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Photoinhibition by solar radiation in the Mediterranean alga *Peyssonnelia squamata* measured on site

Donat-P. Häder[1], Markus Porst[1] & Regas Santas[2]
[1]*Institut für Botanik und Pharmazeutische Biologie, Friedrich-Alexander-Universität, Staudtstr. 5, D-91058 Erlangen, Germany;* [2]*Oikotechnics, Athens Helioupolis 16342, Keffalenias 50, Greece*

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Abstract

Photoinhibition of photosynthesis, defined as reversible decrease in the effective photosynthetic quantum yield, was measured in the Mediterranean red alga, *Peyssonnelia squamata*, using pulse amplitude modulation (PAM) chlorophyll fluorescence and oxygen production on site. This alga is adapted to very low fluence rates of solar radiation and is easily inhibited by exposure to excessive radiation. At high solar angles its photosynthetic capacity is impaired even in its natural habitat, in the protective shade of overhanging rocks. Oxygen production was maximal at 5 m depth and decreased to almost zero at the surface. When exposed at the surface oxygen production ceased within 16 min. The optimal photosynthetic quantum yield, defined as F_v/F_m, was about 0.45 in dark-adapted specimens. After 30 min of exposure to unattenuated solar radiation the (effective, F'_v/F'_m) quantum yield decreased to below 0.1. Removing solar UV (especially UV-B) significantly reduced photoinhibition: the quantum yield of a sample exposed under a UV-B cut-off filter was double that of a sample exposed to full solar radiation after 30 min exposure. Recovery from photoinhibition took several hours and was not complete after prolonged exposure (1.5 h) to direct solar radiation. The degree of photoinhibition depended on the depth at which the thalli were exposed. Recovery from photoinhibition was complete within 2 h except when the algae were exposed at the surface. When measured over the whole day, the effective photosynthetic quantum yield significantly decreased by about 25% from initially high values toward early afternoon and rose again towards evening. The data indicate that this alga is adapted to very low irradiances and is easily inhibited by excessive solar radiation; solar UV contributes substantially to the observed photoinhibition.

Abbreviations and symbols:
F_o – initial fluorescence in the dark adapted state, all reaction centers are in the open state (oxidized);
F_m – maximal fluorescence in the dark adapted state, all reaction centers are closed (reduced);
F_v – variable fluorescence, calculated as 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 (400–700 nm);
qP – photochemical quenching of chlorophyll fluorescence determined as $q^P = (F'_m - F_t)/(F'_m - F'_o)$;
qN – non-photochemical quenching of chlorophyll fluorescence calculated by $q^N = 1 - (F'_m - F'_o)/(F_m - F_o)$.

Introduction

Most macroalgae are sessile organisms which occupy a defined depth band on the coast. While some algae can be found at or even above the high tide mark (supralittoral) and tolerate being exposed in rock pools where they are exposed to unattenuated solar radiation, others inhabit the tidal zone (eulittoral). Especially

rhodophyta are often found below the level of the lowest tides (sublittoral) or in the understory of kelp or other macroalgae (Lüning 1985). It is believed that the vertical distribution is mainly controlled by the availability of light (Lüning 1985). The range in the exposure can be substantial, from over 1000 W m^{-2} on clear days at the surface to less than a percent of this which reaches the understory of a kelp habitat. Some rhodophyta have been found at a record depth of 268 m in the Bahamas (0.001% of the surface light); however, growth in these organisms has been recorded to be extremely slow resulting in the production of only a few cells per year (Lüning 1985). The mediterranean *Peyssonnelia squamata* is adapted to very low irradiances and thrives only in crevices or under overhanging rocks where light exposure is limited to a small fraction of direct radiation. Obviously, the different algal divisions have developed effective mechanisms to optimize their photosynthetic apparatus to the changing light conditions and the very different average irradiances encountered at different depths (Büchel & Wilhelm 1993; Ting & Owens 1992).

While in the past ecophysiological measurements of photosynthesis and light stress physiology were limited to the laboratory, the recent development of portable and versatile instrumentation makes it now possible to determine relevant parameters on site which minimizes artifacts due to the removal and transport of the organisms.

