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Aquatic Botany 56 (1997) 253–264

Aquatic botany

Photosynthetic fluorescence induction and oxygen production in two Mediterranean *Cladophora* species measured on site

Donat-P. Häder [a,*], Heike Herrmann [a], Jochen Schäfer [a], Regas Santas [b]

[a] *Institut für Botanik und Pharmazeutische Biologie, Friedrich-Alexander-Universität, Staudtstr. 5, D-91058 Erlangen, Germany*

[b] *OikoTechnics, Athens Helioupolis 16342, Kefallenias 50, Greece*

Accepted 31 October 1996

Abstract

Photoinhibition under solar radiation was measured in the green macroalgae *Cladophora prolifera* and *C. pellucida*, using pulse amplitude modulation fluorescence and oxygen production *in situ*. Photosynthetic oxygen production is inhibited within about 1 h and the quantum yield follows with similar time kinetics. Samples were collected from a habitat protected from exposure to direct solar radiation except for a few hours per day. Therefore, individuals of both species were adapted to low irradiance and readily inhibited by exposure to excessive radiation. Impairment occurred even at their natural habitat during the few hours of exposure to direct solar radiation of the day. Recovery from photoinhibition of both the photosynthetic quantum yield, defined as F'_v/F'_m, and oxygen production took several hours and was not complete. Judging from both parameters indicated above, *C. pellucida* showed a higher degree of photoinhibition than *C. prolifera*. These results suggest that photoinhibition may be a significant factor in determining the spatial distribution of these benthic macroalgae.

Keywords: Chlorophyta; Oxygen measurements; PAM fluorescence; Photoinhibition; Solar radiation

Abbreviations: F_o: initial fluorescence in the dark adapted state, all reaction centers are open (oxidized); F_m: maximal fluorescence in the dark adapted state, all reaction centers are closed (reduced); F_v: variable fluorescence $= F_m - F_o$; F'_o, F'_m and F'_v: the same for the light-adapted state; F_t: current steady state fluorescence; PAM: pulse amplitude modulated fluorometer; PAR: photosynthetic active radiation; qP: photochemical quenching of chlorophyll fluorescence determined by $qP = (F'_m - F_t)/(F'_m - F'_o)$; qN: non-photochemical quenching of chlorophyll fluorescence calculated by $qN = 1 - (F'_m - F'_o)/(F_m - F_o)$

* Corresponding author.

PII S0304-3770(96)01107-2

1. Introduction

Photosynthesis in macroalgae has been measured in the laboratory for many years. This requires transferring the plants from their habitat to the laboratory and subjecting them to highly artificial conditions which may impose severe stress factors on the organisms including changes in temperature, irradiance and salinity. The advent of portable and versatile instrumentation made possible the determination of photosynthetic parameters in the field. One of the more recent developments in photophysiology is a portable instrument measuring pulse amplitude modulation (PAM) fluorescence. The instrument can be used in the field, at the growing site of the algae, eliminating the stress of transferring and handling the specimens under laboratory conditions (Schreiber and Bilger, 1993; Schreiber et al., 1994).

The technique is based on the measurement of chlorophyll *a* fluorescence most of which is believed to derive from photosystem II. Initially, a constant-output, weak red light source induces a background chlorophyll fluorescence signal F_o which is measured in a dark-adapted specimen; under these conditions all photosystem II reaction centers are in the open (reduced) state. Next the application of a saturating pulse induces maximal fluorescence F_m; at this time all PS II reaction centers are closed. After light adaptation maximal fluorescence (now called F'_m) usually decreases and F'_o either increases or decreases.

Measurement of these signals is the basis of the technique of fluorescence quenching analysis. This approach assumes that two different processes reduce the maximal fluorescence yield F_m: photochemical quenching is partly caused by permanent damage to PSII and is repaired by replacement of D1. Non-photochemical quenching is partly due to the build-up of charge and acidity across the thylakoid, which is thought to promote the xanthophyll cycle and de-energization through heat generation (Schreiber et al., 1995; Krause and Weis, 1991). Genty et al. (1989) and Weis and Berry (1987) developed empirical expressions for the quantum yield based on the fluorescence parameters measured during quenching analysis. Their approach does not require previous knowledge of the dark fluorescence parameters F_o and F_m. The validity of this approach has been supported by concomitant gas exchange measurements (Schreiber and Bilger, 1993).

