



Photosynthetic performance of outdoor *Nannochloropsis* mass cultures under a wide range of environmental conditions

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ABSTRACT: The unicellular alga *Nannochloropsis* sp. (Eustigmatophyta) is a rich source of lipids, polyunsaturated fatty acids (such as eicosapentaenoic acid) and carotenoids (violaxanthin), which makes it valuable for human consumption, aquaculture and biofuel production. Mass production of *Nannochloropsis* sp. can be easily achieved in high-rate algal ponds (HRAP), in flat panel photobioreactors (FPP) or in tubular photobioreactors. While easy to operate, these systems are prone to unfavorable growth conditions, which affect productivity. In the present study, we cultivated *Nannochloropsis* sp. in 2 outdoor production systems and monitored photosynthetic activity. Unfavorable conditions (stressors), such as high temperature and high pH in combination with high irradiance, were induced in FPP, causing a substantial reduction in the photosynthetic rate. The measurements of *Nannochloropsis* sp. photosynthetic activity using several chlorophyll fluorescence techniques as well as oxygen production measurements showed that this species is able to withstand high irradiance levels. Although some photodamage due to high irradiance was found, the cultures rapidly recovered. *Nannochloropsis* sp. coped well with high pH conditions under physiological temperatures. However, a temperature rise above 32°C was detrimental, with repair processes being unable to keep up with the rate of damage. The cultures in the FPP were more prone to damage by extreme temperatures than those in the HRAP due to the high surface:volume ratio, which complicated temperature regulation within the physiological range.

KEY WORDS: Chlorophyll fluorescence · Photobioreactor · *Nannochloropsis* · Mass culture · Photosynthetic rate · Unfavorable conditions · Quantum yield

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INTRODUCTION

The unicellular alga *Nannochloropsis* sp. (Eustigmatophyta; hereafter *Nannochloropsis*) is recognized as an excellent source of eicosapentaenoic acid (EPA)

(20:5 ω 3, an essential polyunsaturated fatty acid) and carotenoids (especially violaxanthin) for human consumption as well as for animal feeds (aquaculture). Recently, there has also been an increased interest in this genus for biofuel production due to its high lipid

content. Mass production of *Nannochloropsis* can be easily achieved in high-rate algal ponds (HRAP) (Sukenik 1999), in vertical flat panel photobioreactors (FPP) (Zuo & Richmond 1999) or in tubular photobioreactors (Chini Zittelli et al. 1999). All these production systems may be maintained outdoors, and operational conditions can be optimized to provide maximum production rates for extended periods (Tredici & Chini Zittelli 1998, Borowitzka 1999, Ugwu et al. 2008). However, these cultivation systems are prone to technical failures such as temporary malfunctioning of control devices (i.e. temperature and pH control, regulation of CO₂ supply), and these can have effects on the physiology of the algae that may be exacerbated by high irradiance at certain times of the day or under particular climatic conditions. Such failures could cause abrupt changes in environmental conditions that could lead to reduced production. Unfavorable growth conditions can be monitored (using various devices such as pH meters, thermocouples, etc.) and their effect on productivity assessed by measurements of photosynthetic activity. Rapid restoration after operational failure can lead to gradual recovery of photosynthetic activity and growth rate. However, it is important to understand the extent to which fluctuations in conditions can perturb photobioreactor systems and how fast the algae can recover after normal conditions are restored.

We exposed the alga *Nannochloropsis* to a wide range of stress conditions in 2 outdoor production systems and monitored the cultures' photosynthetic activity upon stress introduction and during the subsequent recovery phase. Stressors, such as high temperature and high pH in combination with high irradiance, were induced in outdoor cultures, and their effect on photosynthesis was monitored by pulse amplitude modulated (PAM) fluorometry.

MATERIALS AND METHODS

Algal mass production systems. Field experiments were carried out at the facilities of the National Mariculture Center (NCM), Israel Oceanographic and Limnological Research, Eilat, Israel (29° 33' 5.48" N, 34° 58' 25.27" E) for 3 consecutive days from 1–3 April 2008. Outdoor cultures of the alga *Nannochloropsis* were cultivated in 2 mass production systems. A high-rate algal pond (HRAP), which is a concrete raceway pond of 60 m² that is equipped with a paddle wheel, was maintained at a water level of 35 cm and provided with CO₂ via a solenoid valve activated by a pH controller set to pH 8.4 (see Fig. 3.5 in Sukenik 1999). The culture was diluted daily by replacing 10% of the HRAP volume with fresh

medium and evaporation was compensated for by the addition of tap water. The culture medium was composed of 70% seawater and 30% tap water to produce a salinity of 28 ppt, and was fertilized with 2 mM (NH₄)₂SO₄, 0.2 mM H₃PO₄, and 0.02 mM Fe₂(SO₄)₃. The HRAP was operated continuously for 4 wk prior to the measurements. The other production system was comprised of a flat panel photobioreactor (FPP) made of a metal frame that held a flat plastic bag (5 × 200 × 100 cm, w × l × h) with a total volume of 100 l (Boussiba & Zarka 2005). The *Nannochloropsis* culture from the HRAP was used to inoculate the FPP a week prior to the measurements. The FPP culture was bubbled with CO₂-enriched (~0.5%) air at the rate of 5 l min⁻¹ to maintain turbulence and to keep the pH between 7.5 and 8.2. In an attempt to avoid extreme temperatures in the FPP culture, ambient seawater was circulated in 'cooling fingers' immersed in the culture. The culture pH and temperature were monitored continuously. Incident solar irradiance was measured with a pyranometer (Li-Cor) and data were logged every 5 min with a data logger (Li-Cor 1000). A conversion factor of 1 W m⁻², equaling 4.5 μmol photon m⁻² s⁻¹, and a contribution of 40% of photosynthetically active radiation (PAR) to total solar irradiance, were used to express the data as PAR in μmol photon m⁻² s⁻¹.

Measurements of photosynthetic activity. Samples from both HRAP and FPP cultures were collected at 6 to 8 h intervals for 51 h, which represented >2 complete consecutive diurnal cycles. The photosynthetic activity of the culture was measured using a continuous bypass from the cultivation units to a flow-through Water PAM fluorometer (Water PAM, Heinz Walz) programmed to run rapid light-response curves (RLC). Samples were simultaneously collected from both HRAP and FPP, dark adapted for 15 to 20 min, and evaluated for their photosynthetic capacity using 2 other fluorometers: the Dual PAM (Heinz Walz) to estimate Photosystem I and II (PSI and PSII) yields, and the Aquapen AP 100 (Photon Systems Instruments) to measure chlorophyll fluorescence induction curves. Subsamples were obtained for the measurement of photosynthetic oxygen evolution using an oxygen electrode in conjunction with a light source that provided PAR at different intensities (Dubinsky et al. 1987).