A valuable tool for the determination of the ecophysiological properties of algal photosynthesis is the assessment of oxygen exchange *in situ*. For this purpose, a portable instrument has been developed which allows real time, computer-controlled measurements of net oxygen production at various depths in the water column using solar radiation as an actinic light source (Häder & Schäfer 1994a,b).

Another recent development is the miniaturization of instrumentation for pulse amplitude modulation (PAM) fluorescence measurements (Schreiber et al. 1986). This technique provides valuable information on the physiological status of the photosynthetic apparatus. The effects of stress factors such as excessive radiation can thus be assessed (Briantais et al. 1986; Renger & Schreiber 1986; Krause & Weis 1991). While PAM data interpretation is still under discussion past research has led to a better understanding of this complex process (Quick & Horton 1984; Schreiber et al. 1986; Walker 1992; Schreiber & Bilger 1993: Schreiber et al. 1994). PAM chlorophyll fluorescence analysis can be used to determine the photosynthetic quantum yield (in the PAM literature defined as optimal quantum yield, F_v/F_m, in dark-adapted specimens and as effective quantum yield, F'_v/F'_m, in irradiated samples). From these data the degree of photoinhibition can be determined. Photoinhibition is defined as the reversible decrease in the photosynthetic quantum yield in contrast to photodestruction which is not reversible within a few hours.

The underlying assumption of fluorescence quenching analysis is that two different processes can reduce the maximal fluorescence yield. The first is photochemical quenching which can be suppressed by the application of a short saturating pulse which closes all reaction centers of PS II. The second process is non-photochemical quenching which is thought to be mainly based on the energization of the thylakoid membrane (Schreiber et al. 1995; Krause & Weis 1991).

To obtain quantitative information on the physiological status of the photosynthetic apparatus Genty et al. (1989) and Weis & Berry (1987) developed empirical expressions for the quantum yield based on the measured fluorescence parameters. This analysis does not require previous knowledge of the dark fluorescence parameters and has been validated by concomitant gas exchange measurements.

The aim of the present paper is to assess photoinhibition by solar radiation in the Mediterranean red alga *Peyssonnelia squamata* on site by determining oxygen production and calculating photosynthetic quantum yield obtained from PAM chlorophyll fluorescence measurements. The specific question addressed is whether ambient irradiation levels impair photosynthesis in these algae in their natural habitat.

Materials and methods

Plant material

The experiments were carried out during the summer of 1996 at an east-facing rocky shore of Saronikos Gulf, near Korinth, Greece (37°58′ N, 23°0′ E). Thalli of the mediterranean red alga *Peyssonnelia squamata* (Peyssonneliaceae) were used for all experiments. The specimens were collected from underneath overhanging rocks at a depth of about 6 m where they were never exposed to direct sunlight.

Oxygen exchange measurements

Oxygen exchange was measured under solar radiation at the water surface or at different depths in the water column with a submersible device described by Häder & Schäfer (1994a,b). This device monitors dissolved oxygen concentration with a Clark type electrode in a closed chamber filled with sea water and containing the specimen. PAR irradiance, temperature and depth are measured simultaneously. After amplification the signals are routed to an analog/digital card located in a laptop computer. A computer program samples the data, calculates and plots mean values and stores them on the hard disk. Linear regression is applied in order to determine net oxygen production per unit time. Care was taken to avoid depletion of carbon dioxide in the closed cuvette by adding carbonate (10 mM) and limiting the experimental time.

Photoinhibition was determined by exposing thalli to solar radiation immediately after collection. After an initial measurement of dark respiration, net oxygen production was assayed continuously until it ceased due to a massive decrease in the effective quantum yield. At the end of all experimental runs the exposed area of the thalli was measured as well as their dry weight.

Measurements of PAM fluorescence

A portable pulse amplitude modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany) was used to determine *in vivo* chlorophyll fluorescence on site (Schreiber et al. 1986). The PAM fluorometer can be programmed for running experimental sequences. Using this feature it is possible, e.g., to correlate the fluorescence parameters to the actinic irradiance provided by a red light emitting diode (LED). Thalli collected by diving were immediately subjected to fluorescence measurements in chambers made of Plexiglas transparent to both UV and PAR. The specimens were dark adapted by covering the chambers with a dark cloth and leaving them in shallow waters for avoiding sample overheating. The fluorescence parameters were then measured and the optimal photosynthetic quantum yield determined. Subsequently the specimens were exposed to solar radiation and the fluorescence parameters measured again. Recovery of photosynthetic quantum yield was analyzed in the following hours during which the talli were kept under shaded conditions.