PAM fluorescence techniques have been first used for higher plants to evaluate their ecophysiological conditions in the field; later they were extended to unicellular and macroscopic algae. The limited sensitivity of the early commercial models, however, hampered successful measurements in, for example, dilute suspensions of algae. In addition, several algal groups showed a qualitatively different behavior than higher plants, a phenomenon which has been attributed to the existence of different regulatory mechanisms in various taxonomic algal groups (Büchel and Wilhelm, 1993; Ting and Owens, 1992). Recent investigations have shown that it may be appropriate to use shorter saturating pulses for several groups of algae and cyanobacteria than for higher plants (Schreiber et al., 1995).

Another important technique to evaluate the ecophysiological properties of algal photosynthesis is the measurement of oxygen exchange. As for fluorescence measurements, instrumentation for this purpose was bulky, limiting its use to the laboratory. A

portable and submersible device has been developed which allows on-line, computer-controlled measurements in the water column under solar irradiation (Häder and Schäfer, 1994a,b).

The aim of the present paper was to determine the photosynthetic parameters of two Mediterranean *Cladophora* species under solar irradiation on site. A specific question addressed was whether the natural irradiance in the habitat impaired the photosynthesis of these macroalgae.

2. Materials and methods

2.1. Plant material

Specimens of the common Mediterranean green algae *Cladophora prolifera* (Roth) Kütz. and *C. pellucida* (Huds.) Kütz. (Cladophoraceae) were used for the experiments. The algae were collected from a rocky, east-facing shore of Saronikos Gulf, near Korinth, Greece (37°58′ N, 23°0′ E). Both species seem to be adapted to shaded conditions as they were found only in crevices and light-protected habitats where exposure to direct sunlight was limited to a few hours per day. The experiments were carried out during the summers of 1994 and 1995.

2.2. Measurements of PAM fluorescence

A portable PAM fluorometer (PAM 2000, Waltz, Effeltrich, Germany) was used to determine *in vivo* chlorophyll fluorescence on site (Schreiber et al., 1986). Thalli were harvested immediately before use and mounted in custom-made open UV-B translucent Plexiglas frames (GS 2458, Röhm and Haas, Darmstadt, Germany) submerged in shallow water of a rock pool on site in the shade for 30 min. After this dark adaptation period, PAM fluorescence was determined and the optimal photosynthetic quantum yield calculated. Following this treatment the specimens were exposed to solar radiation during local noon time in shallow water to induce photoinhibition as indicated by a decrease in the effective photosynthetic quantum yield. After 30 min of solar radiation PAM fluorescence was evaluated again. Subsequently, the samples were transferred back into the shade, and the recovery of the quantum yield was estimated at predefined time intervals for up to 6 h.

In order to follow the natural daily course of photoinhibition, thalli were collected every hour from sunrise to sunset, and the fluorescence parameters were estimated immediately after harvest (within less than 5 min). The PAM instrument also allows to run preprogrammed experimental sequences. Using this feature, the dependence of the fluorescence parameters on the irradiance of the actinic light was determined.

2.3. Oxygen exchange measurements

Photosynthetic oxygen production and respiratory oxygen uptake were measured at the surface or in the water column with a submersible device (Häder and Schäfer,

1994a,b) using solar radiation as the actinic light source. The oxygen concentration was determined with a Clark type electrode. Simultaneously, photosynthetic active radiation (PAR), temperature and depth were measured. After amplification the signals were routed to an analog/digital converter housed in a laptop computer. A computer program was developed to poll the data at frequent time intervals, determine mean values, display the data and store them on the hard disk drive. Linear regression of the oxygen concentration was calculated on line in order to evaluate oxygen production or consumption per unit time.

The time course of photoinhibition was determined in thalli exposed to solar radiation. Immediately after harvest dark respiration was measured, and then net oxygen production was assayed until oxygen production had ceased. Recovery of photosynthetic oxygen production was induced by storing the samples at 5 m depth for a predefined period of time in a translucent container. Additional data were collected by exposing the thalli to ambient radiation at various depths in the water column and oxygen production determined. The exposed surface area of the thalli was measured as well as their dry weight for each experimental run.

