Figure 1A) and at midday (Figure 1B) with respect to the PSII photochemical yield $\Delta F / F_M'$ as calculated from the response to saturating light pulse. In the sample taken at 08:30 h, we found the quenching of maximum fluorescence yield (F_M') in the lowest light intensities (the first saturating pulse at 7 µmol quanta $m^{-2}\,s^{-1}$ in Figure 1A), and in the midday sample, below 28 µmol quanta $m^{-2}\,s^{-1}$ (between the third and forth saturating pulse in Figure 1B). The observed quenching can be ascribed to the State 2 dependent quenching, which is eliminated at higher light intensities when the cells enter State 1.

The left-hand panels in Figure 2 show semi-logarithmic plots of the light-response curves of PSOE and ETR at various times of the day (08:30, 10:30, 12:00, 14:00 and 17:00 h). The 08:30 sample (panel A in Figure 2) was taken when the sampling place in the fish pond was still half shaded (about 450 µmol quanta $m^{-2}\,s^{-1}$). At this time, we measured the highest P_{max} [over 800 µmol O_2 mg^{-1} (Chl *a*) h^{-1}] for the day and there was no inhibition of PSOE at high irradiances. The light response curves in the morning revealed the highest efficiency of light utilization for PSOE as well as a high ETR_{max} value. The regression coefficient κ (calculated from the plot of PSOE versus ETR, Figure 2, right-hand panels) was the highest in the early morning sample (κ = 9.2), exhibiting an almost linear relationship between ETR and PSOE (Figure 2F). The highest ETR value was found at 17:00 h (more than 120 µmol e^- $m^{-2}\,s^{-1}$) when high irradiance stress relaxed (Figure 2E). At 10:30 h (Figure 2B), we found the highest inhibition of photosynthesis during the day, with a P_{max} of 360 µmol O_2 mg^{-1} (Chl *a*) h^{-1}, which was less than half of the value measured at 08:30 h. ETR was high at 10:30 h, and there was no light saturation at high irradiance as was seen in the 08:30 h sample. We also found the coefficient κ to be significantly smaller (κ = 5.6) than at 08:30 h. At 10:30 h, the relation between ETR and PSOE became

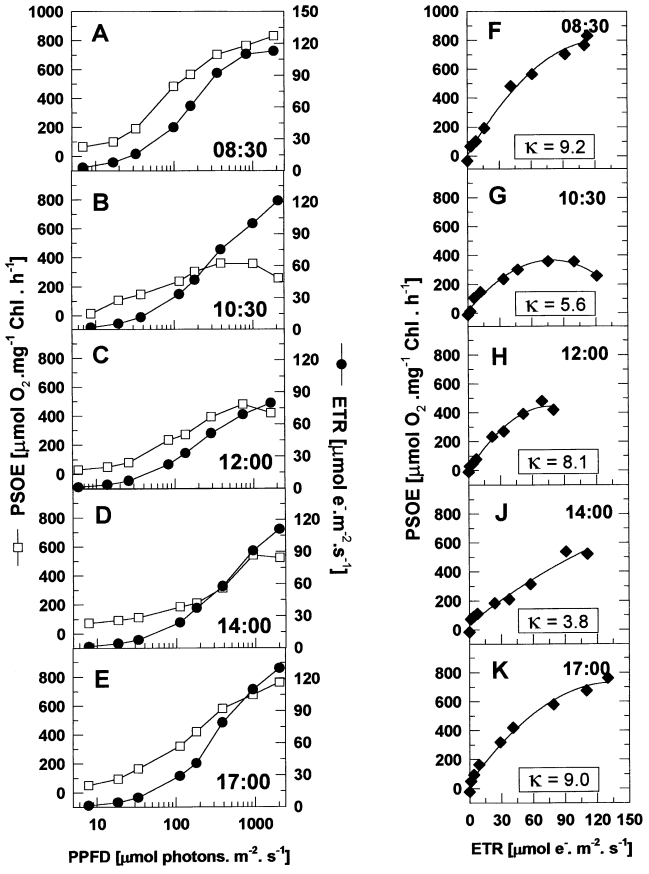

Fig. 2. Semi-logarithmic plots of the light-response curves of PSOE (photosynthetic oxygen evolution activity) and ETR (relative rate of electron transport) at various times of the day: 08:30, 10:30, 12:00, 14:00 and 17:00 h (panels **A–E**) and the relationship between ETR and PSOE (panels **F–K**). The parameter κ (efficiency of electron use in photosynthesis = photosynthetic efficiency) of the ETR/PSOE ratio was calculated as linear regression of the first five light-points on the light-response curve.