Flow-through Water PAM fluorometry: This technique involves the use of a highly sensitive chlorophyll fluorometer designed for continuous monitoring of phytoplankton photosynthetic activity. The fluorometer consists of a waterproof measuring head covered by a flow-through chamber and is operated in conjunction with the standard PAM control unit via a personal computer using the Win Control software (Heinz Walz). The flow-through chamber was connected to a peri-

staltic pump that continuously pumped algal culture from the photobioreactor into the measuring head. *In situ* RLCs were acquired by saturating-pulse analysis under increasing irradiances (using a 30 s illumination period). This procedure provided a series of photosynthetic quantum yields (PQY), which were measured in the ambient light-adapted state, F_v'/F_m' , as a function of the actinic light intensity. F_v'/F_m' is equal to $(F_m' - F_0')/F_m'$, where F_v' is the variable fluorescence in light-acclimated cells, F_m' is the maximum fluorescence from light-acclimated cells, and F_0' is the minimum fluorescence from light-acclimated cells. Three replicate RLCs were measured for both production systems at each sampling time. On several occasions, *in situ* variations in F_v'/F_m' were followed by a series of repetitive saturating pulses separated from each other by 2 to 3 min.

PSI and PSII efficiency: Changes in absorption efficiency of PSI and PSII were measured simultaneously based on the pulse modulation principle with the dual wavelength approach using the Dual PAM fluorometer (Klughammer & Schreiber 2008). Because the PSI signal was rather low, the measurement required ~10-fold sample concentration using a benchtop centrifuge (6 min at $1000 \times g$). The complete operation of RLC recording took 15 min and was performed on samples after dark adaptation. Thus, the obtained RLCs did not show the *in situ* activity but were more likely to express potential photosynthetic activity (White & Critchley 1999). At each sampling time, 3 replicate RLCs were measured using increasing actinic light steps, each lasting 30 s. All RLCs were fitted using an exponential model (Webb et al. 1974). After dark adaptation, the maximum quantum efficiency of PSII in the dark-adapted state, F_v/F_m (where F_v is the variable fluorescence in dark-acclimated cells and F_m is the maximum fluorescence from dark-acclimated cells), was calculated. The effective quantum efficiencies of PSI and PSII (Φ_{PSI} and Φ_{PSII}), measured with the Dual PAM fluorometer, were calculated as $\Delta F/F_m' = (F_m' - F_s)/F_m'$, where ΔF is the change in fluorescence emission from dark-acclimated cells and F_s is the fluorescence emitted due to the Dual PAM measuring light. The relative electron transport rates (rETR) of PSI and PSII were calculated as Φ_{PSI} or $\Phi_{\text{PSII}} \times \text{PAR}$ (Kromkamp & Forster 2003).

Fluorescence induction curves: The rapid fluorescence induction kinetics (the so-called Kautsky curve) of all oxygenic photosynthetic organisms show a polyphasic rise (chlorophyll fluorescence transient) between the initial (F_0) and the maximum (F_m) fluorescence yield during the first second of illumination (Neubauer & Schreiber 1987). These phases were designated as O, J, I and P (or the inflection points on the fluorescence induction curve), and can be visualized

using a logarithmic time scale (Strasser et al. 1995). The inflection point J (2 ms) represents the double-reduction of electron carriers Ph, Q_A , and Q_B , while the I step (30 ms) is connected to a 3-electron reduction of the PSII electron carriers to different redox states of the reaction center complex, which reduces the PQ pool (Strasser et al. 2004, Lazár 2006). The I step probably reflects the heterogeneity of the PQ pool, with fast-reducing and slow-reducing PSII centers. The drop in the induction curve beyond the P step indicates that the PQ pool is being re-oxidized due to the demand for reducing equivalents from the Calvin-Benson cycle.

A portable fluorometer (Aquapen AP 100) was used to follow the polyphasic rise of chlorophyll fluorescence in both outdoor cultures. Dark-adapted (15 min) samples were diluted to a final concentration of $\sim 200 \text{ mg dw l}^{-1}$ ($\sim 3.5 \text{ mg chl l}^{-1}$; see 'Chlorophyll concentration and other biochemical parameters') and were placed in a 3 ml fluorescence cuvette that was mounted in front of the detector, while red light-emitting diodes (LEDs) supplied saturating light intensity from both sides of the cuvette perpendicular to the detector. Rapid fluorescence induction curves were recorded in the time range between 50 μs and 2 s from the onset of the saturation light level. Three curves were recorded and averaged for each sample.

Photosynthetic oxygen evolution: Photosynthesis and dark respiration rates in response to irradiance levels were measured as changes in oxygen concentration using a polarographic oxygen electrode (Clark type, Yellow Springs 5331), while illumination of the algal sample was provided by a Light Pipette (Brammer, Illuminova) as previously described by Dubinsky et al. (1987). The light intensity was measured with a light meter (LI-250A) connected to a quantum sensor (LI-190, Li-Cor). Samples collected from the HRAP or FPP were diluted twice with fresh medium, bubbled with a stream of N_2 to reduce the initial oxygen concentration, inserted into the incubation chamber ($\sim 15 \text{ ml}$) and stirred for temperature equilibration (25°C) in the dark. After 20 min of dark incubation, respiration was measured for 120 s. Net photosynthetic rates were measured in the incubation chamber at a sequence of 9 light intensities (6, 44, 80, 130, 166, 280, 350, 580 and $740 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$) provided by the Light Pipette as described by Ben-Zion & Dubinsky (1988). Each illumination period lasted for 120 s. Gross photosynthetic rate was calculated from average values for the oxygen evolution rate during each illumination period and dark respiration rate measured prior to the illumination. Photosynthetic parameters (α , P_{max} , and I_k) were calculated by fitting the measured P vs. I data to the Platt & Jassby (1976) hyperbolic tangent model.

Chlorophyll concentration and other biochemical parameters. Subsamples were collected from both the

FPP and HRAP systems. Photosynthetic pigments were extracted from cells using 100% methanol and collected after centrifugation. Spectra of the supernatants were determined using a UV/VIS spectrophotometer (Shimadzu UV 1240) and chlorophyll *a* (chl *a*) and carotenoid concentrations were calculated according to Lichtenthaler (1987). Absorption coefficients of the cells were measured as described by Shibata et al. (1954) using standard A4 paper mounted in front of a cuvette holder as a light diffuser. Cells were counted with a haemocytometer. Biomass dry weights were estimated by filtering a known volume of culture through pre-dried and pre-weighed GF/C filters (Whatman). The filters with the sample were weighed after drying for 1 h at 110°C.