2.4. Statistics

Mean and standard deviation values were calculated for a minimum of eight independent PAM fluorescence measurements taken from different parts of the same thallus or from different thalli of the same species collected at the same site and depth. Photosynthetic oxygen exchange was measured at least three times in independent samples for each treatment. All experimental runs were repeated several times and Student's *t*-tests were performed where appropriate.

2.5. Measurement of solar radiation

During the experiments solar irradiance was measured in three wavelength bands (UV-B, 280–315 nm; UV-A, 315–400 nm; PAR, 400–700 nm) using a newly developed filter instrument (ELDONET, Real Time Computer, Möhrendorf, Germany). The dosimeter takes readings in each channel at 1 s intervals and averages them over 1 min intervals. After amplification and analog/digital conversion the signals are graphically displayed and simultaneously stored on a computer. Doses are calculated on an hourly and daily basis for each channel.

3. Results

3.1. PAM fluorescence measurements

Typical irradiances under clear skies were 390 W m^{-2} for PAR, 38 W m^{-2} for UV-A and 0.95 W m^{-2} for UV-B at local noon under cloudless skies. In preparation of the photoinhibition experiments, the fluorescence parameters of both algae were determined as a function of the actinic irradiation (Fig. 1). Samples were harvested, and F_o

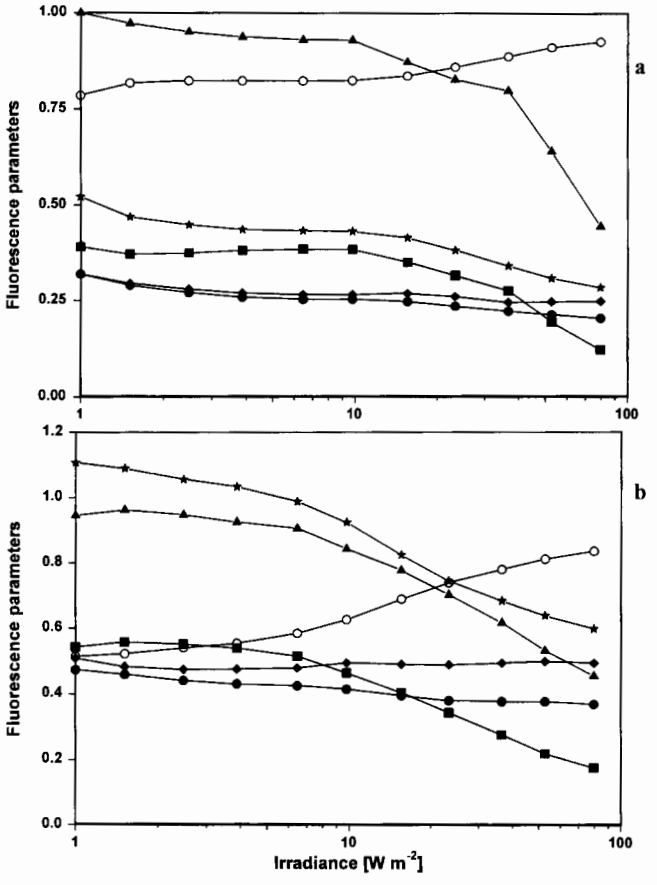

Fig. 1. Fluorescence parameters measured in *C. prolifera* (a) and *C. pellucida* (b) in dependence of the fluence rate of the actinic red light. Before the experiments the thalli were adapted to an intermediate fluence rate of 23 W m^{-2} for 10 min and then exposed to increasing irradiances for periods of 6.5 min each. At the end of each period the fluorescence parameters were determined. ◆, F_t; ■, photosynthetic quantum yield; ▲, photochemical quenching; ∘, non-photochemical quenching; ★, F'_m; ·, F'_o.