Short wavelength radiation was removed by a series of UV cutoff filters (Schott & Gen., Mainz, Germany). In another type of assay specimens were collected every hour from sunrise to sunset, and the photosynthetic quantum yield determined immediately after harvest.

Statistics

Photosynthetic oxygen exchange was measured in a minimum of three replicates per treatment. Eight replicate PAM fluorescence measurements were taken at minimum using different parts of the thallus or different thalli. Mean values and standard deviation were calculated. All experimental runs were repeated several times and Student's *t*-tests were performed where appropriate.

Measurement of solar radiation

Solar irradiance was recorded during the whole measurement period with a recently developed dosimeter (ELDONET, Real Time Computer, Möhrendorf, Germany) in three wavelengths bands (UV-B, 280–315 nm, UV-A, 315–400 nm, PAR, 400–700 nm). The instrument takes readings at 1-s intervals for each channel and the software calculates mean values over 1-min intervals. Hourly or daily doses can be calculated from the stored data. 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.

Results

Figure 1a shows the oxygen exchange curve of *Peyssonnelia squamata*. After initial measurement of dark respiration photosynthetic oxygen production was determined at different depths from 5 to 1 m. Even though the irradiance at 5 m was about half that of the surface, photosynthetic oxygen production was highest at the lowest depth and decreased to almost zero at 1 m. When kept submerged just under the surface of the water, net oxygen production was constant for about 8 min and then started to decrease (Figure 1b). After 16 min, net photosynthetic oxygen production dropped to zero, while negative values were recorded thereafter.

Using PAM the dependence of chlorophyll fluorescence parameters on the irradiance of the actinic light was determined (Figure 2). First the thalli were kept in darkness for at least 30 min, and F_o and F_m were measured. Then the thalli were exposed to white light