RESULTS AND DISCUSSION

Physicochemical parameters and stress conditions

Diurnal variations in PAR during the 3 d study period are presented in Fig. 1A, indicating typical spring radiation at the study site, with a maximum PAR of 2000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at 13:00 h local time. The temperature of the HRAP culture fluctuated between 11°C in the early morning and 23°C during the early afternoon (13:00 h). The pH varied between 7.0 at night and 8.3 (the controller setting point) at noon and during the early afternoon (Fig. 1B,C). The temperature of the FPP culture, on the other hand, increased to 40°C during the early afternoon on the third day, as the cooling system's capacity failed to cope with the extreme heat flux. During the early morning hours, the temperature of the FPP culture decreased to 15°C. During the daylight hours of the first and second day, the FPP culture pH was allowed to increase (due to photosynthetic consumption of CO_2) and reached pH 10 (at 17:00 h on Day 2), but was maintained at 8.2 during the third experimental day by the addition of CO_2 enriched air. At night, the pH gradually decreased to a minimum value of 7.9 (Fig. 1B,C). The relatively stable pH and temperature conditions in the HRAP culture were compared with those of the FPP culture; the differences between the 2 systems are shown in the top panels of Fig. 1B,C. During the afternoon hours of Day 3, this comparison indicated extremely high temperature conditions in the FPP culture while 'normal pH conditions' were maintained. Thus, it was possible to separate temperature stress from high pH/low CO_2 stress. In addition, potential stress conditions from the combined effects of supra-optimal temperature and pH were identified in the FPP culture during the afternoon of Day 2.

Photosynthetic quantum yields (PQY)

Photosynthetic responses to the wide range of environmental conditions that developed in the 2 production systems are presented in Fig. 2, which compares *in situ* PQY values (acquired using flow-through Water PAM fluorometry and expressed as F_v'/F_m') simultaneously measured in the FPP and HRAP cultures. The greatest differences in the *in situ* PQY between the FPP and HRAP cultures were observed between 12:00 and 18:00 h (on Days 2 & 3). During these sampling times, the *in situ* PQY of the FPP culture was much lower than that of the HRAP culture. Both high irradiances and high temperatures could have caused this drop in F_v'/F_m' between 14:00 and 17:00 h in the FPP. While highest irradiances were observed at 13:00 h, the PAR values dropped from 1911 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at 14:00 h to 875 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at 17:00 h, suggesting that the rise in temperature was the main factor responsible for the large drop in F_v'/F_m' observed in the FPP. Morris & Kromkamp (2003) studied the effect of temperature on the photosynthetic performance of the benthic diatom *Cylindrotheca closterium* and reported a sharp drop in F_v/F_m and a less sharp decrease in maximum photosynthetic oxygen evolution (P_{max}) at a temperature $>30^\circ\text{C}$. The cumulative data of *in situ* PQY presented on a log scale against culture temperature indicate the temperature tolerance of *Nannochloropsis* (Fig. 3). Relatively high *in situ* PQY values were maintained over a wide range of temperatures (from 11 to 32°C). However, PQY values declined significantly at the higher temperatures that were measured in the FPP culture (Fig. 3) between 14:00 and 17:00 h on Day 3.

Photosynthetic fluorescence parameters were measured using the Dual PAM fluorometer after dark adaptation for 15 min at room temperature (Fig. 4). The F_v/F_m values (PQ4 yield of PSII in the dark-adapted state) measured by this procedure for the 2 production systems were similar to, or higher than, the corresponding values measured *in situ* with the Water PAM fluorometer. Similar F_v/F_m values were also recorded for the FPP and HRAP samples collected at night or in the early morning. During the afternoon on Days 1 and 2, the dark adaptation period was sufficient to allow full recovery of the culture to their dark F_v/F_m values. The differences between the HRAP and the FPP culture were minor and both culture systems showed similar trends after dark adaptation. This showed that the decrease in F_v'/F_m' measured with the flow-through Water PAM fluorometer at midday was most likely a combination of dynamic downregulation, which is probably related to the xanthophyll cycle causing energy quenching (qE), and chronic downregulation (qI, see 'In-line chlorophyll fluorescence and fluores-