and F_m were measured after dark adaptation. Subsequently, the thalli were allowed to adapt to an irradiance of 23 W m^{-2} using the red light emitting diode built in the PAM instrument. After these initial measurements the actinic light irradiance was increased in 11 steps from 1 to 79 W m^{-2} and the fluorescence parameters measured using an automatic experimental run. In *C. prolifera* the steady state fluorescence, F_t, dropped slightly from an initial value of 0.32 (Fig. 1a). F'_o followed a similar pattern at slightly lower values. F'_m decreased from a value of 0.52 to about 0.28. The photosynthetic quantum yield remained about constant up to 10 W m^{-2} and then declined steadily. The photochemical quenching dropped only slightly from its initial value close to 1 up to an irradiance of 10 W m^{-2} and then decreased to less than half the initial value. The non-photochemical quenching rose slightly from values near 0.8. Thalli of *C. pellucida*

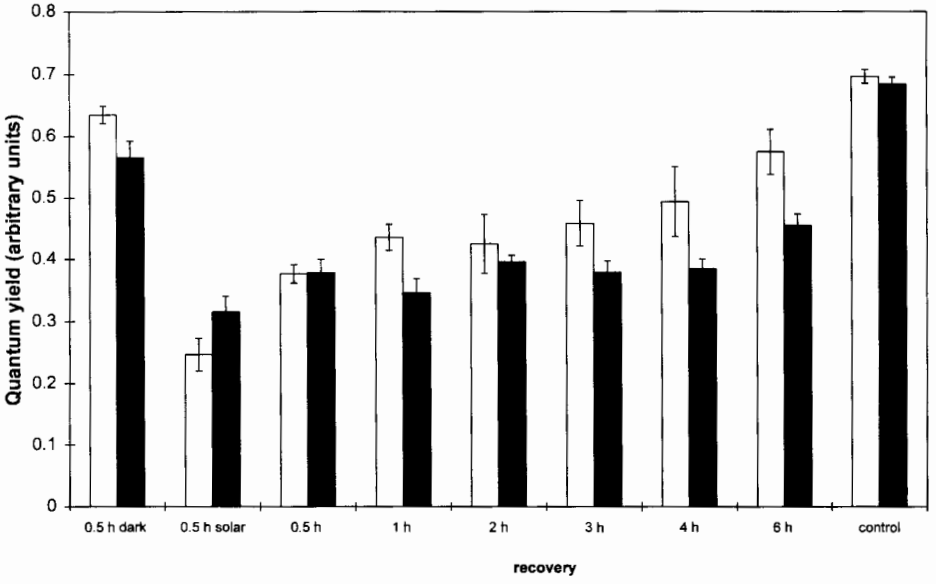

Fig. 2. Photosynthetic quantum yield of *C. prolifera* (open bars) and *C. pellucida* (closed bars) measured after dark adaptation, after exposure to solar radiation in a rock pool and during recovery (in the shade) calculated as $(F'_m - F_t)/F'_m$. For each data point at least eight measurements were averaged and the standard deviation calculated.

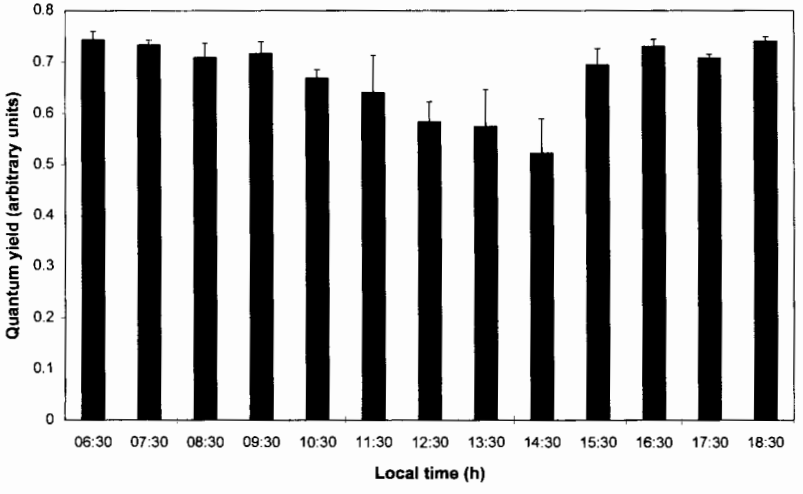

Fig. 3. Photosynthetic quantum yield of *C. prolifera* measured at intervals of 1 h, from dawn to dusk. Thalli were retrieved from their growth site and measured immediately after harvest. For each data point at least eight measurements were averaged and standard deviation calculated.

were subjected to the same experimental run (Fig. 1b). Both F_t and F'_o were slightly higher than in *C. prolifera* and remained almost constant with increasing irradiances. F'_m started from a significantly higher value and decreased to values above 0.6. The quantum yield started at slightly higher values but followed a similar course as in *C. prolifera*. The photochemical quenching showed a similar behavior in both algae, but the non-photochemical quenching rose from a lower value (0.5) in *C. pellucida*.

