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Photoinhibition in the Mediterranean Green Alga *Acetabularia mediterranea* Measured in the Field under Solar Irradiation

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Summary

Photoinhibition of photosynthesis was investigated in the Mediterranean green macroalga *Acetabularia mediterranea* using pulse amplitude modulated (PAM) chlorophyll fluorescence and oxygen evolution measurements *in situ* under solar radiation. Fluorescence parameters measured during the course of the day showed that moderate photoinhibition occured even in the natural environment of this alga when the sun was at high angles, but photosynthetic capacity was almost fully restored until sunset. A drastic decline in effective quantum yield was monitored when plants collected at 5 m depth were exposed to high irradiance close to the surface. Removal of the short wavelength band from solar radiation using cut-off filters revealed that UV has an overproportional inhibitory effect on photosynthesis. Especially the UV-B part of the spectrum contributes to photoinhibition whereas UV-A seems to be less effective in *A. mediterranea*. Total recovery of PAM chlorophyll fluorescence was obtained after 2 h of shading in all samples, indicating reversible photoinhibition rather than non-reversible photodamage. Oxygen evolution showed slower kinetics of inhibition.

Key words: Acetabularia mediterranea – Chlorophyta – Oxygen measurement – PAM fluorescence – Photoinhibition – Solar radiation – Ultraviolet 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_o' and F_m' = the same for the light-adapted state; F_v = variable fluorescence, calculated as F_m-F_o; ΔF = variable fluorescence in the light-adapted state, calculated as F_m'-F; F = current steady state fluorescence; PAM = pulse amplitude modulated fluorimeter; PAR = photosynthetic active radiation; qP = photochemical quenching of chlorophyll fluorescence; qN = non-photochemical quenching of chlorophyll fluorescence; UV-B = ultraviolet-B radiation (280–315 nm); UV-A = ultraviolet-A radiation (315–400 nm).

Introduction

Despite the fact that about half of the primary biomass production occurs in aquatic ecosystems, most ecophysiological investigations of photoinhibition have been restricted to higher plants, whereas considerably less data are available on algae. This imbalance may be due to complicated data interpretation as removing aquatic plants from their natural environment creates several stress factors and thus gives rise to a number of artifacts. The development of portable instruments, however, made it possible to obtain essential photosynthetic parameters *in situ*. A miniaturized and computer-

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controlled fluorometer provides satisfactory data for quenching analysis by the saturation pulse method (Quick and Horton, 1984; Dietz et al., 1985). With the pulse-amplitude-modulation (PAM) system (Schreiber et al., 1986) an easy and sensitive determination of PS II effective quantum yield (Φ II) is possible under field conditions as the empirically developed expression $\Phi II = (F_m'-F)/F_m' = \Delta F/F_m'$ (Genty et al., 1989) does not require previous knowledge of fluorescence parameters from dark-adapted plants. The linear relationship between Φ II and the quantum yield of CO_2 fixation has been demonstrated (Weis and Berry, 1987; Schreiber and Bilger, 1993). The evaluation of photosynthetic electron transport rates and the information on the status of the photosynthetic apparatus under natural conditions yield valuable insights into plant stress conditions and contribute to a better understanding of the mechanisms of regulated energy dissipation. Algae of different taxonomic groups show a distinctly different behavior upon illumination (Häder et al., 1996 a–d; Hanelt, 1992), and especially the ability to cope with enhanced UV radiation varies widely among species. Therefore a broad survey is necessary to understand photosynthesis in aquatic ecosystems and its interaction with enhanced solar UV radiation.

Another device for the evaluation of ecophysiological properties of algal photosynthesis is a portable and submersible chamber for oxygen exchange measurements (Häder and Schäfer, 1994 a, b). This device allows computer-controlled monitoring of oxygen exchange *in situ*.

The aim of this paper is to describe the effects of natural solar UV radiation on the photosynthetic capacity and an estimation of the effectiveness of different wavelength bands on the photoinhibition of the Mediterranean alga *Acetabularia mediterranea*.

Materials and Methods

Plant material

Thalli of the common Mediterranean alga *Acetabularia mediterranea* were collected from a depth of 5 m on the coast of Saronikos Gulf, near Korinth, Greece (37° 58′ N, 23° 0′ E). The algae grew on pieces of rock in front of a rocky shore facing east. The thalli were collected while still attached to the stones, and transported in a light-tight container, which also prevented any mechanical disturbance; they were then immediately subjected to the measurements. The experiments were carried out in the summers of 1995 and 1996.