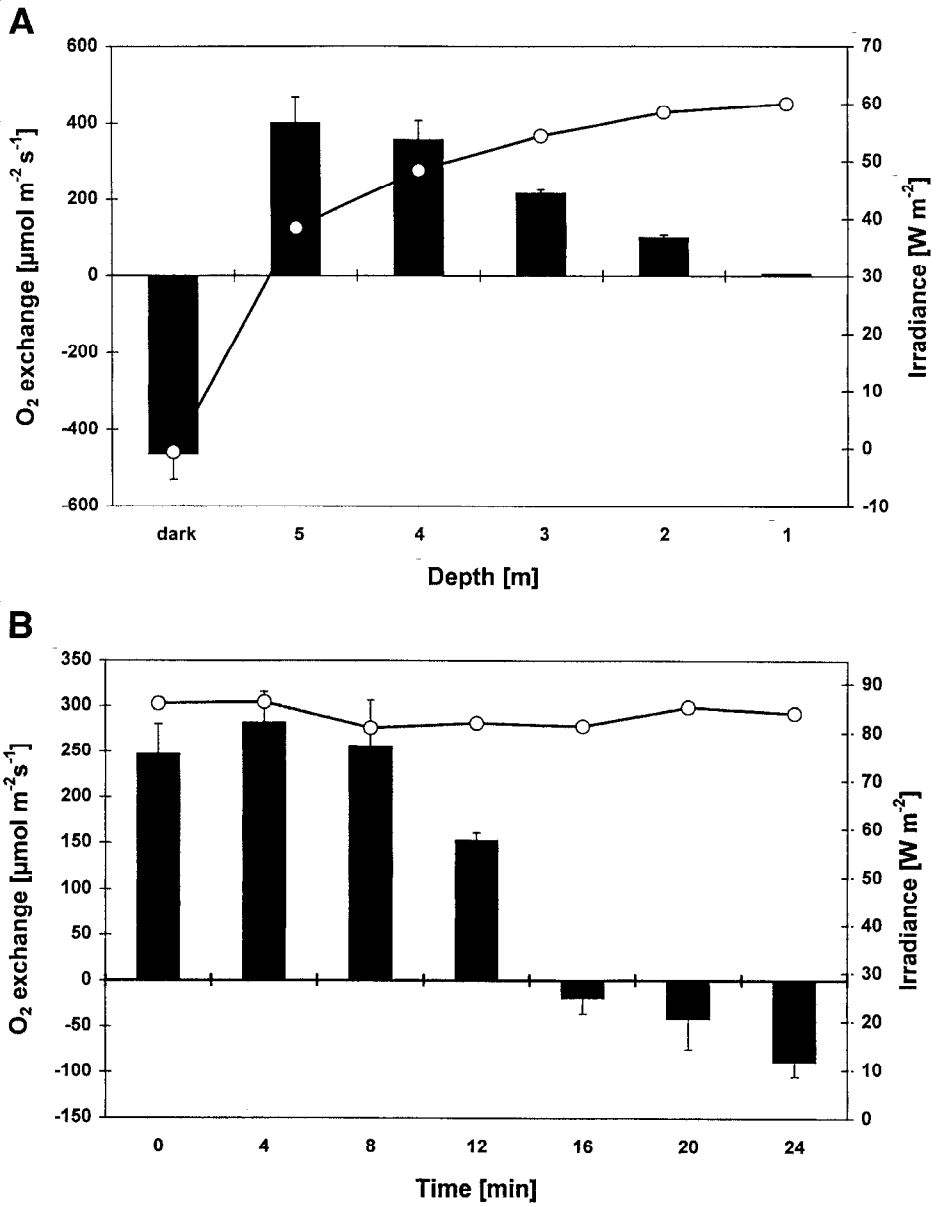

Figure 1. (A) Photosynthetic oxygen exchange of *Peyssonnelia squamata* measured under solar radiation at different depths (B) and at the surface in dependence of the exposure to solar irradiance (open circles, solid line). Before exposure, dark respiration was determined. The data are means of three independent measurements ± S.E.

at an irradiance of 23.3 W m^{-2} to activate the Calvin cycle enzymes. Subsequently, the actinic light irradiance was increased in 11 steps from 1 to 79 W m^{-2}. The steady state fluorescence, F_t, remained constant at a value of about 0.5 to 0.6. F_o' followed a similar pattern at slightly lower values. In contrast, as irradiance increased, F_m' dropped from an initial value near 1 to about 0.6. The photosynthetic yield declined with increasing irradiances to values close to zero. The photochemical quenching dropped from its initial value of close to 1 to about 0.2, while the non-photochemical quenching rose concomitantly from values near 0.1 to about 0.75.

Thalli were harvested and kept in a shallow rock pool suitable for on-site measurements with the PAM instrument. The optimal quantum yield was deter-

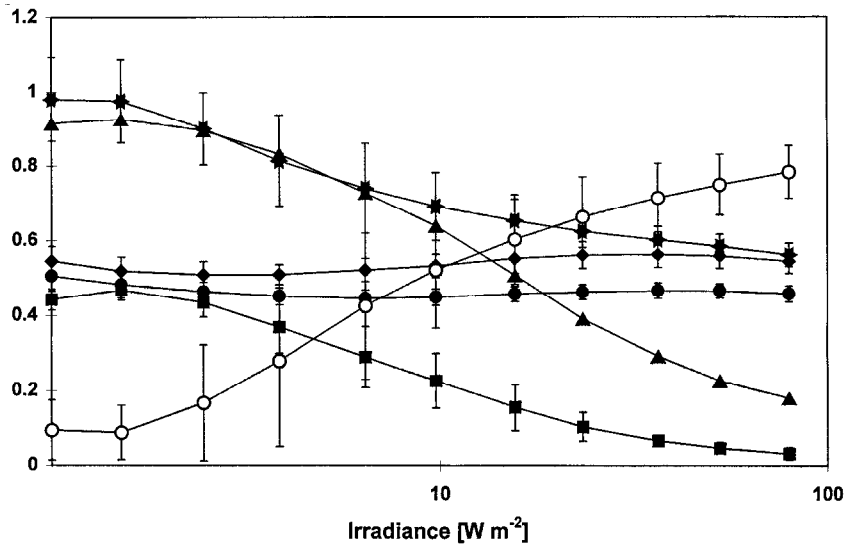

Figure 2. Fluorescence parameters measured in *Peyssonnelia squamata* in dependence of the fluence rate of the actinic red light. Before the experiments the thallus was adapted to an intermediate fluence rate of 23 W m^{-2} for 10 min and then exposed to increasing irradiances for periods of 6.5 min each. At the end of each period the fluorescence parameters were determined. The results shown here are means of three independent measurements ± S.E. Diamonds: F_t, squares: photosynthetic quantum yield, triangles: photochemical quenching, open circles: nonphotochemical quenching, asterisks: F'_m and closed circles: F'_o.