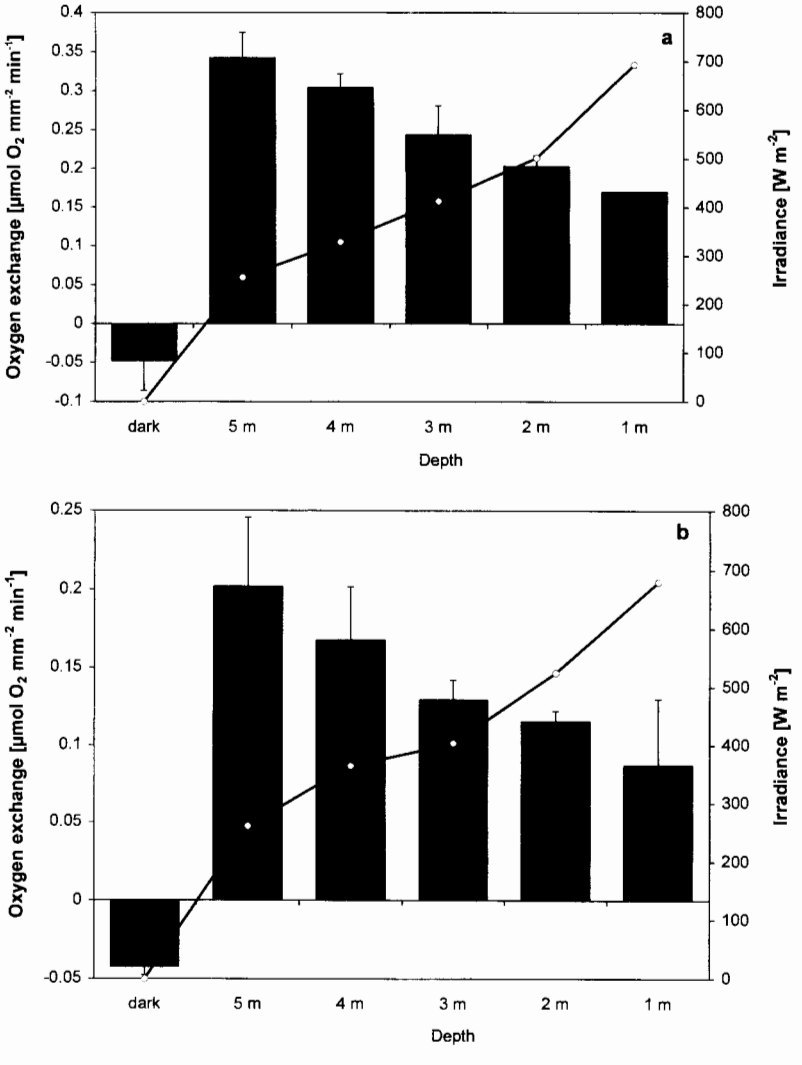

Fig. 4. Photosynthetic oxygen exchange of *C. prolifera* (a) and *C. pellucida* (b) measured under solar radiation between 5 m and 1 m depth in comparison to the illuminance measured at that depth. Before exposure, dark respiration was determined and then oxygen exchange measured integrated over periods of 2 min each. Temperature was 23°C.

Thalli of *C. prolifera* were harvested from their growth site and transferred into a UV-transmitting Plexiglas container which kept the algae in place so that exposure and measurement area could be controlled; sea water circulated through the container which was kept in a shallow rock pool in order to expose the samples to high solar radiation. First the samples were dark-adapted for 30 min to determine the optimal quantum yield (Fig. 2), and then they were exposed to solar radiation for 30 min; this treatment significantly decreased the photosynthetic quantum yield. After exposure the thalli were transferred into the shade and recovery was estimated at predetermined intervals. After 6 h of recovery the quantum had almost reached its initial value. At this time the quantum yield was also measured in algae subjected to the same experimental treatment except for exposure to solar radiation, in order to determine whether there were any other stress factors, in addition to high solar irradiance, affecting the quantum yield. The high quantum yield value indicated that the experimental handling had no effect on the photosynthetic capacity of the algae. The same experiment was repeated with *C. pellucida* which followed the same pattern but showed slightly lower values during recovery.