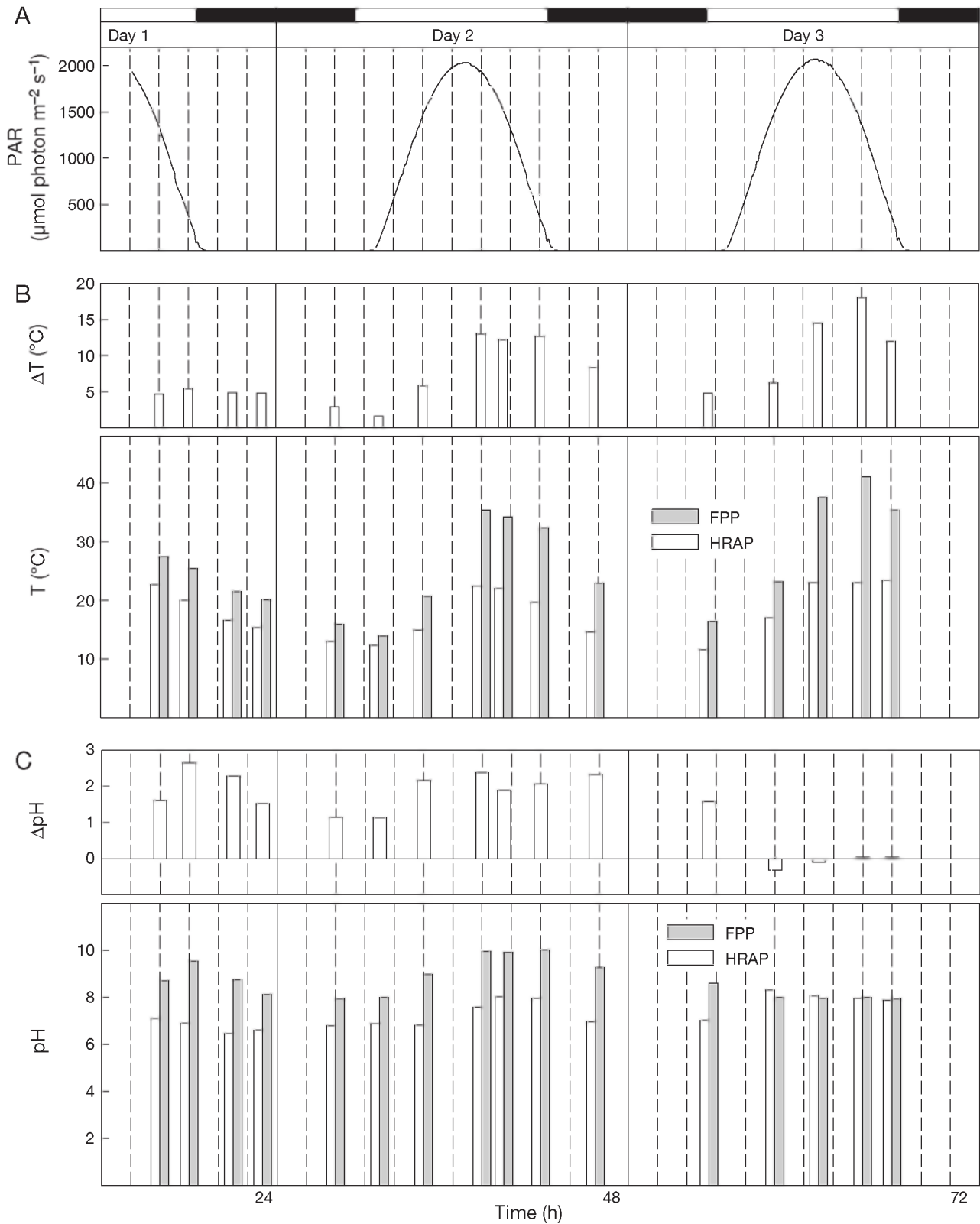


Fig. 1. Diurnal changes in (A) solar photosynthetically available radiation (PAR), (B) culture temperature, and (C) culture pH measured at various time intervals during 3 consecutive days (1–3 April 2008) in the flat panel photobioreactor (FPP) and the high rate algal pond (HRAP). The differences in temperature and pH between the 2 cultivation systems (FPP – HRAP) are presented as ΔT or ΔpH in (B) and (C). The bar on top indicates day (white) and night (black) periods; the vertical dashes indicate 2 h intervals

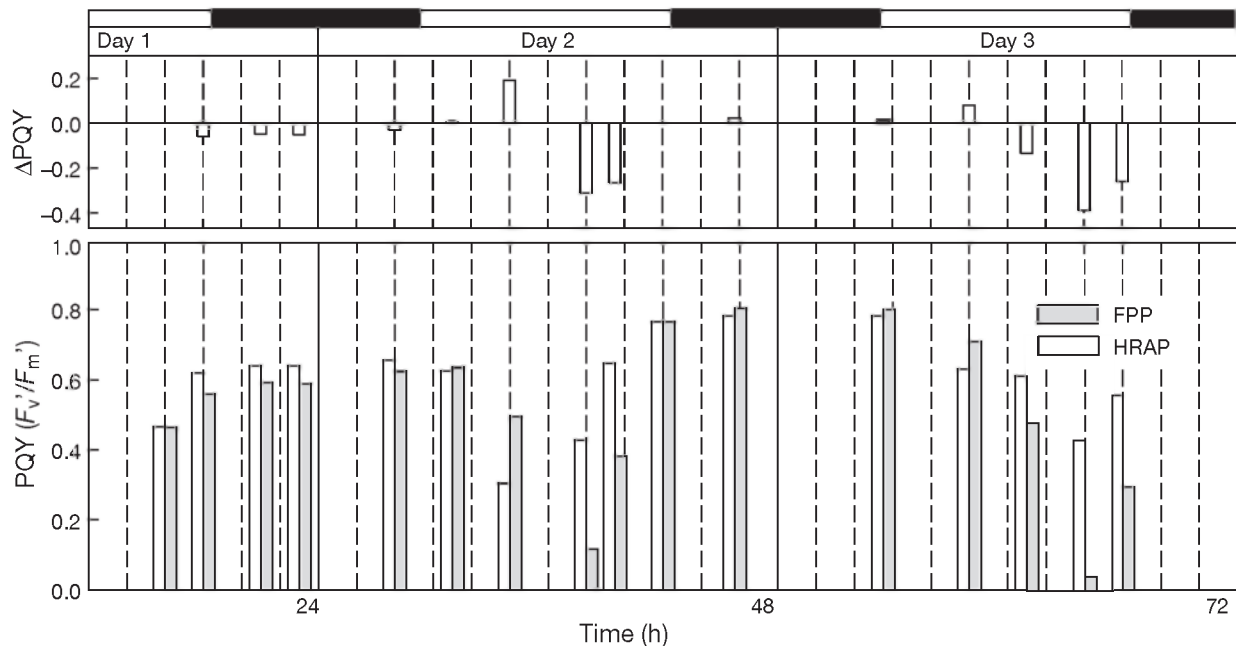


Fig. 2. *Nannochloropsis* sp. Diurnal changes in photosynthetic quantum yield ($PQY = F_v'/F_m'$) in ambient-light-adapted cultures measured *in situ* using flow-through Water PAM (pulse amplitude modulation) fluorometry at various time intervals over 3 consecutive days. Cultures were maintained in a flat panel photobioreactor (FPP) and a high rate algal pond (HRAP). The differences in PQY between the 2 bioreactor systems are shown as ΔPQY (FPP – HRAP) in the top panel. The bar on top indicates day (white) and night (black) periods; the vertical dashes indicate 2 h intervals

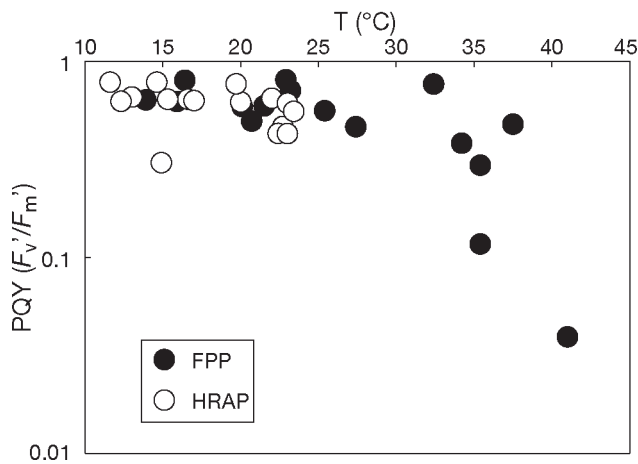


Fig. 3. Relationship between temperature and photosynthetic quantum yields ($PQY = F_v'/F_m'$) measured *in situ* in ambient-light-adapted *Nannochloropsis* cultures cultivated in a flat-panel photobioreactor (FPP) and in a high rate algal pond (HRAP). *In situ* measurements were done using flow-through Water PAM (pulse amplitude modulation) fluorometry. Note that the PQY values are presented on a logarithmic scale

cence induction kinetics'). However, the dark adaptation time was rather short to allow full relaxation of qE . If any damage occurred, it did not last long, since high values of F_v'/F_m' were already measured again by the evening. However, much lower F_v'/F_m' values were

recorded in the FPP samples collected during the afternoon of Day 3. Different to the HRAP samples, FPP samples collected at 16:00 and 18:00 h were unable to fully recover after 15 min of dark adaptation, thereby showing signs of true photodamage (Fig. 4).