Measurements of fluorescence induction

In vivo induced chlorophyll fluorescence was measured on site with a portable pulse amplitude modulated fluorometer (PAM 2000, Waltz, Effeltrich, Germany) as described by Schreiber and Bilger (1993). To follow the changes in fluorescence parameters upon excessive radiation, plants from 5 m depth were brought to a shallow rock pool. The fluence rate close to the surface is about twice as high as at the natural growing site (Häder et al., 1996 b). To allow an estimation of the damage of the photosynthetic apparatus on the basis of F and F_m' measurements, all experiments were performed on cloudless days in the time between 11:30 and 14:30, as during that time the change in light intensity was less than 10%. Incoming waves provided a temperature constance of less than 1 °C compared with the original habitat. After collection, F and F_m' were measured immediately, and the algae were covered for 30 min. After dark adaptation the quantum yield was determined again. Three parallel samples were exposed 2.5 h under different cut-off filters, 10 × 10 cm each, which removed short wavelengths (WG 295, WG 335, and GG 400, all from Schott & Gen., Mainz, Germany). As a control, one sample was exposed without any filter. Measurements of the effective quantum yield were performed every 30 min. Subsequently, the samples were covered again and the recovery of photosynthetic quantum yield was determined periodically during the following hours.

To assess the daily variation in the effective quantum yield at the growth site, thalli were collected every hour from sunrise to sunset, and the fluorescence parameters were measured in the shade immediately after collection.

The PAM fluorometer provides preprogrammed experimental runs that can also be modified by the user. Using this feature the dependence of the fluorescence parameters on the actinic fluence rate was determined. First, F_m and F_o were measured in the dark-adapted sample. Then the sample was exposed to an intermediate irradiance (23 W m^{-2}) of red light produced from an array of light emitting diodes for 10 min for activation of the Calvin cycle enzymes; subsequently, a series of 11 levels of irradiation, starting from the lowest, were applied, lasting 6.5 min each. At the end of each irradiation period a saturating white light pulse was given to determine the fluorescence parameters, F, F_m' and the effective quantum yield. For correct determination of qP and qN a far red light pulse was given before the saturating light pulse to determine F_o'.

To assure comparability between all samples, the intensity of measuring light, pulse length and distance were kept constant.

Oxygen exchange measurements

Samples were transferred from the dark container into the submersible chamber for oxygen exchange measurements in the water column under solar radiation. Care was taken during handling to avoid any damage to the algae. Dark respiration was measured first, followed by measurements of net oxygen production for 4 min at various depths in the water column. Subsequently, the sample was exposed close to the surface, but still submerged, until photoinhibition was reached. In another type of experiment the kinetics of photoinhibition were investigated in thalli immediately after collection. After exposure, the thallus area and the dry weight were measured.

Statistics

For PAM measurements at least 8 individual organisms were measured (except for the actinic irradiance series) from which mean values and standard deviation were calculated. For the filter experiments Student's t-tests were performed to evaluate significant differences between the treatments. Photosynthetic oxygen exchange was measured at least three times for each irradiation. All experimental runs were repeated at least 2 times.

Measurement of solar radiation

Solar irradiance (W m^{-2}) at the surface was measured in three wavelength bands (UV-B 280–315 nm; UV-A 315–400 nm; PAR 400–700 nm) using a permanently installed instrument (ELDONET, Real Time Computer, Möhrendorf, Germany). The instrument includes commercially available, waterproof filter sensors (Gröbel, Ettlingen, Germany). Readings were taken at 1 s intervals and were averaged over 1 min. After amplification and analog/digital conversion the data in the three channels were graphically displayed and stored in a computer. Hourly and daily doses were calculated for each wavelength band. Typical irradiances under clear skies at noon were 390 W m^{-2} for PAR, 38 W m^{-2} for UV-A and 0.95 W m^{-2} for UV-B.

Results

PAM fluorescence measurements

For experiments in which the effective quantum yield after excessive irradiation was determined, the recorded value of PS II effective quantum yield immediately after collection was around 0.65 (Fig. 1). The slight differences between the mean values of the population of one rock can be ascribed to the fact that under natural conditions irradiation varies slightly. After adaptation for 30 min under a shading cover with only dim light coming from the sides, all samples had recovered to almost the optimal quantum yield measured early in the morning (see below). Also standard deviation decreased to minimal values since in contrast to variable growth conditions the dark exposure was identical for all organisms in the population. Exposure to solar radiation caused a drastic decline in the yield within 30 min. Organisms that received unfiltered sunlight and those that were covered by a WG 295 filter showed a more pronounced decline in effective quantum yield than plants under the WG 335 or GG 400 filters. After 2.5 h, the quantum yield had dropped by 35 % in the populations exposed to partially UV-depleted radiation and by 55 % in the group that received the full solar spectrum. The differences between the plants that received full sunlight and those covered by the WG 295 filter were negligible. Algae that were exposed to UV-B and UV-A depleted radiation showed mostly a higher yield than those exposed to radiation depleted only of UV-B, but these differences were always below the significance level. The fast recovery after 2 h in the shade to almost optimal quantum yield in all samples indicates that no irreversible photodamage had occurred during exposition. The recovery reveals differences between exposure to sunlight with or without the UV-B component, as plants without UV-B treatment reached the initial values after 1 h whereas the others needed at least twice as long.