In the experiments described above, the specimens were exposed to direct solar radiation in a shallow rock pool with a significantly higher irradiation than at the growth site of the algae. In order to determine whether photoinhibition also occurs at their natural growth site, the photosynthetic quantum yield was measured in specimens immediately after harvest. This experiment was carried out from dawn to dusk at

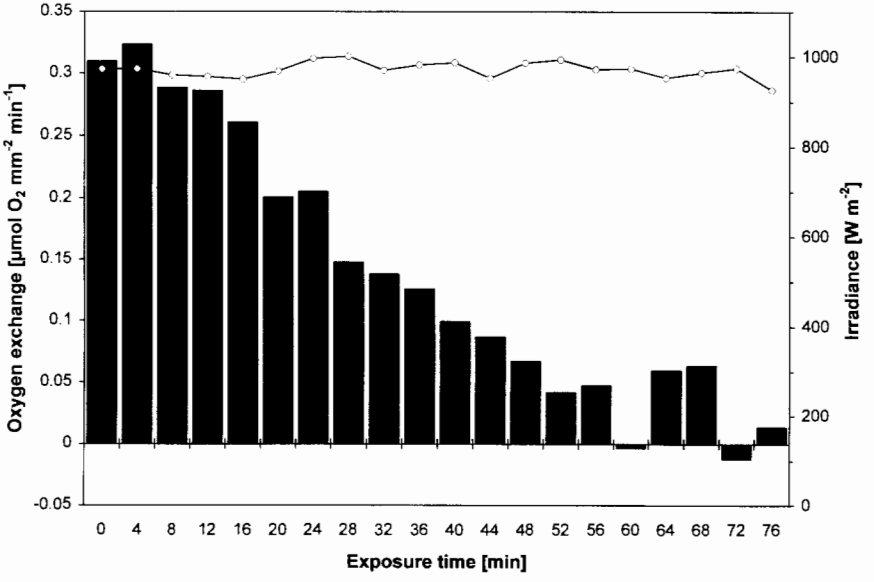

Fig. 5. Oxygen exchange (bars ± SD) in *C. prolifera* as affected by solar radiation at the surface in comparison to the PAR irradiance (open circles and solid line). Temperature was 23°C.

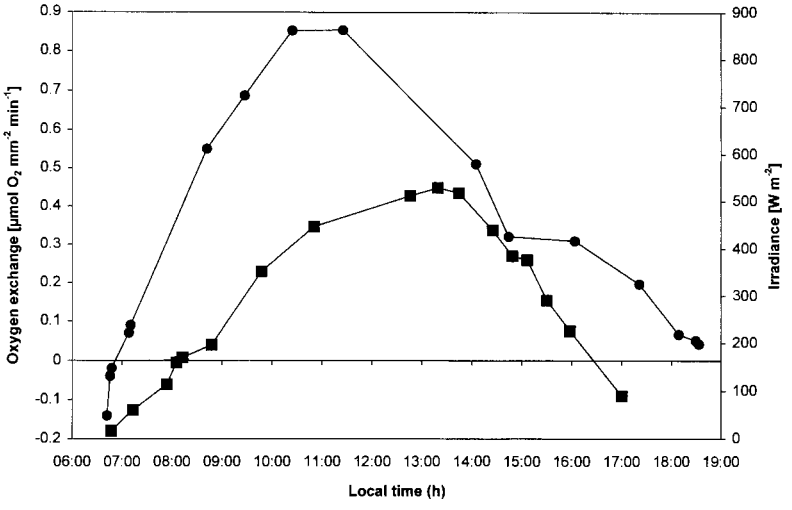

Fig. 6. Oxygen exchange (circles ± SD) in *C. prolifera* measured at 2 m depth under solar radiation from dawn to dusk in comparison to the PAR irradiance in comparison to the irradiance measured at the surface (squares). Temperature was 23°C.

intervals of 1 h, in order to follow their natural daily course. At their growth site the algae received direct solar radiation only between 12:30 and 14:30 local time. The quantum yield values decreased substantially from values above 0.7 to about 0.5 (Fig. 3); after the end of direct solar radiation the quantum yield returned to the initial values within 1 h. *C. pellucida* followed a similar pattern (data not shown).