In-line chlorophyll fluorescence and fluorescence induction kinetics

The *in situ* recovery process of the FPP culture (on Day 3 at 16:00 h) exposed to high temperature (42°C) extended for a long period, as demonstrated by the fluorescence record of repetitive saturation pulses imposed at 150 s intervals (Fig. 5). The fluorescence emission from light-acclimated cells (F' values) gradually increased, concomitant with an increase in the F_m' values, as the culture temperature decreased later that evening and the recovery process continued. The recovery kinetics of F' and F_m' revealed a biphasic pattern, which was especially visible in the pattern of PQY values and the relaxation of nonphotochemical quenching (NPQ; Fig. 5C). The first fast phase of recovery might be related to the relaxation of energy quenching, presumably facilitated by the xanthophyll cycle. The slower phase is most likely due to PSII repair processes. Photodamage to PSII is known to quench PSII fluorescence and is therefore also called

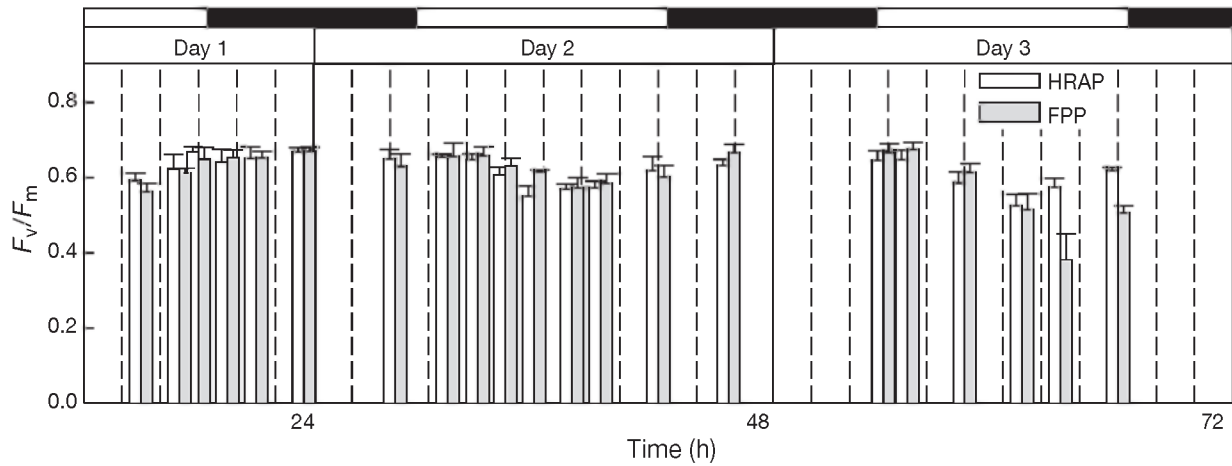


Fig. 4. *Nannochloropsis* sp. Diurnal changes in the maximum photosynthetic yield of dark-adapted samples (F_v/F_m) measured after 15 to 20 min of dark adaptation using Dual PAM (pulse amplitude modulation) fluorometry at various time intervals during 3 consecutive days, in the flat panel photobioreactor (FPP) and the high rate algal pond (HRAP). Data are means (\pm SDs) of 3 measurements. The bar on top indicates day (white) and night (black) periods; the vertical dashes indicate 2 h intervals