To determine to which extent photoinhibition occurs at the natural growing site, algae were collected every hour from dawn to dusk and their quantum yield was measured immediately. The optimal quantum yield recorded before sunrise was around 0.75 (Fig. 2). The effective quantum yield monitored later in the day gradually declined with increasing solar radiation and reached its minimum effective quantum yield of 0.52 around 15:00 h. The quick recovery within the next hour to values above 0.6 is due to the fact that the growing site of the algae was in the shade after 15:15 h. The recovery to almost the optimal quantum yield is achieved within 2 h, even before sunset.

A comparison of the fluorescence parameters F and F_m' (Fig. 3) reveals that at the natural growth site F shows only

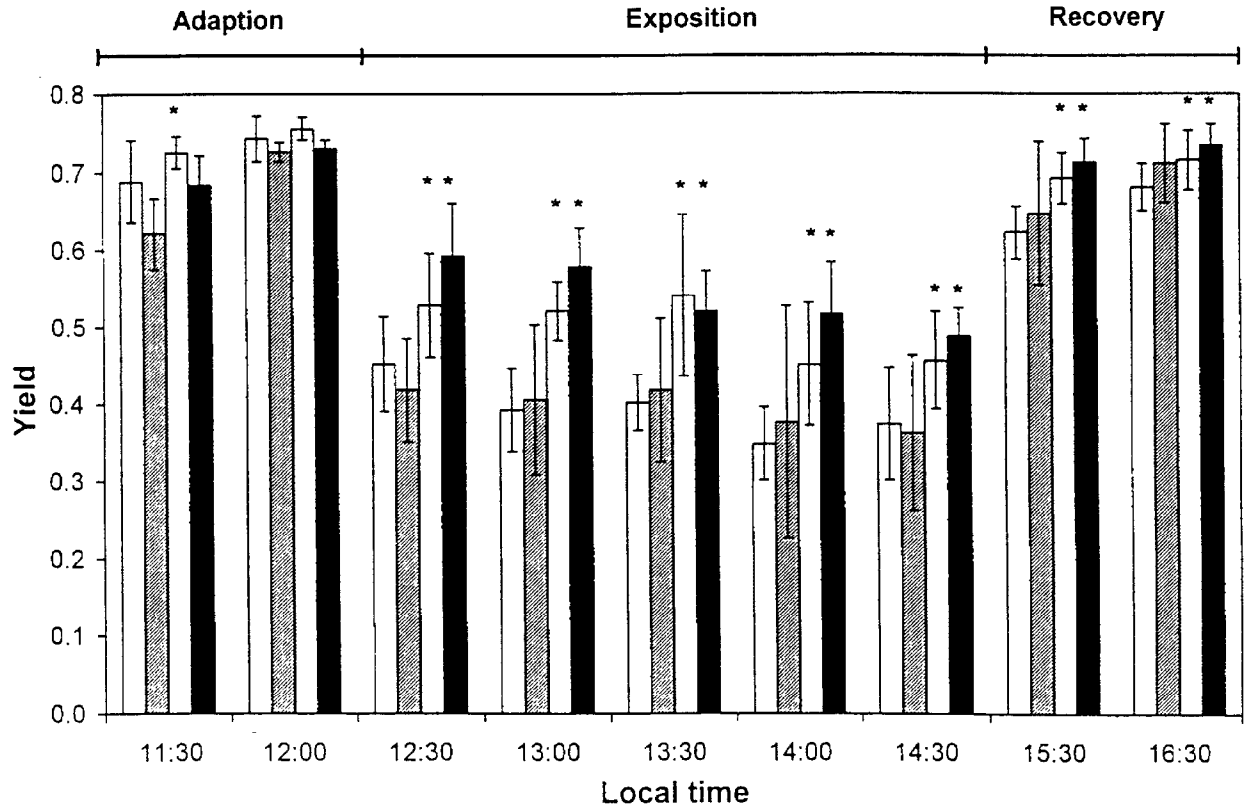

Fig. 1: Photosynthetic quantum yield of *A. mediterranea* harvested from 5 m was measured after collection (11:30 h), after 30 min of dark adaptation, during 2.5 h of exposure to solar radiation close to the surface and during recovery in the shade. The thalli were exposed to unfiltered solar radiation (open bars) or under cut-off filters: WG 295 (hatched bars), WG 335 (dotted bars) and GG 400 (solid bars). For each data point at least eight measurements were averaged and the standard deviation calculated. Asterisks indicate those values that significantly ($p<0.05$) deviate from the values measured for the unfiltered exposition in each time group.