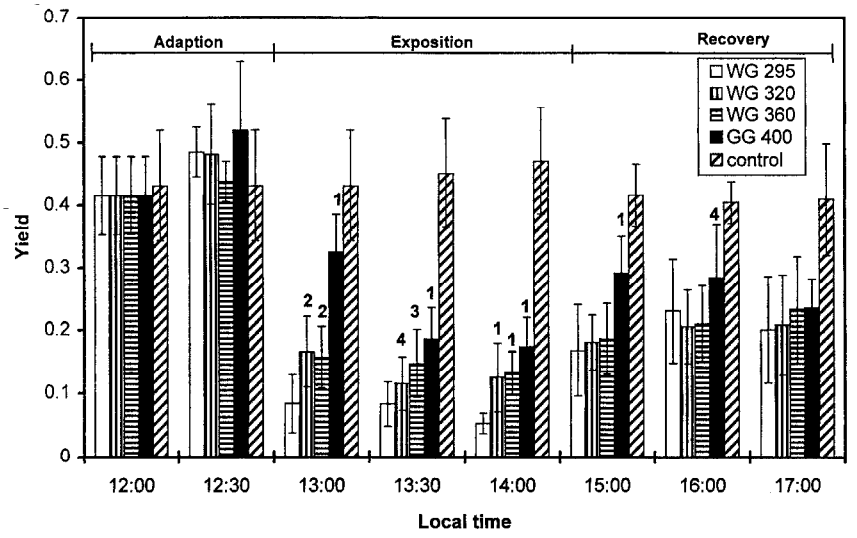

Figure 3. Photosynthetic quantum yield of *Peyssonnelia squamata* measured after adaptation in the shade, after exposure to solar radiation in a rock pool and during recovery (in the shade). Local time is indicated below each set of data as a reference. For each data point at least eight measurements were averaged and the standard deviation calculated. During exposition and recovery values are statistically significantly different from the solar exposure (WG 295) with $p < 0.005$ (1), < 0.01 (2), < 0.025 (3) or < 0.05 (4) as indicated by the Student-t test.

mined immediately after harvest and after 30 min of dark adaptation (Figure 3). Then the thalli were exposed to unfiltered solar radiation (open bars) at the surface for 90 min and the quantum yield was measured at 30-min intervals. After 30 min the effective photosynthetic quantum yield had already decreased to below 0.1. After the exposure period the thalli were transferred to the shade and the photosynthetic yield measured after 1, 2 and 3 h. The same experiment was simultaneously carried out in parallel with specimens exposed under different UV cut-off filters which removed increasing portions of short wavelengths. The WG 295 filter did not remove any solar radiation and served as a control, the WG 320 filter removed all