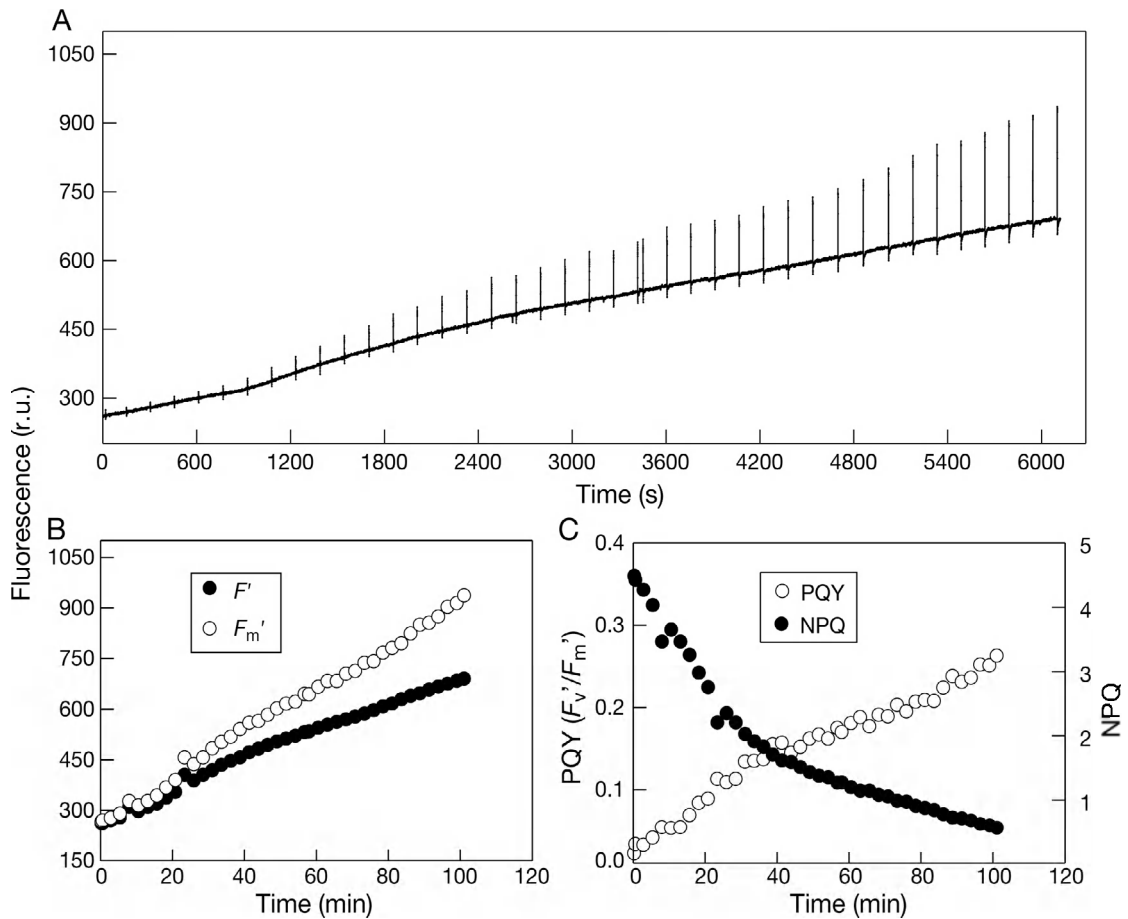


Fig. 5. *Nannochloropsis* sp. Recovery of the culture from the high-temperature stress imposed in the flat panel photobioreactor. (A) Trace of the fluorescence signal recorded *in situ* using flow-through Water PAM (pulse amplitude modulation) fluorometry from 16:20 to 18:00 h on Day 3, as the culture temperature declined from 42 to 35°C. Saturation pulses were triggered at 150 s intervals. Note the 'low waves' in chlorophyll fluorescence trace following each saturation pulse. (B) Increase in F' and F_m' values during the recovery process. (C) Changes in the photosynthetic quantum yield (PQ4) in the ambient-light-adapted state (F_v'/F_m') and the nonphotochemical quenching (NPQ) component during the recovery process. r.u.: relative units

chronic downregulation or qI. In this case, the higher amount of the modified PSII centers on the afternoon of Day 3 must thus have been caused by the higher temperature values, as the irradiances on all experimental days were similar. These modified PSII centers, which are inactive in electron transport, can actually provide some protection against high light because they dissipate absorbed light as heat (Chow et al. 2002). In addition, a downshift in fluorescence was observed following each saturation pulse. The PQY values increased as the culture temperature decreased by 5.6°C (from 41 to 35.4°C; Fig. 1B) during the 2 h after 16:00 h. During this time, there was an exponential decrease in NPQ, although no steady state values were attained by the end of the measurements (Fig. 5C). The increase in the F' signal is attributed to high-temperature stress. The brief drop in chlorophyll fluorescence (so-called low waves) immediately after application of the saturating light pulse (Fig. 5A) usually appears under stress (high temperature, lack of CO₂) and might be caused by an interplay between PSII and PSI electron transport. The primary cause is usually a limitation in carbon fixation (Calvin-Benson cycle) as the low waves in chlorophyll fluorescence kinetics indicate deprivation of bicarbonate in the reaction center of PSII (Xyländer & Hagen 2002). Can such a local deprivation be induced by high temperature? A HCO₃⁻ deficiency could be more pronounced at higher temperatures due to the lower solubility of CO₂ and temperature inactivation of internal carbonic anhydrase.

Fluorescence induction curves of *Nannochloropsis* samples collected from the FPP culture on the afternoon of Day 3 (13:00 and 15:00 h samples), when the culture was exposed to high temperature (42°C), exhibited maximum chlorophyll fluorescence-induced signals lasting between 20 and 90 ms (data not shown), indicating strong over-reduction of the PSII acceptor side. At 18:00 h on Day 3, the chlorophyll fluorescence induction curve measured in the FPP culture did not show significant recovery as on the previous day, since no F_p peak (i.e. the normalized fluorescence intensity of the P point in the OJIP transient) was detected (Fig. 6). Similarly, the V_j and V_i parameters (or the chlorophyll fluorescence yields at the corresponding points on the fluorescence induction curve) did not recover towards dusk at 18:00 h on Day 3 (i.e. they remained relatively high; Fig 7B) compared to the fluorescence induction curve recorded on Day 2 (Fig. 7A). These results suggest that high-temperature stress-induced damage had not been fully repaired and the Q_B-nonreducing centers persisted. The patterns of the V_j , V_i and F_v/F_m parameters on both days showed the most significant photoinhibitory stress at 15:00 h, concomitant with the temperature maxima in the cultures. This observation can be attributed to continuous dam-

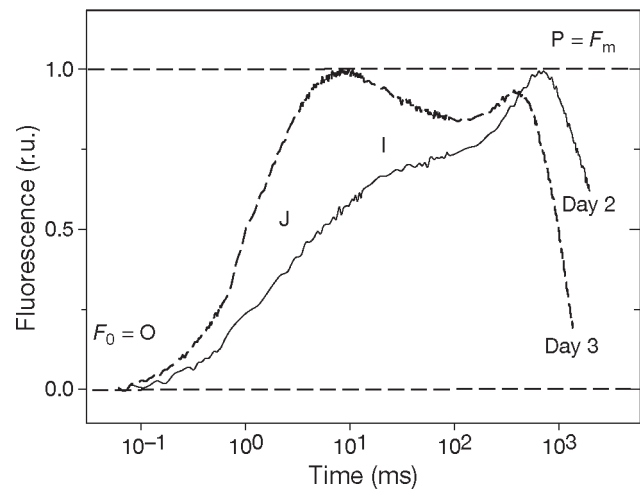


Fig. 6. *Nannochloropsis* sp. Comparison of rapid chlorophyll fluorescence induction kinetics measured in the culture from the flat panel photobioreactor (FPP) at 18:00 h on Days 2 and 3. The curves were 'double-normalized' to the same level of F_0 and F_m in order to distinguish differences in the intermediate steps designated J and I, which represent various reduction states of the electron carriers of the PSII complex. r.u.: relative units

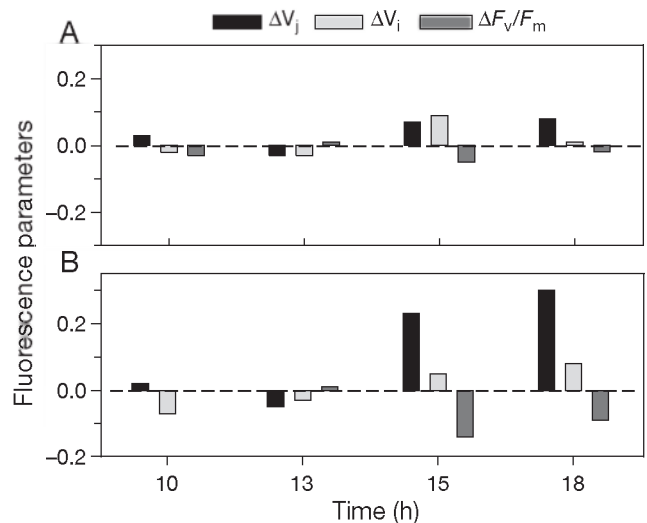


Fig. 7. Differences between the flat panel photobioreactor (FPP) and the high rate algal pond (HRAP) in the fluorescence parameters V_j , V_i and F_v/F_m of *Nannochloropsis* cultures measured at various times on (A) Day 2 and (B) Day 3. Differences are expressed as ΔV_j , ΔV_i , $\Delta F_v/F_m$ (FPP – HRAP). The parameters V_j and V_i were calculated as $V_j = (F_j/F_0)/(F_m/F_0)$ and $V_i = (F_i - F_0)/(F_m - F_0)$, respectively, where F_0 , F_j , F_i and F_m represent chlorophyll fluorescence yields at the corresponding points on the fluorescence induction curve

age to the D1 protein that is sluggishly repaired during the early afternoon hours (Takahashi & Murata 2008, Zhao et al. 2008). Thus, the 'JIP' test can be used as a rapid monitor of photosynthetic activity, including the effects of various stressors on this process.