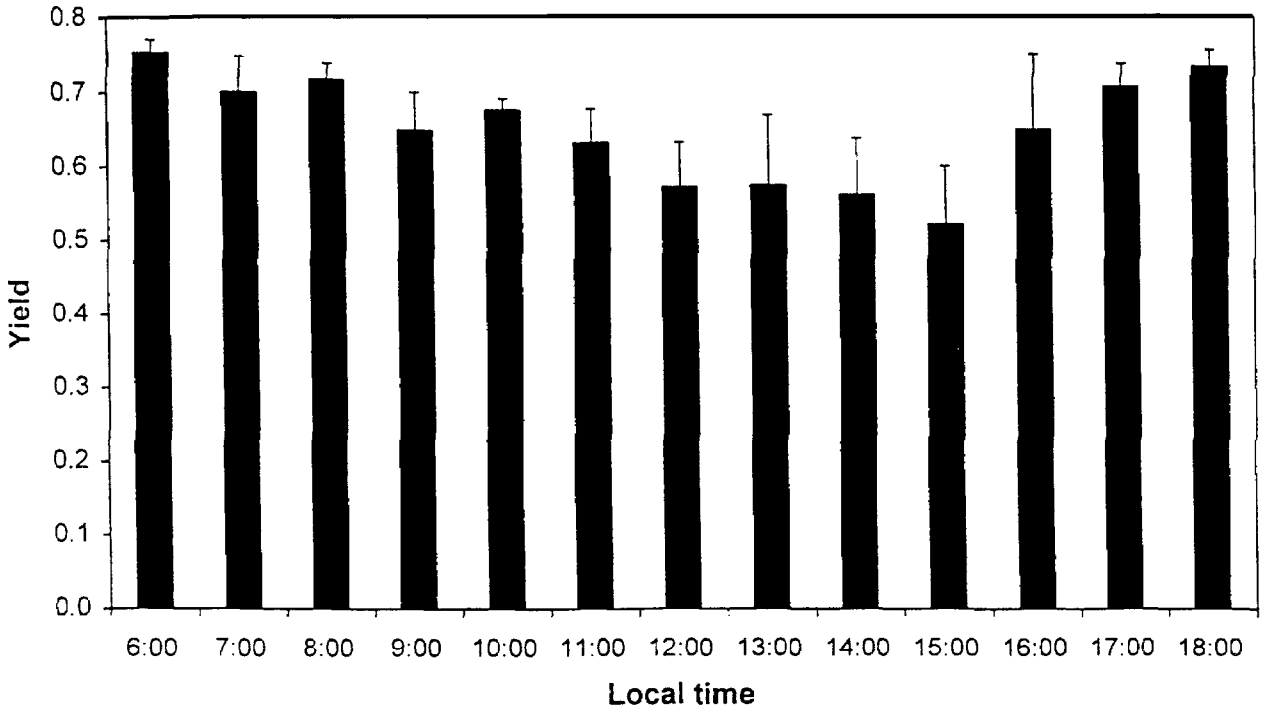

Fig. 2: Photosynthetic quantum yield from dawn to dusk of *A. mediterranea* harvested from 5 m. Thalli were retrieved from their growing site and measured immediately after collection. For each data point at least eight measurements were averaged and the standard deviation calculated.