strongly curvilinear at the irradiance of 200 µmol quanta m^{-2} s^{-1} when PSOE showed light saturation (Figure 2G). At 12:00 h, the maximum value of ETR was lower by 30% (Figure 2C) than at 08:30 h (Figure 2A). The PSOE activity partially recovered compared with the 10:30 h sample, but was still much lower than in the 08:30 h sample (compare Figure 2A, 2B and 2C). The relationship between ETR and PSOE was slightly curvilinear, starting from about 800 µmol quanta m^{-2} s^{-1} (Figure 2H). A decrease in the maximum ETR between 10:30 and 12:00 h indicated the down-regulation of PSII electron transport efficiency as the photosynthetic activity was decreasing. At 14:00 h, ETR increased almost to the morning value and PSOE activity further increased compared with the values at 10:00 and 12:00 h (compare Figure 2B, 2C and 2D). The relationship between ETR and PSOE at 14:00 h became linear because there was probably a partial down-regulation of ETR and, at the same time, PSOE was recovering from the earlier depression (Figure 2J). At 17:00 h when the irradiance became similar (600 µmol quanta m^{-2} s^{-1}) to that at 08:30 h, the ETR and PSOE values were close to those measured in the morning (compare Figure 2A and 2E). The relationship between ETR and PSOE was linear until about 200 µmol quanta m^{-2} s^{-1}, and it was similar to the 08:30 h sample (compare Figure 2F and 2K). A detailed analysis of the light-response curves revealed an almost linear relationship between ETR and PSOE up to 400–800 µmol quanta m^{-2} s^{-1}, except in the 10:30 h sample. In all cases, the relationship became curvilinear close to saturating irradiances for PSOE. Thus, the limit of linearity is well above the acclimated growth irradiance (maximum 100 µmol quanta m^{-2} s^{-1}) as indicated by the irradiance level corresponding to the minimum of NPQ (Figure 3).

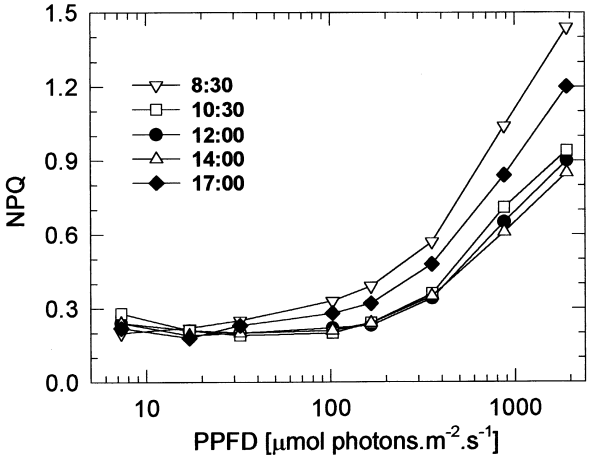

Fig. 3. Semi-logarithmic plots of the light-response curves of NPQ (non-photochemical quenching calculated according to Stern-Volmer formalism, $F_M/F_M' - 1$) at various times of the day: 08:30, 10:30, 12:00, 14:00 and 17:00 h.

Table I summarizes the following photosynthetic parameters: the maximum capacity of ETR_{max} and $PSOE_{max}$, the efficiency α (ETR and PSOE), and the saturating light intensity I_k calculated from light-response curves of ETR and PSOE. The experimental data were fitted using the model of Platt *et al.* (Platt *et al.*, 1980), according to which P (PSOE or ETR) = $[1 - \exp(-\alpha*I/P_t)]*\exp(-\beta*I/P_t)$, where P_t is a theoretical parameter and β is an inhibition coefficient. A major difference was found between the 08:30 and 10:30 h PSOE and ETR measurements. $PSOE_{max}$ decreased from 828 µmol O_2 mg^{-1} (Chl *a*) h^{-1} at 08:30 h to 361 µmol O_2 mg^{-1} (Chl *a*) h^{-1} at 10:30 h, while ETR_{max} increased slightly from 113 µmol e^- m^{-2} s^{-1} at 08:30 h to 119 µmol e^- m^{-2} s^{-1} at 10:30 h. The highest efficiency α ($\alpha = \sigma_{PSII} \times n$) of both ETR and PSOE was found in the light-limited period of the day at 08:30 h and the lowest at 14:00 h.