Effective quantum efficiencies of PSII and PSI

For photosynthesis to be efficient, a close coupling between PSII and PSI activity is expected. Thus, we compared the effective photon yields of PSI and PSII as a function of irradiance measured via RLC (Fig. 8). Despite the relative noise in the data (due to rather low signals attributed to low cell concentration), they show that PSI activity was higher than PSII activity, as almost all values lie above the 1:1 line (with a few exceptions being evident in the HRAP Day 2 data). This suggests that the rate of electron transport in PSI might be higher than that in PSII, presumably due to cyclic electron transport around PSI. However, whether this is the case also depends on the total rate of light absorption of PSI relative to PSII. The

slope of the line did not deviate significantly from 1:1 in most cases (ANOVA linear regression analyses) on Day 2. However, on Day 3 we found a different pattern in the FPP cultures at 15:00 h compared to those at 18:00 h. When the quantum efficiencies of PSI were larger than ~0.6, there was no increase in the PSII efficiency. Hence, the high-temperature stress, on top of the high irradiances, probably generated 2 populations of PSII reaction centers: one that showed a normal variable fluorescence and another that showed a low variable fluorescence. The latter were likely to be the Q_B -nonreducing (inactive) PSII centers. We calculated the relative rate of electron transport (rETR) of PSI and PSII by multiplying the irradiance of the RLC with the measured efficiency of the respective photosystems of the FPP on Day 3 (Fig. 9).

Results show a biphasic pattern of rETR: in the 07:00 h sample, there was a good linear relationship between rETR(II) and rETR(I). However, as a result of the combined light and temperature stress, the linear relationship broke down: rETR(II) hardly increased any further, while rETR(I) exceeded a value of 150 relative units (r.u.). This does not mean that only PSII was affected by the stress conditions. While the maximum rate of rETR(II) decreased 2-fold from ~225 to 110 r.u., the maximum rETR(I) decreased ~70% from 350–400 to 230–300 r.u. Whether this indicates damage to PSI or active downregulation remains unclear.

Photosynthetic oxygen evolution

Photosynthetic capacity of the *Nannochloropsis* cultures maintained in the 2 production systems was measured under a wide range of irradiance levels. The P vs. I experiments were run at various time intervals for 3 consecutive days. The results suggest that the HRAP culture was acclimated to low light conditions, with P_{max} values that were lower than those calculated for the FPP culture (Fig. 10). Lower light saturation parameter (I_k) values for the HRAP culture further indicated culture acclimation to low light conditions (data not shown). Furthermore, the HRAP culture showed a relatively stable photosynthetic perfor-

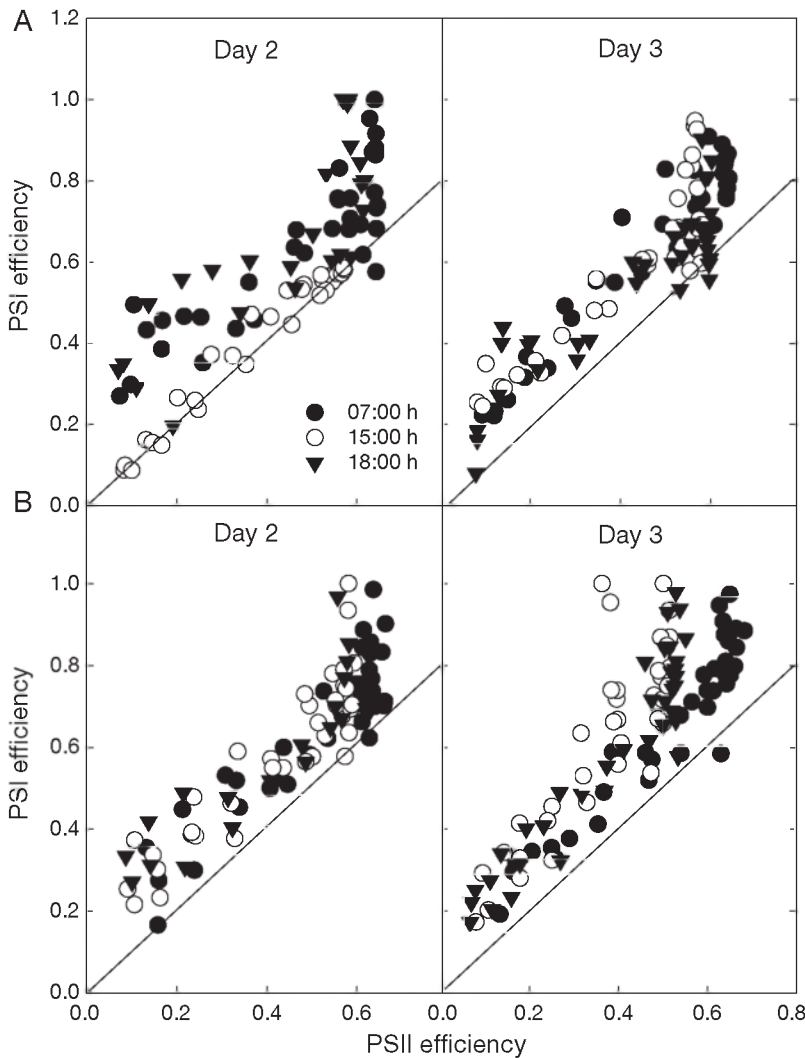


Fig. 8. Effective efficiencies of PSII and PSI measured in *Nannochloropsis* sp. cultures in (A) the high rate algal pond (HRAP) and (B) the flat panel photobioreactor (FPP) systems. Data are presented for samples collected at various times on Days 2 and 3. The line denotes the 1:1 ratio

