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Variable fluorescence parameters in the filamentous Patagonian rhodophytes, *Callithamnion gaudichaudii* and *Ceramium* sp. under solar radiation

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Abstract

The filamentous rhodophytes *Callithamnion gaudichaudi* Agardh and *Ceramium* sp. were utilized to study the effects of solar radiation (PAR, 400–700 nm, UV-B, 280–315 nm and UV-A, 315–400 nm) on the photosynthetic performance in situ in Patagonia waters (Argentina). A pulse amplitude modulated (PAM) fluorometer was used to determine the fluorescence parameters. The two species grew in different habitats in the eulittoral: *Ceramium* sp. was found only in rock pools while *C. gaudichaudii* grew on exposed rocks and fell dry during low tide. Both species differed in their fluorescence parameters and their sensitivity to solar radiation exposure. The photosynthetic quantum yield had its lowest values at noon, but it recovered in the afternoon/evening hours, when irradiances were lower. PAR (irradiance of about 400 W m^{-2} at noon) was responsible for most of the decrease in the yield on clear days, especially in *Ceramium* sp., but UVR (280–400 nm) also accounted for a significant decrease. Fluence rate response curves indicated that both species were adapted to low fluence rates and showed a pronounced non-photochemical quenching at intermediate and higher irradiances. Both species showed a rapid adaptation during measurement of fast induction kinetics but differed significantly in their fluorescence components. All photosynthetic pigments were bleached after 8 h exposure to solar radiation over a full day. Strong absorption in the UV-A range, most likely due to mycosporine-like amino acids, was detected in both strains. The pronounced sensitivity to solar radiation in situ and the recovery capacity of these two filamentous Rhodophyte species, as well as the presence of protective compounds, suggests that these algae have the ability to adapt to the relatively high radiation levels and changes in irradiance found in the Patagonia waters.

Keywords: Rhodophyceae; *Callithamnion gaudichaudii*; *Ceramium* sp.; PAM fluorescence; Photoinhibition; Photosynthetic yield; Solar ultraviolet radiation

1. Introduction

Aquatic ecosystems are responsible for about 50% of the biomass production on Earth, equaling the productivity of all terrestrial ecosystems taken together [1,2]. Most of the aquatic productivity is due to phytoplankton [3,4] but macroalgae have a significant share particularly in coastal areas. In addition, they are exploited commercially on a large scale and form habitats for larval stages of fish and other ecologically and economically important animals [5].

Almost all macroalgae are sessile and thus cannot escape deleterious irradiation by vertical migration in the water column [6–9]. Solar irradiation is one of the most important factors controlling the vertical distribution of macroalgae in their habitat [10–12]. Some species are adapted to the supralittoral (zone above the high water mark) where they are exposed only to the wave spray. Most species inhabit the eulittoral (intertidal zone) characterized by the regular temporal change in the tides [13]. On the Patagonian coast this zone is fairly wide because the tidal range exceeds 2–3 m and the

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shore has a shallow slope. Other algae are restricted to the sublittoral zone where they are rarely exposed to the air. The adaptation capacity and the protective mechanisms of a species define its upper growth limit in the water column. In the top layers, macroalgae are exposed to almost unfiltered solar PAR as well as to UV radiation especially during low tides.

Being photosynthetic organisms, algae, like terrestrial plants, show strong photoinhibition when exposed to excessive solar radiation or even irreversible damage [14,15]. Chronic photoinhibition is due to the photodegradation of the D1 protein in photosystem II (PS II) which results in a decrease in the photosynthetic electron transport [16,17] and protects the photosynthetic apparatus from excessive radiation. Dynamic photoinhibition is operated through the xanthophyll cycle identified in higher plants and in many macroalgae with the exception of the red algae [9,18].

During excessive solar radiation the photosynthetic quantum yield of PS II decreases and the excess excitation energy is dissipated thermally. After a moderate exposure the reduction in quantum yield may be reversible within minutes or hours, but long-term exposure may cause irreversible damage. Photoinhibition has been reported in a variety of macroalgal groups including chlorophyceae, phaeophyceae and rhodophyceae [5,19–21]; it has been studied in major seas and oceans of the world including the Atlantic and Pacific Oceans, the Mediterranean, the North and Baltic Seas [22–25] and from the Arctic and Antarctic to the Tropics [26–28]. Adaptive processes of the photosynthetic apparatus in algae have been summarized in recent reviews [7,9].