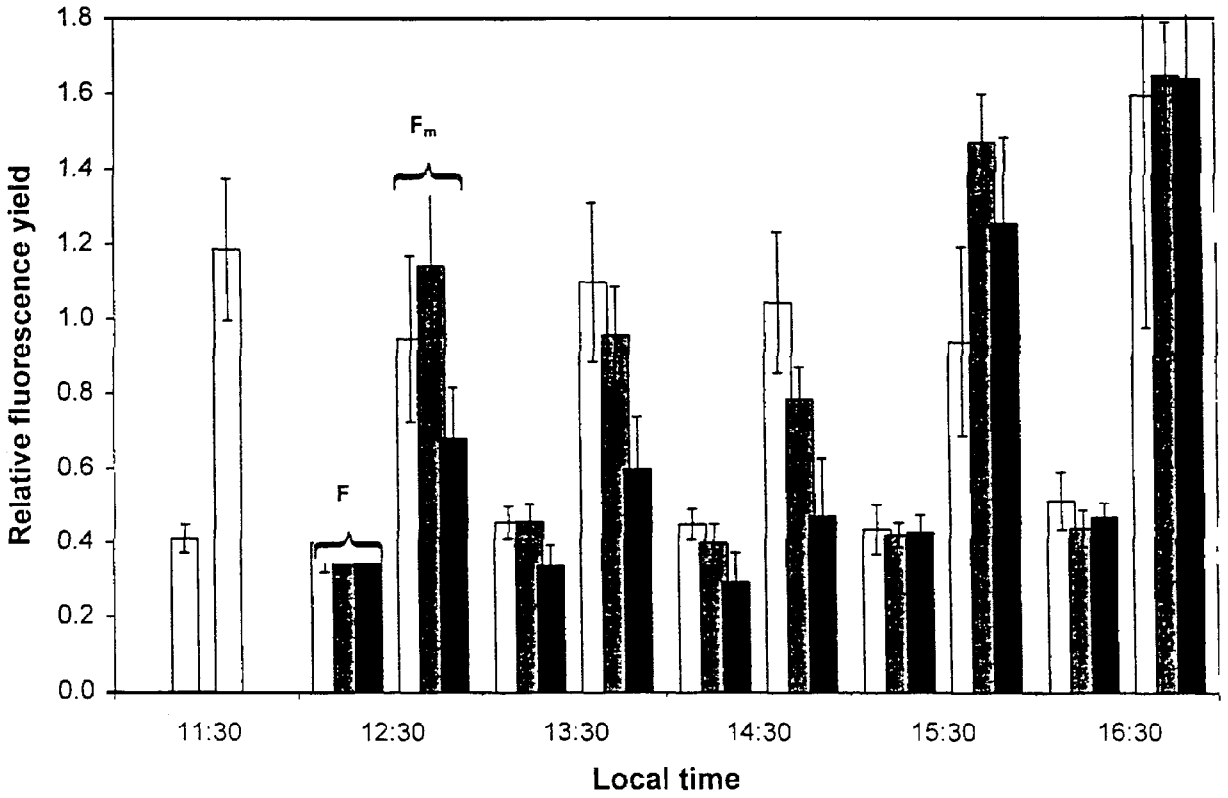

Fig. 3: Fluorescence parameters F (left bars) and F_m' (right bars) immediately after harvest at 11:30 local time and after different illumination: at the natural growing site (5 m depth, open bars) or close to the surface under GG 400 (hatched bars) and WG 295 (solid bars) cut-off filters. Plants under filters were shaded after 14:30 h for recovery. For each data point at least eight measurements were averaged.