When the light-response curves of non-photochemical quenching at various times of the day were plotted against

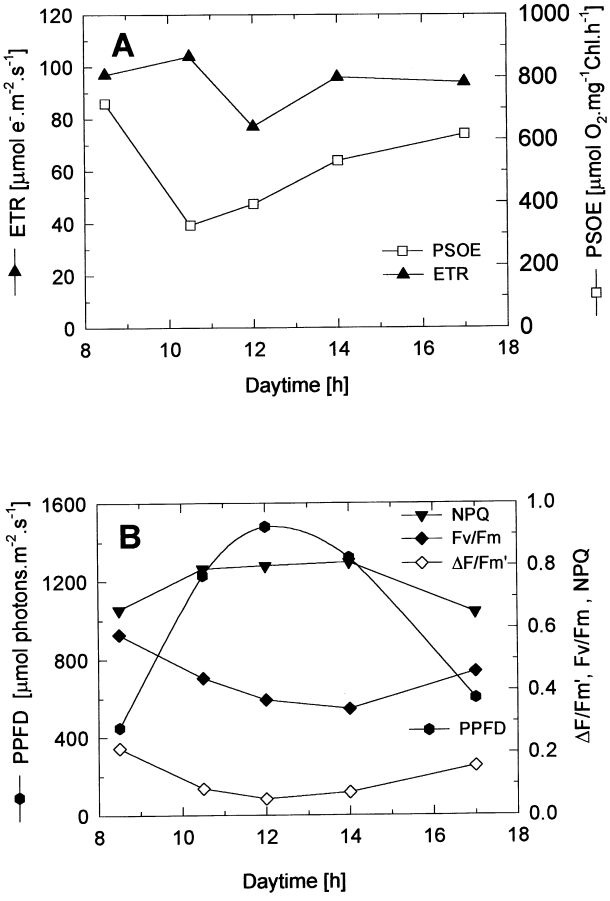

Fig. 4. Diel curves of PSOE and ETR (panel **A**), and NPQ, Fv/F_M and $\Delta F/F_M'$ (panel **B**) calculated for irradiance intensity at the water surface (curve with solid hexagons).

Table I: The photosynthetic parameters—maximum capacity (ETR_{max} or P_{max}), efficiency (α) and saturating light intensity (I_k)—were calculated from light-response curves of the relative electron transport rate (ETR) and photosynthetic oxygen evolution (PSOE) according to Platt et al. (Platt et al., 1980); ±SE was calculated for all the measurements

	Light response curve of ETR			Light response curve of PSOE		
Time of day (h)	ETR_{max} [µmol e^- m^{-2} s^{-1}]	α_{ETR}	I_k [µmol quanta m^{-2} s^{-1}]	$PSOE_{max}$ [µmol O_2 mg^{-1} (Chl *a*) h^{-1}]	α_{PSOE}	I_k [µmol quanta m^{-2} s^{-1}]
08:30	113 ±1.4	0.53 ± 0.009	212 ± 1.0	828 ± 26.1	7.88 ± 0.695	105 ± 5.7
10:00	119 ± 1.3	0.36 ± 0.008	331 ± 3.7	361 ± 45.3	3.47 ± 0.774	104 ± 9.1
12:00	83 ± 0.7	0.33 ± 0.005	256 ± 1.7	468 ± 45.9	3.03 ± 0.043	154 ± 12.9
14:00	111 ± 5.4	0.24 ± 0.013	456 ± 2.1	551 ± 58.4	1.50 ± 0.066	367 ± 22.3
17:00	130 ± 9.0	0.28 ± 0.021	427 ± 2.4	761 ± 35.6	3.94 ± 0.454	193 ± 12.5

PPFD, we found minima in NPQ [State 1 (Rouag and Dominy, 1994)] between 15 and 100 µmol quanta m^{-2} s^{-1} (Figure 3). It has been suggested that this value corresponds to the growth irradiance to which the cyanobacterial population is acclimated (Campbell and Öquist, 1996). In the morning, the waterbloom population was acclimated to low irradiances (the minimum of NPQ at 7 µmol quanta m^{-2} s^{-1}). Even at midday, the average cells of the waterbloom population found in the 0.5 cm layer were acclimated to relatively low irradiance of about 100 µmol quanta m^{-2} s^{-1}, despite being exposed to high surface irradiance.