In many intertidal algae a pronounced decrease in the photosynthetic yield is observed between noon and early afternoon resulting from the excessive radiation exposure [29,30]. This is modulated by the tidal rhythm, and most excessive radiation stress occurs when low tides and high solar angles coincide [31,32]. These two external factors lead to a complicated pattern of photosynthetic activity which is further altered by the changing cloud cover.

In both algae and higher plants, the largest share in photoinhibition is caused by the visible radiation [13]. However, solar UV and especially the short-wavelength UV-B causes a considerable effect [33–35] although UV-B constitutes only about 1% of the radiation energy reaching the Earth's surface. Because of the strong absorption of short wavelength radiation in the water column, UV-related photoinhibition is higher in algae inhabiting the eu- and supralittoral than those in the sublittoral.

This contribution characterizes the photosynthetic performance of common red macroalgae under natural conditions at their growth site on the Patagonian coast, Argentina. While there are several descriptive studies on the macroalgae flora of Patagonia, Argentina [36–38] there have been no studies relating to the photosynthetic responses of these algae under natural conditions [39]. The aim of the present work is to evaluate and compare the adaptation of the photosynthetic apparatus in the widespread red macroalgae, *Callithamnion gaudichaudii* Agardh and *Ceramium* sp. in response to solar radiation under natural conditions. Most of the studies discussed above investigated the short-term photoinhibition and recovery of macroalgae to light stress. In contrast, the present study extends the investigation to long-term (days) monitoring. Another novel aspect of this study includes the analysis of fast induction and recovery kinetics to characterize the collaborative behavior of the redox components in the photosynthetic electron transport chain. These measurements have never been published before for these macroalgae.

2. Materials and methods

2.1. Plant material

This work was carried out on two rocky shores of the Patagonian coast in the Chubut Province: Playa Barrancas Blancas, Bahía Engaño (43°S, 65°W) and Playa del Amor, Bahía Bustamante (45°S, 66.5°W), Argentina in March 2000. Playa Barrancas Blancas has a ~200 m rocky shore which is completely exposed to solar radiation during low tide. Playa del Amor is characterized by a steeper shoreline, and most of the intertidal algae are found in rock pools of different sizes and depths. The organisms used in this study were two filamentous branched red algae growing in the lower eulittoral. *Ceramium* sp. was found attached in rock pools, while *Callithamnion gaudichaudii* grew at the top edges of channels cut into exposed rocky surfaces. During the experimental period the water temperature was around 17 °C and the salinity 34‰.

2.2. Measurements of PAM fluorescence

A portable pulse amplitude modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany) was used to analyze the in vivo chlorophyll fluorescence parameters as described by Schreiber and Bilger [40]. PAM fluorescence measurements are based on the determination of the ground fluorescence F_0 induced by a weak, constant irradiation of a dark-adapted sample (all reaction centers in the open state). Upon a single saturating flash, maximal fluorescence, F_m, is measured indicating that all reaction centers are closed. The variable fluorescence, F_v, is calculated from the difference between F_0 and F_m. The optimal quantum yield can be calculated from the ratio F_v/F_m [41]. Irradiation with high fluence rates of the previously dark-adapted sample leads to a decrease

of F_m, now called F'_m and an increase or a decrease in F_0, leading to F'_0. The effective quantum yield of photochemical energy conversion can be determined from $(F'_m - F_t)/F'_m$ [42,43]. From these measured values and the currently encountered fluorescence, F_t, the photochemical quenching q_P can be calculated [44], i.e., the amount of excitation energy funneled into the photochemical processes.

$$q_P = (F'_m - F_t)/(F'_m - F'_0).$$

The non-photochemical quenching q_N is calculated from

$$q_N = 1 - (F'_m - F'_0)/(F_m - F_0).$$

The non-photochemical quenching includes all non-radiative processes dissipating excitation energy; but the underlying molecular mechanism is still controversial [44].

The PAM instrument can be used to program experimental sequences. Using this feature, induction curves were calculated with on-line quenching analysis at 10 ms sampling rate. Initially the measuring light was switched on and subsequently saturation pulses were elicited every 20 s for the determination of F_0 and F_m followed by the kinetic recording of the fluorescence yield. After every saturating pulse the quantum yield, q_N and q_P were calculated. The relaxation kinetics of q_N was determined immediately following the induction curve to warrant that the specimen had reached a steady-state fluorescence and developed non-photochemical fluorescence quenching. The built-in halogen lamp was used instead of the red light-emitting diode (LED) in order to induce a strong q_N. The thalli were exposed to the actinic light, and F_0 and F_m were determined every 30 ms. Initially, two saturating light pulses were applied to determine the quenching parameters in the light-adapted state before the actinic light was turned off. Then saturating light pulses were applied with exponentially increasing time intervals between consecutive pulses according to the function $t = 10 \times 1.2^{n-1}$.