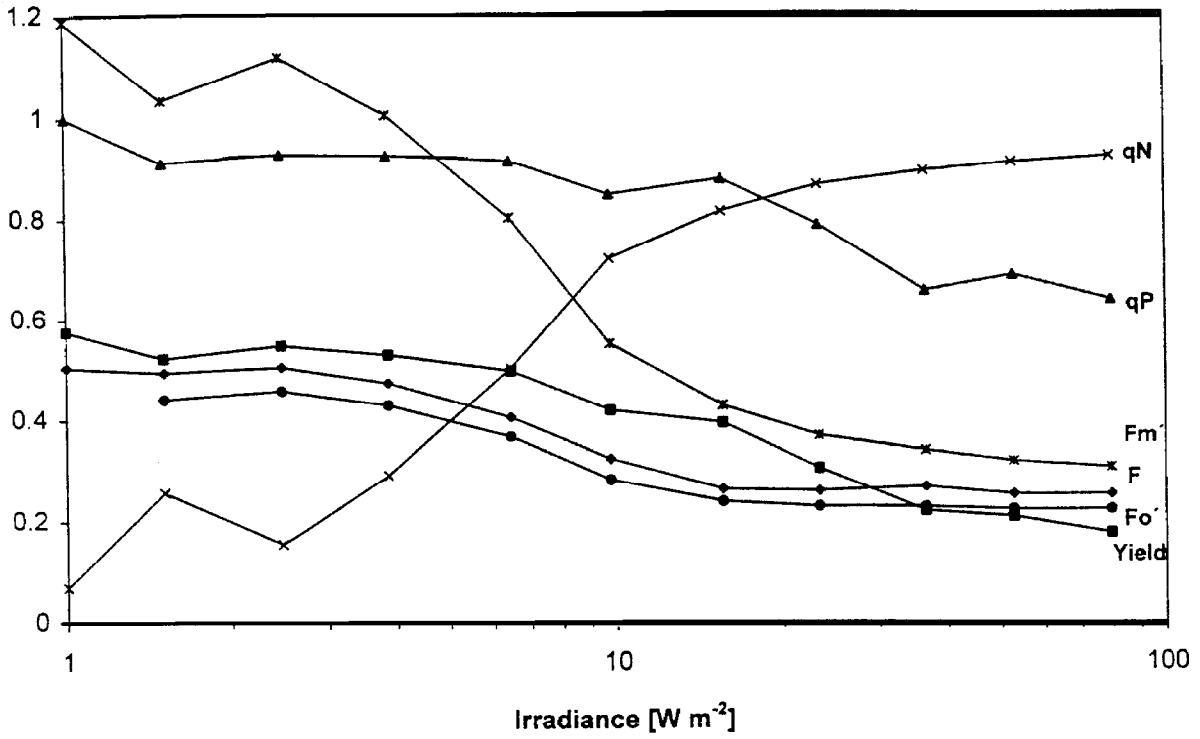

Fig. 4: Fluorescence parameters measured in *A. mediterranea* harvested from 5 m depth in dependence of the fluence rate of the actinic red light. Before the experiments the thallus was adapted to an intermediate fluence rate of 23 W m^{-2} for 10 min and then exposed to increasing irradiances for periods of 6.5 min each. At the end of each period the fluorescence parameters were determined. Diamonds: F, squares: photosynthetic quantum yield, triangles: photochemical quenching, crosses: non-photochemical quenching, asterisks: F_m' and circles: F_o'.

minor variations throughout the day, whereas F_m' drops to 70 % of early morning values (data not shown) when the sun is at high angles. Plants exposed close to the surface, which received the full solar spectrum, not only show a drastic decline of the F_m' parameter to less than half of the values measured at the growth site but also show a reduced steady state fluorescence. Excess PAR without the UV part of the spectrum leads to a less pronounced reduction of F_m' and has only little effect on F.

To determine the dependence of the fluorescence parameters on irradiation with actinic light, an automatic run was performed on freshly harvested samples (Fig. 4). The sample was first dark-adapted, and F_o and F_m were measured (data not shown). Then the thallus was adapted to light using the built-in red light emitting diode with an irradiance of 23 W m^{-2} for 10 min. Subsequently, the actinic light irradiance was increased in 11 steps from 1 to 79 W m^{-2}. The effective quantum yield gradually dropped from 0.59 to below 0.2, countered by an increase of the non-photochemical quenching qN from 0.05 to values close to 1, representing minimal quantum yield. In contrast, the photochemical quenching qP started with a value of 1, indicating optimal energy exploitation for photosynthesis, and decreased to values around 0.6. Also the maximal fluorescence level, after a saturating light pulse, F_m' showed a drastic decline upon increasing fluence rates, whereas both the steady state fluorescence, F, and the minimal fluorescence yield, F_o' showed only small decreases.

Oxygen exchange measurements

Thalli harvested from 5 m depth showed a pronounced respiration when transferred into darkness (Fig. 5). Exposure to sunlight at different depths between 5 m and 1 m caused a net oxygen production ranging from 0.25 µmol O_2 mm^{-2} min^{-1} to 0.35 µmol O_2 mm^{-2} min^{-1}. Despite the fact that the plants were adapted to irradiances at 5 m, the highest net oxygen production was found close to the surface with an evolution of 4.8 µmol O_2 mm^{-2} min^{-1}.

To determine the kinetics and the extent of photoinhibition at the level of oxygen production, freshly harvested plants from 5 m depth were exposed to solar radiation just below the water surface (Fig. 6). The net oxygen production remained rather constant for 30 min. Thereafter the oxygen evolution was more and more inhibited, and a zero net production was detected after 150 min of exposure.

Discussion

Photoinhibition, the light-dependent reduction in photosynthetic efficiency (Kok, 1956), is a widespread phenomenon in oxygenic photosynthetic organisms and can result in considerable decreases in photosynthetic productivity. Its nat-