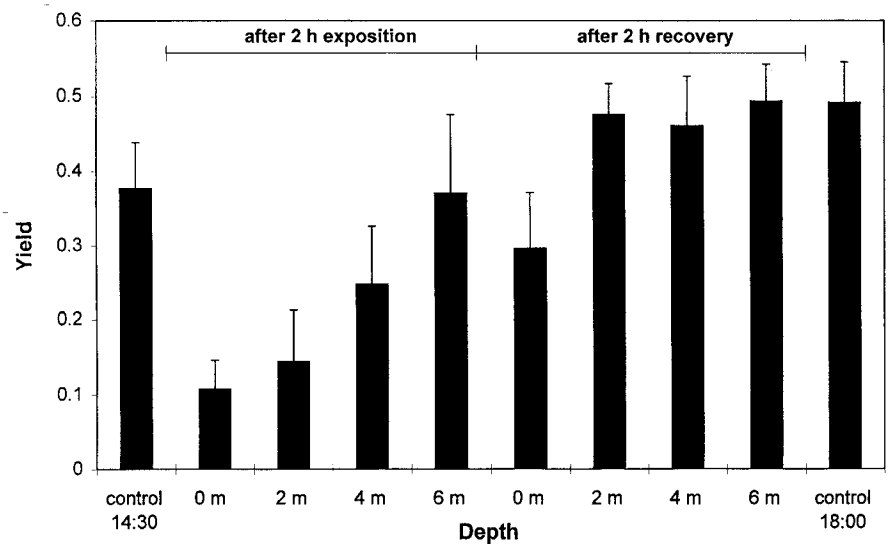

Figure 4. Photosynthetic quantum yield of *Peyssonnelia squamata* after exposure for 2 h at different depths and subsequent recovery for 2 h at the same depth.

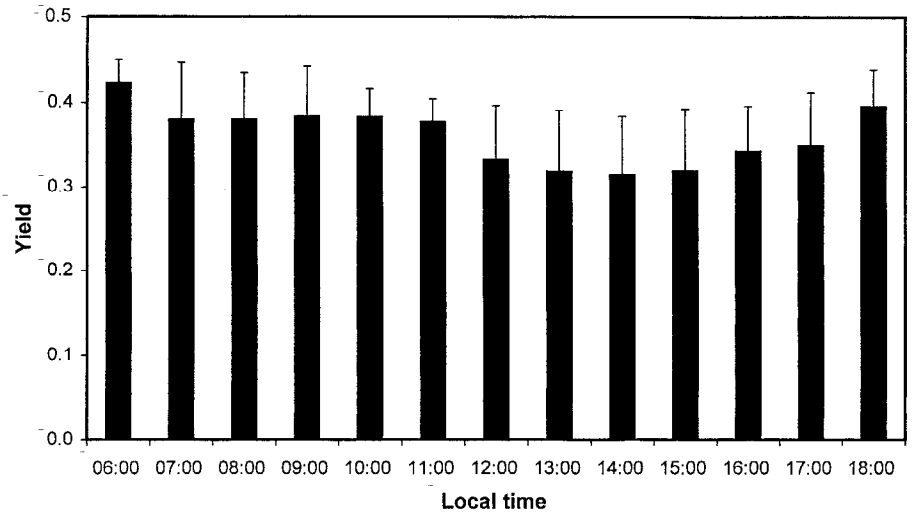

Figure 5. Photosynthetic quantum yield of *Peysonnelia squamata* measured from dawn to dusk. Thalli were retrieved from their growing site and fluorescence parameters measured immediately after harvest. For each data point at least eight measurements were averaged and the standard deviation calculated.

of UV-B, the WG 360 filter removed half of the UV-A (and all of UV-B) and the WG 400 filter removed all UV radiation and passed only PAR and infrared radiation. It is interesting to note that the photosynthetic quantum yield dropped less the more of the short wavelength radiation was excluded. The statistical significance is indicated in the figure. During the subsequent recovery period treatments continued to manifest differences, even though not so pronounced as during the exposure. Control samples were subjected to identical experimental conditions except for exposure to solar radiation and therefore kept in the shade. The photosynthetic quantum yield of the control samples was not altered significantly as compared to the initial measurement indicating that the experimental procedure did not pose any stress on the organisms.

In order to determine the stress posed by solar radiation in the water column, freshly harvested thalli were exposed at different depths below the surface for

2 h (Figure 4). Subsequently, the specimens were allowed to recover for another 2 h in the shade, and the photosynthetic quantum yield was measured again. It is interesting to note that a) there was no inhibition during the 2 h exposure at 6 m and b) all specimens had recovered from photoinhibition within the following 2-h period except the sample exposed immediately below the surface. Algae from their (shaded) habitat at 6 m served as controls.

The final experiment was designed to determine whether photoinhibition occurs also at the natural habitat of the organisms. For this purpose thalli were collected from dawn to dusk at 1-h intervals, and the photosynthetic quantum yield was determined immediately after harvest. The specimens showed maximal yield values of about 0.4 for the first few hours (Figure 5). Then the photosynthetic quantum yield dropped significantly even though the algae were never exposed to direct solar radiation at their growth site; the photosynthetic quantum yield returned to initial levels later in the afternoon.