3.2. Oxygen measurements

Thalli of *C. prolifera* were harvested and immediately transferred into the instrument to determine oxygen exchange. After measuring dark respiration they were exposed at different depths between 5 m and 1 m starting at the lowest level (Fig. 4a). It is interesting to note that the highest net photosynthetic oxygen production was found at a depth of 5 m, even though the irradiance was about 50% lower at 5 m than at the surface. A similar result was found for *C. pellucida* (Fig. 4b). When exposed to solar radiation close to the surface, oxygen production started to decline after a few minutes of exposure, and negative values were recorded after about 1 h of exposure (Fig. 5). The algae were then stored in a translucent container at a depth of 5 m for 2.5 h. Subsequently, photosynthetic oxygen production was determined again. In none of the two species was a noticeable recovery observed (data not shown). In a final experiment oxygen production was measured from dawn to dusk at 2 m depth (Fig. 6). Water was changed at regular time intervals (2 h) in order to avoid oxygen saturation in the limited volume of the chamber. Optimal oxygen production was observed well before local noon (13:30 h).

4. Discussion

High fluence rates of solar radiation induce photoinhibition in higher plants (Björkman and Demmig, 1987; Schreiber et al., 1994), macroalgae (Hanelt et al., 1992, 1993; Franklin et al., 1992; Larkum and Wood, 1993) and phytoplankton (Helbling et al., 1992; Leverenz et al., 1990; Herrmann et al., 1997). The mechanism of photoinhibition is still under debate (Crofts and Yerkes, 1994), but it is regarded as an active regulatory process to protect the photosynthetic apparatus from excessive radiation. During photoinhibition both photosynthetic quantum yield and photochemical quenching decrease, and often the non-photochemical quenching increases. A central role of this process is the turnover of the D1 protein located in photosystem II (Sundby et al., 1993). During intensive solar radiation active oxygen species are produced by transfer of excessive excitation energy from excited chlorophyll molecules to ground state (triplet) oxygen molecules (Foyer et al., 1994).

Both *Cladophora* species used in this study are found mainly in shaded habitats and seem to be adapted to moderate solar irradiance. The algae share almost the same habitat, with *C. pellucida* even more restricted to the shade than *C. prolifera* (Riedl, 1970). This habitat preference is reflected by the physiological parameters determined in this study: direct solar radiation of as short as 30 min causes massive photoinhibition from which the algae took about 6 h to recover. In contrast, other algae adapted to direct solar radiation recover from exposure to direct sunlight much faster (Häder et al., 1996, 1997). Another important result is that the photosynthetic quantum yield decreased even at the natural habitat of the algae.

Photoinhibition as measured by the fluorescence parameters was paralleled by the inhibition of oxygen production. A similar result was found in the brown alga *Dictyota dichotoma* (Hanelt et al., 1994): High white light fluence rates caused a decrease of oxygen production and a concomitant decrease in the photosynthetic quantum yield. In this alga a simultaneous increase in zeaxanthin was found, which is thought to play an important role in the photoprotection mechanisms under light stress (Uhrmacher et al., 1995).

The results described here are based on short-term experiments. Therefore they do not allow conclusions on possible adaptation phenomena. Future experiments are planned to transplant specimens to habitats with both higher and lower irradiance and follow the oxygen production as well as quantum yield over longer periods of time. Further investigations, including inhibition and recovery kinetics are necessary to elucidate the molecular mechanisms of photoinhibition and photodamage in algae.

Acknowledgements

This work was supported by financial support from the European Union (EV5V-CT91-0026; EV5V-CT94-0425; DG XII, Environmental Programme) to D.-P.H. and R.S. The authors gratefully acknowledge the skillful technical assistance of H. Wagner.

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