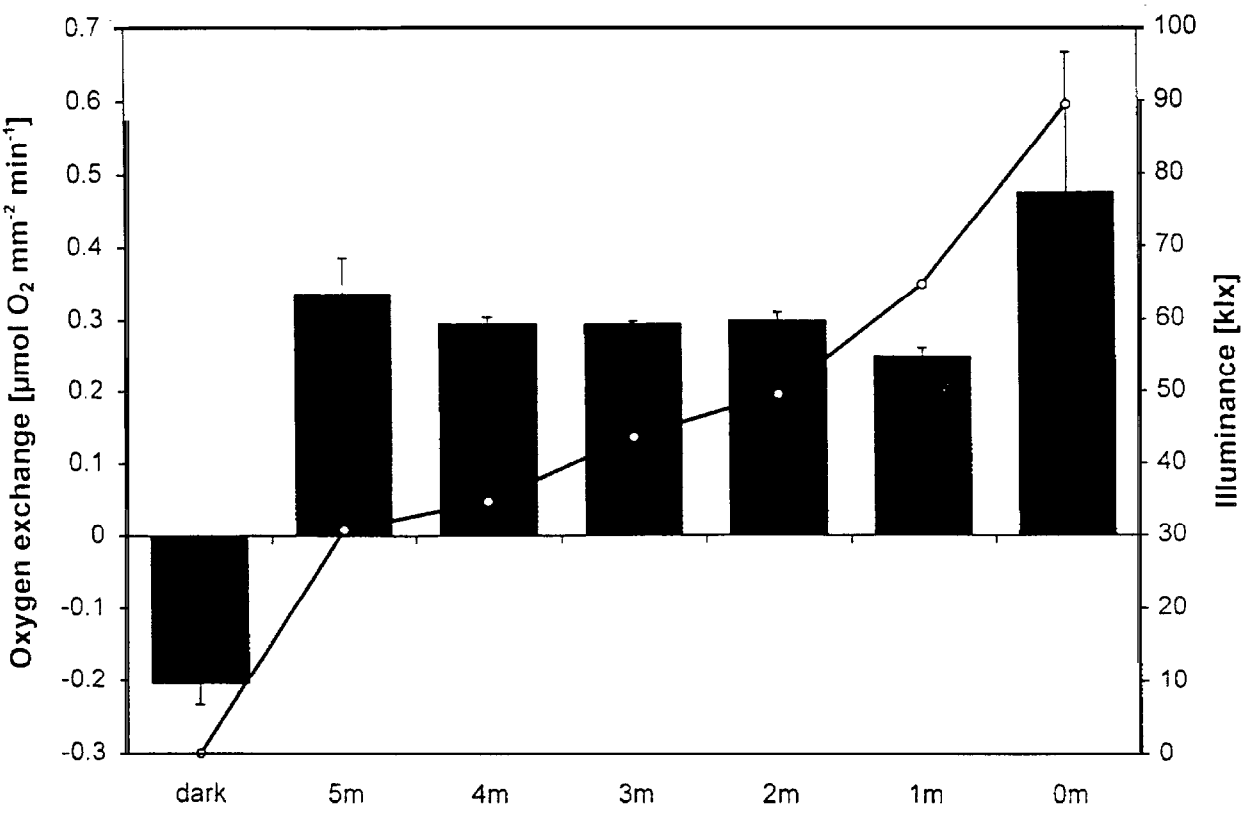

Fig. 5: Photosynthetic oxygen exchange of *A. mediterranea* collected at 5 m depth in darkness and subsequently at decreasing depths from 5 m to 0 m for 4 min each.

ural occurrence has been demonstrated in higher plants (Björkman and Demmig, 1987; Schreiber et al., 1994), macroalgae (Hanelt, 1992; Hanelt et al., 1992; Franklin et al., 1992) and phytoplankton (Helbling et al., 1992; Leverenz et al., 1990; Herrmann et al., 1996). The mechanism of photoinhibition is still controversial, but it is widely regarded as an intrinsic feature of photosynthetic organisms to protect their photosynthetic apparatus from excessive radiation.

Moderate photoinhibition in *A. mediterranea* was found at its natural growing site, comparable with findings in other green algae (Häder et al., 1996 a, b). The decline in the effective quantum yield followed the encountered photon fluence rate and was not due to a circadian periodicity, since measurements on a cloudy day showed less effect (data not shown). The quick recovery after shading indicates that no photodamage had occurred.

The photosynthetic yield of *A. mediterranea* harvested from 5 m and exposed to unfiltered solar radiation close to the surface dropped drastically to 50 % of the initial values. The minimal value of the yield parameter at the natural growing site never fell below 70 %, not even in algae growing naturally in shallow waters (data not shown). This increased sensitivity reflects an adaptation to the lower irradiances found at 5 m, which is about half of the surface radiation. By an almost complete recovery after 2 h of shading these algae demonstrate their capacity to cope with a wide range of fluence rates, in contrast to some green and red algae that did not recover after a comparable treatment (Häder et al., 1996 c, d). As the steady state fluorescence F is affected under stress conditions a linear relationship between Y and the photosynthetic electron transport is not warranted any more. Decreasing F values under excess radiation might arise from reversible regulatory mechanisms such as photoprotection via thermal dissipation. A loss of F due to photobleaching seems not very likely as after shading F recovers quickly. An increase in F_o', which would be indicative for photodamage to PS II (Demmig-Adams, 1990; Franklin et al., 1992) and has been found in some red algae (Hanelt, 1992; Häder et al., 1996 c), was never seen in *A. mediterranea*.

The removal of wavelengths shorter than 335 nm resulted in a significantly reduced degree of photoinhibition, but additional removal of longer wavelengths did not further diminish photoinhibition. Similar results were reported for other algae (Helbling et al., 1992; Larkum and Wood, 1993; Herrmann et al., 1995; Häder et al., 1996 b), but cannot be generalized as findings in phytoplankton showed a similar or even higher photoinhibitory effect of the UV-A component (Holm-Hansen et al., 1993).