From the measured light-response curves, the values of ETR, PSOE and NPQ corresponding to PPFD at the surface of the fish pond reservoir (curve with solid hexagons, Figure 4B) were determined by extrapolation. ETR changed little during the day, varying between 90 and 100 µmol e^- m^{-2} s^{-1} with a depression to 77 µmol e^- m^{-2} s^{-1} at midday (80% of the morning value) when the irradiance intensity reached almost 1500 µmol quanta m^{-2} s^{-1} (Figure 4A). The course of PSOE was similar to that of ETR, except that it decreased to 46% of the initial value at 10:30 h. From 10:30 h onwards, PSOE increased until late afternoon when it reached 86% of the 08:30 h value (Figure 4A). The curve of NPQ calculated for surface irradiancies revealed an increase from 0.66 to 0.79 between 08:30 and 10:30 h (Figure 4B). This was followed by a slight increase until 14:00 h. It is important to note that NPQ was higher at 14:00 h than at midday when the irradiance was highest. As also seen in the light-response curves (Figure 3), NPQ at 17:00 h was lower than at 14:00 h, indicating a recovery of the photosynthetic apparatus from high-irradiance stress. The F_V/F_M ratio, which is also an indicator of stress, showed a continuous decrease from 08:30 h onwards until 14:00 h when it was less than 40% of the morning value (Figure 4B). After 14:00 h, the F_V/F_M ratio recovered. The same trend was seen for $\Delta F/F_M'$.

DISCUSSION

Using natural, mixed, surface waterblooms of cyanobacteria, we investigated the relationship between photosynthetic oxygen evolution (PSOE) and electron transport rate (ETR) estimated from chlorophyll fluorescence measurement. The correlation between PSOE and ETR over the whole range of irradiances was poor during some periods of the day while at other times, a good correlation was found.

There are two possible sources of the discrepancy when estimating the rates of electron transport and net photosynthesis (measured as O_2 evolution) in cyanobacteria. Firstly, the use of the $\Delta F/F_M'$ ratio is based on several simplifying assumptions, as discussed by Genty *et al.* (Genty *et al.*, 1989). According to these authors, the reasons are (i) no change in the optical cross-section, (ii) a negligible contribution of other fluorescing systems to the measured signal, and (iii) all non-photochemical quenching supposedly originates in the antennae (no quenching in the reaction centre). In cyanobacteria, the validity of these assumptions has not been completely justified. There is a significant contribution of PSI and phycobiline emission to F_0 (Büchel and Wilhelm, 1993), forming more than 50% of the signal (Koblížek, unpublished data). The potential source of discrepancy between the rates of electron transport and photosynthesis in cyanobacteria relates to state-transition mechanisms. This causes large changes in the PSII functional cross-section at lower irradiances

(Koblížek *et al*., 1997). Taking into account potential changes in the optical cross-section, the model of Kolber and Falkowski (Kolber and Falkowski, 1993), using the functional cross-section for calculation of light harvesting efficiency, can give a better estimate of the energy utilized than the application of the $\Delta F/F_M'$ parameter described by Genty *et al.* (Genty *et al*., 1989). However, the distortion of ETR by state-changes is likely to be insignificant because the excitation is mostly transferred by a spill-over mechanism (which is reflected in the $\Delta F/F_M'$ ratio), and the change of the optical cross-section caused by the phycobilisome detachment is only small (Koblížek *et al*., 1998). In any case, all the factors discussed above cause an underestimation of the PSII electron transport rate. Thus, the higher values of ETR in comparison with PSOE are unlikely to be a result of this underestimation.

The second source of discrepancy may originate from the fact that the ETR parameter reflects the gross PSII-dependent electron transport while PSOE represents net photosynthesis (ETR minus losses). It is possible that a part of the electrons is utilized in oxygen consuming processes, which can play a significant role, especially at higher irradiance. Hoch *et al.* found that at such irradiances, the oxygen uptake was significantly accelerated in the cyanobacterium *Anacystis nidulans* (Hoch *et al.*, 1963). In the cyanobacterium *Synechocystis* sp. PCC 6803, it was shown that oxygen-dependent flow (when the Calvin cycle is blocked) can be as high as 50–70% of the normal, CO_2-dependent electron flow (Goosney and Miller, 1997). However, the mechanism responsible for this process is still unknown.

In the light, oxygen uptake in cyanobacterial waterblooms can be caused by several processes, i.e. (dark) respiration, photorespiration, cyclic electron flow around PSII and/or the Mehler reaction (reduction of O_2 by PSI). Dark respiration will probably be insignificant because in cyanobacteria, this process is suppressed in the light (Schmetterer, personal communication; Scherer, 1990). Photorespiration, the oxygenase activity of RuBisCO, depends on the relative concentrations of oxygen and C_i, where a high O_2/CO_2 ratio stimulates photorespiration. In cyanobacteria, photorespiratory activity is relatively low due to effective CO_2-concentrating mechanisms (Ogren, 1984; Kaplan *et al.*, 1995). However, Ibelings and Maberly (Ibelings and Maberly, 1998) found that C_i could be depleted in surface waterblooms, causing a high O_2/CO_2 ratio and thus high photorespiration rates.