Discussion

It has been shown that exposure to high levels of solar irradiation causes photoinhibition in higher plants (Björkman & Demmig 1987; Schreiber et al. 1994), macroalgae (Hanelt et al. 1992, 1993; Franklin et al. 1992; Larkum & Wood 1993; Häder et al. 1996a–d) and phytoplankton (Helbing et al. 1992; Leverenz et al. 1990; Herrmann et al. 1996). The mechanism of photoinhibition is still under debate (Crofts & Yerkes 1994). It can be regarded as a regulatory process protecting the photosynthetic apparatus from excessive radiation. In the center of this active physiological regulation is the breakdown of the D1 protein in PS II (Sundby et al. 1993). Through this process the non-cyclic electron transport decreases. Both the effective photosynthetic quantum yield and the photochemical quenching decrease during photoinhibition and often the non-photochemical quenching increases. Part of the inhibition has also been attributed to photooxidative stress due to light-dependent generation of active oxygen species (Foyer et al. 1994).

The red alga *Peyssonnelia squamata* used for this study is adapted to extremely low solar irradiances and thrives in the shade of overhanging rocks. At the research site the species was never found in a habitat exposed to direct sunlight. This is reflected by the fact that photosynthetic oxygen production was highest at 5 m, near the depth where the algae grew. Also the short time to induce complete inhibition of oxygen production indicates adaptation to very low light levels.

In contrast to other algae adapted to direct solar radiation, recovery from exposure to direct sunlight was slower and not complete (Häder et al. 1996a,b). While visible radiation is responsible for part of the photoinhibition a considerable fraction is due to short wavelength solar radiation and this fraction is much higher than the energy distribution in the solar emission spectrum would account for. Another important result is that partial photoinhibition even occurred at the natural growth site of the organisms during several hours of the day when the sun was at high zenith angles even though the organisms were protected from direct sunshine throughout the day.

Other target sites for solar UV radiation are the quinones Q_A and plastoquinone which may in turn induce the degradation or damage of the D1 protein of PS II (Greenberg et al. 1989; Melis et al. 1992). Consequently, the exposure of organisms adapted to low solar irradiance to full sunlight may result in an increase of photoinhibition or photodamage.

As indicated in the introduction the vertical distribution of macroalgae is believed to be mainly governed by the available irradiance. Differences in exposure to solar radiation are far higher than for higher plants since deep water algae are exposed to much less light than, e.g., typical shade plants. Therefore it is ecologically interesting to study photoinhibition in algal species which are adapted to very different light regimes. Algae adapted to being exposed to unattenuated solar radiation at or near the surface show photoinhibition after considerably longer times, and recovery after photoinhibition is much faster and more effective than in deep water algae and those protected from direct solar radiation (Häder et al. 1996a–d). The red alga *Peyssonnelia squamata* is obviously adapted to very low levels of irradiance; this is documented by the fact that non-photochemical quenching increases while photochemical quenching and the photosynthetic quantum yield decrease at irradiances <2 W m^{-2}. In their natural habitat the algae show signs of photoinhibition in the early afternoon hours even though the plants are protected from direct solar radiation. It is interesting to note that the substantial photoinhibition by PAR is enhanced even further by UV and especially UV-B radiation even though this wavelength band represents about 0.1% of the total energy in solar radiation.

Algae seem to differ in several respects from higher plants in their regulatory mechanisms and capacity (Büchel & Wilhelm 1993). Red algae have plastids with two envelopes. The thylakoids are not stacked since the phycobilisomes attached to them act as spacers. Therefore, all thylakoid membranes are biochemically and functionally equal (no lateral heterogeneity as in higher plants or green algae). Nevertheless, red algae can regulate the allocation of excitation energy between the two photosystems very efficiently. In state 1 light energy absorbed by the phycobilisomes are effectively transferred to PS II, which PS I uses energy directly harvested by chlorophyll. In state 2 part of the energy absorbed by the phycobilisomes is funneled to PS I. Currently three models are discussed to explain this adaptive mechanisms: (1) the phycobilisome moves from PS II to PS I, (2) PS II transmits excessive energy to PS I (spill over) and (3) the phycobilisome detaches from PS II and thus effectively reduces the absorption cross section of PS II.

Further investigations, including inhibition and recovery kinetics are necessary to elucidate the mechanisms of photoinhibition and photodamage.

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