Measurements of oxygen evolution under high light conditions close to the surface showed slower inhibitory kinetics than PAM measurements. The net oxygen production was hardly affected during the first 30 min. In more sensitive algae the net production declined rapidly after exposure to high fluence rates (Häder et al., 1996 d), again indicating that

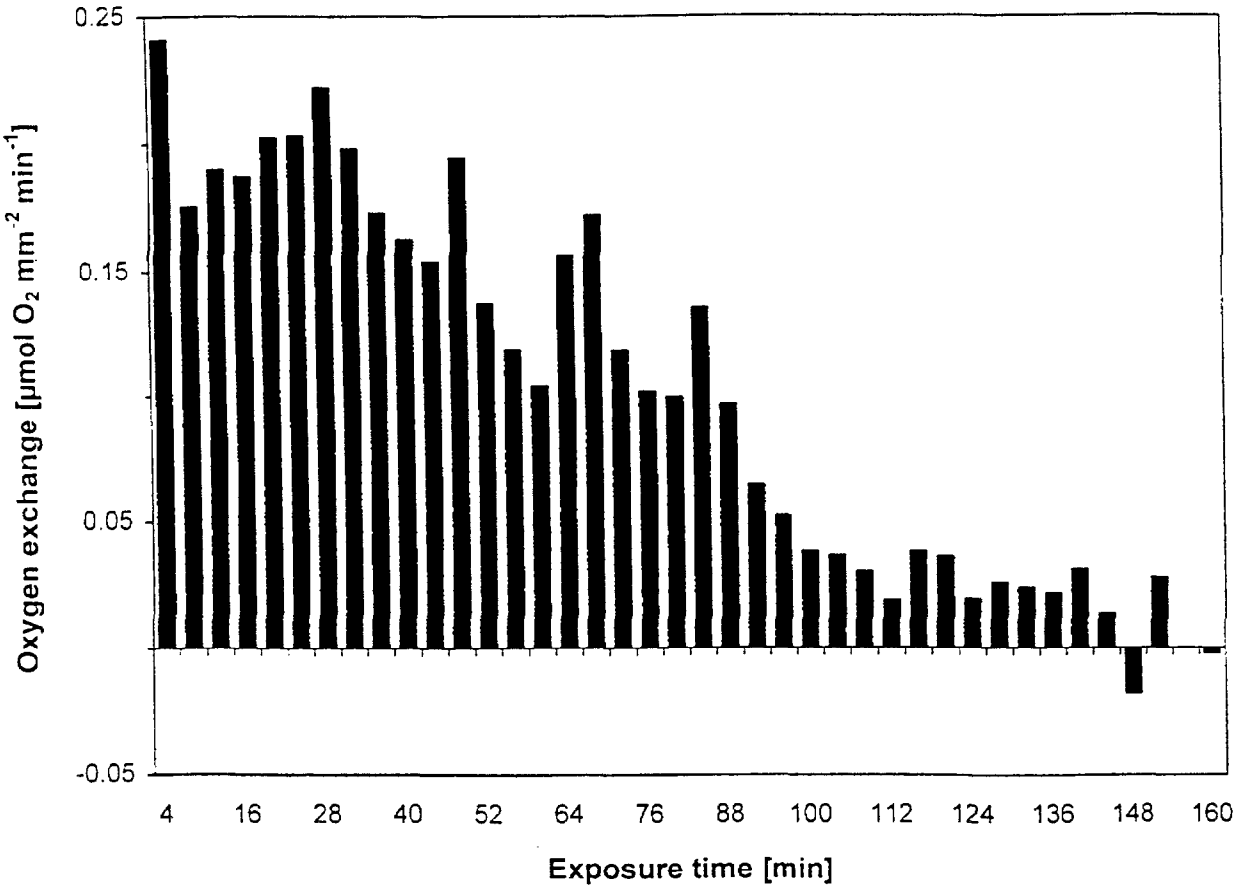

Fig. 6: Photosynthetic oxygen exchange of *A. mediterranea* harvested from 5 m and exposed close to the surface.

A. mediterranea is relatively insensitive to enhanced radiation. The fact that after 150 min of exposure zero net oxygen production was reached although the quantum yield maintained 50 % might be due to an increased respiration.

Different inhibitory kinetics for effective quantum yield and oxygen production after excess radiation have been reported under artificial conditions for green (Herrmann et al., 1995) and red algae (Hanelt et al., 1992) and may be due to a regulation at different levels. Although donor side photoinhibition has not yet been demonstrated to be a feature of photoinhibition under physiological conditions, the different onset of inhibition suggests this explanation. It seems that regulatory mechanisms and capacity not only vary between algae of different groups (Büchel and Wilhelm, 1993) but also among different species of green algae. To elucidate the molecular mechanisms and the wavelength dependence of photoinhibition and photodamage further investigations of inhibition and recovery kinetics as well as detailed action spectra are necessary.

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