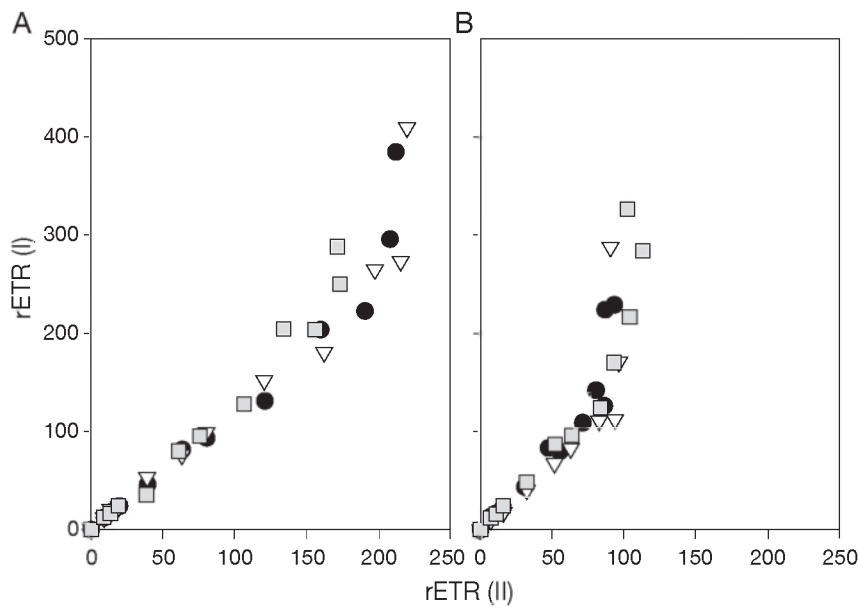


Fig. 9. Relative electron transport rates of PSI, rETR(I), plotted as a function of rETR(II) at (A) 07:00 h and (B) 18:00 h. Different symbols show individual results of triplicate measurements

mance, whereas the P_{\max} values measured in the FPP culture fluctuated substantially during the experimental period, indicating its response to variations in environmental conditions. The FPP culture drastically responded to the high temperature conditions to which it was exposed in the afternoon of Day 3. As depicted in the P vs. I curves, a gradual decrease in the photosynthetic parameters was recorded from 13:00 to 18:00 h (Fig. 11). These results also indicate considerable irreversible damage caused by the high temperature, likely due to the occurrence of the Q_B -nonreducing centers.

was demonstrated earlier (Sukenic et al. 1997). Nevertheless, the temperature rise above 32°C was rather detrimental and repair processes could not keep up with the rate of damage. This is in agreement with the findings of Lubián & Montero (1998). The FPP culture was more prone to supra-optimum temperatures because its high surface-to-volume ratio rendered the maintenance and control of temperature within a tolerable range difficult.

Several chlorophyll fluorescence techniques as well as the oxygen electrode method were shown to be reliable for the measurement of photosynthetic perfor-

CONCLUSIONS

The exposure of *Nannochloropsis* cultures to a wide range of environmental conditions in 2 production systems demonstrated that this species is able to withstand high irradiances. Although some downregulation was seen due to high irradiance (and/or high pH), the cultures recovered quickly. While much of the damage restoration had already occurred by the evening of the day on which the damage was sustained, repair was completed by the morning of the following day. The irradiance conditions in the HRAP diminished the overall rate of damage as the effective level per cell was rather low. In the present study, we imposed high pH values under a wide range of temperatures. It was found that *Nannochloropsis* cultures coped well with high pH values, as

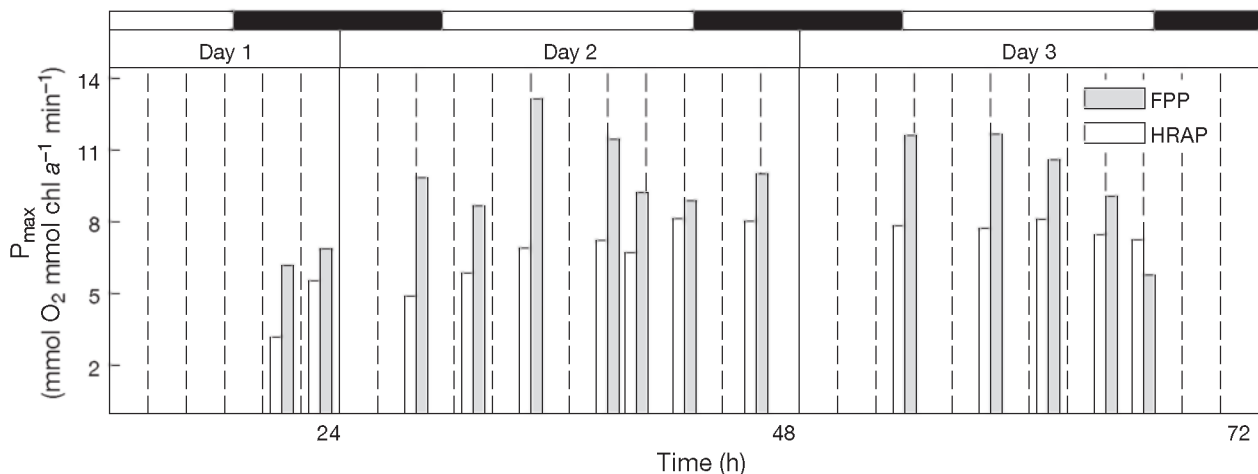


Fig. 10. *Nannochloropsis* sp. Diurnal changes in the maximum photosynthetic rate (P_{\max}) of the cultures, measured using an oxygen electrode at various time intervals during 3 consecutive days in the flat panel photobioreactor (FPP) and the high rate algal pond (HRAP). The bar on top indicates day (white) and night (black) periods; the vertical dashes indicate 2 h intervals

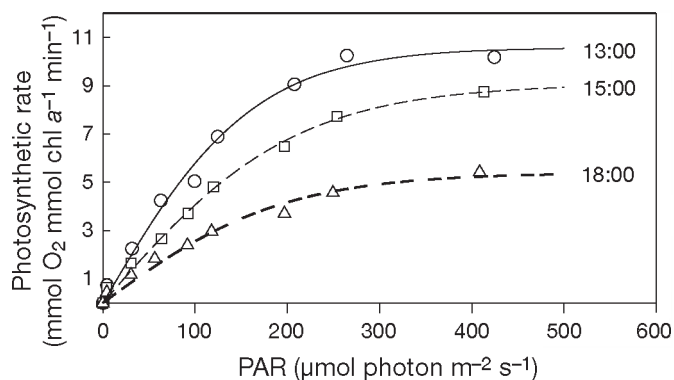


Fig. 11. Photosynthetic rate as a function of photosynthetically active radiation (PAR) in the *Nannochloropsis* sp. culture grown in the flat panel photobioreactor. Measurements were performed on culture samples prior to the high temperature shock (13:00 h) and during the recovery process between 15:00 and 18:00 h as the culture temperature declined from 42 to 35°C

mance of *Nannochloropsis* cultures maintained under a wide range of environmental conditions. The application of flow-through Water PAM fluorometry was found to be preferable for real-time monitoring of the condition of outdoor cultures. Changes in photosynthetic activity were easily monitored and analyzed on a real-time basis, which may help in the optimal operation of outdoor algal production systems.

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