The discrepancy between ETR and PSOE found in diatoms (Geel *et al*., 1997) or some microalgae (Flameling and Kromkamp, 1998) was usually attributed to the Mehler reaction. In this reaction, PSI donates, via ferredoxin, electrons to O_2, which results in the formation of superoxide radicals. These are rapidly converted to hydrogen peroxide, resulting in oxygen uptake [e.g. (Asada and Takahashi, 1987)]. Considering the data of Goosney and Miller (Goosney and Miller, 1997), it is likely that in the case of cyanobacteria, a significant

Table II: Comparison of the coefficients $\kappa = PSOE/ETR$ taken from the literature

Organism	κ	Reference
Green algae		
Chlorella pyrenoidosa	2.1 – 2.6*	Kroon, 1994
Scenedesmus obliquus	2.6 – 4.9	Heinze *et al*., 1996
Scenedesmus protuberans	1.9 – 2.2*	Flameling and Kromkamp, 1998
Spongiochloris sp.	2.9 – 3.7*	Koblížek *et al*., 1999
Cyanobacteria		
Nostoc (*Peltigera canina*)	2.9	Sundberg *et al*., 1997
Waterbloom mixture	3.8 – 9.2	This paper
Diatoms		
Phaeodactylum tricornutum	0.7 – 1.4*	Geel *et al*., 1997
Phaeodactylum tricornutum	6.2 – 6.4*	Flameling and Kromkamp, 1998
Other		
Emiliania huxleyi	6.9 – 13.4*	Flameling and Kromkamp, 1998
Phaeocystis globosa	5.2 – 7.1*	Flameling and Kromkamp, 1998
Rhodomonas sp.	1.9*	Geel *et al*., 1997

*The coefficient κ was recalculated from the data presented in the corresponding reference.

portion of electrons produced by PSII is also taken up in the Mehler reaction.

Falkowski and co-workers (Falkowski *et al*., 1986; Prášil *et al*., 1996) found a deviation of linearity of flash-induced oxygen evolution and the variable fluorescence yield in microalgae. The non-linearity started when photosynthetic rates reached light saturation, and this result was attributed to the occurrence of a cyclic electron flow around PSII (Behrenfeld *et al*., 1998). However, the exact mechanism and its physiological relevance remains obscure.

In our experiments, the ratio between ETR and PSOE not only varied under different light intensities but it also underwent large changes during the day. These diel changes were reflected in the coefficient κ, which ranged from 9.2 in the morning (08:30 h) to 3.8 in the afternoon (14:00 h). These values are compared with data published for other organisms in Table II; most authors have reported relatively narrow ranges of κ for green algae. The range was much larger for cyanobacteria, diatoms or other photosynthesizing organisms. These results suggest that the ETR parameter may be used to estimate gross photosynthesis in green algae, but its application to other species would be problematic and should be approached with caution. However, ETR can provide supplementary information about the status of the photosynthetic apparatus at the level of PSII-dependent electron transport.

Despite variability in the relationship between ETR and PSOE, both parameters reflected a midday decrease in photosynthesis of the phytoplankton bloom population (Figures 2C and 4A). Similar behaviour of phytoplankton or macroalgae has been reported from field and laboratory studies (Marra and Heinemann, 1982; Ibelings *et al*., 1994; Sagert *et al.*, 1997). Henley found that photoinhibition caused a decrease in the α parameter followed by a decrease in P_{max} (Henley, 1993). Our measurements showed a similar pattern characterized by a pronounced decrease during the morning. Similar trends were also observed in marine phytoplankton (Lizon *et al*., 1995). The partial recovery of α_{ETR} and α_{PSOE} could be attributed to the cyanobacterial populations being shade adapted, where recovery from photoinhibition is much slower. It is difficult to reconcile to what extent the decrease in the light-saturated PSOE and α_{PSOE} reflects the oxygen-consuming processes, and what part is actually induced by photoinhibition (PSII photoinactivation).

CONCLUSIONS

Although the relationship between ETR and PSOE varied and was non-linear at very low and very high irradiances, our measurements of photosynthetic oxygen evolution and PSII electron transport during the diel cycle showed that, in some situations, it is possible to relate both parameters. The results clearly reveal that the cyanobacteria became stressed over the midday period, and both ETR and PSOE measurements indicated a suppression of the photosynthetic capacity.

We favour the hypothesis that the discrepancy between the electron transport rate and photosynthetic oxygen evolution can be attributed mainly to the presence of the secondary oxygen-consuming processes such as photorespiration and Mehler reaction.

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