Rapid induction kinetics were measured at a sampling rate of 1 ms. F_0 was determined after the measurement light was switched on and before the actinic light (modulated at 20 kHz) was pulsed for 2 s. The signals were recorded for an additional 2 s to monitor the relaxation kinetics. Switching on a far red background light simultaneously accelerated the reoxidation of the different acceptor pools in PS II.

Fluence rate response curves of important fluorescence parameters as well as photochemical and non-photochemical quenching were measured by increasing the actinic irradiance at regular time intervals of 6.5 min. Before the sequence, F_0 and F_m were determined in dark-adapted thalli followed by a 10-min irradiation at an intermediate irradiance of 23 W m^{-2} to adapt the sample to light and to activate the Calvin cycle enzymes. F'_0 was determined at each irradiance level, and the electron transport chain was oxidized before each measurement by applying a proceeding far red pulse.

2.3. Experimental setup

Algal materials were collected in the early morning when low tide permitted access to their habitat and kept in a large volume of seawater in darkness during transport to the laboratory (approx. 20 min) until being subjected to experimental treatments. The thalli were exposed in flat open plastic containers (500 ml) floating on top of a large open water container to keep the temperature constant. The thalli were either permitted to free float or kept in custom-made UV transparent Plexiglas holders (GS 2458, Röhm and Haas, Darmstadt, Germany) with open sides to allow water to circulate. The organisms were dark-adapted under black plastic foil for at least 30 min (<10 W m^{-2}) and subsequently exposed to (a) full solar radiation (covered with a filter foil with a cut-off at 295 nm, Ultraphan UV transparent, Digefra, Munich, Germany), (b) radiation deprived of UV-B by the application of a cut-off filter removing all wavelengths below 320 nm (Montagefolie, No. 10155099, Folex, Dreieich, Germany) and (c) visible radiation above 395 nm (holders covered with Ultraphan UV Opak, Digefra, Munich, Germany). The transmission spectra of these filter foils have been published by Figueroa et al. [45].

After short-term exposure (15 or 30 min), the samples were transferred back into the shade to monitor recovery of the photosynthetic yield. The photosynthetic parameters were measured with a pulse amplitude modulated (PAM) fluorimeter after the initial dark period, after the exposure time and at predefined times during the recovery period for up to 6 h. Alternatively, the thalli were exposed to solar radiation for extended periods of time (days). Control thalli were subjected to the same treatments without exposure to solar radiation. Parallel experiments were carried out at the same time to warrant the same UV-A, UV-B and PAR doses across all treatments. Eight replicates were measured for each data point.

Another group of experiments was carried out at Bahía Bustamante (250 km south of Rawson) on *Ceramium* sp. in a natural rock pool during low tide without removing the specimens from their growth site. The photosynthetic parameters were measured hourly from 13 to 16 h local time.

2.4. Chlorophyll determinations and absorption spectra

Absorption spectra were recorded in specimens exposed to a full day of solar radiation as well as in control thalli kept in dim light. Equal amounts of fresh weight were extracted in 10 ml of absolute methanol over night at 4 °C. Subsequently, the samples were centrifuged for

10 min at 1000 rpm, and the absorption of the methanolic extract was measured in a UV–Vis spectrophotometer (Hewlett–Packard 8453E). After extraction the specimens were dried at 40 °C for 24 h, the dry weight determined and the chlorophyll concentration calculated [46].

2.5. Measurement of solar radiation

During the experimental period (3–24 March 2000) solar radiation was monitored using a filter radiometer (ELDONET, Real Time Computer, Möhrendorf, Germany) permanently located on the roof of the Estación de Fotobiología Playa Unión [47,48]. The three-channel radiometer automatically records the irradiances as averages every 1-min interval for three wavelength bands, namely UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm). All data were transferred to and are available on the central server of the ELDONET network (http://www.eldonet.org) [49].

2.6. Statistics

For all PAM measurements mean values and standard deviations were determined from eight independent measurements on different thalli. All experiments were repeated three to four times on different days. ANOVA tests were used to determine whether or not the differences between groups were due to the different light treatments. This was followed by an a posteriori test (Tukey's).

3. Results

During the measurement period most days were bright. With the exception of some scattered clouds over noon and in the afternoon the sky was blue throughout the day with maximal irradiances of about 400 W m^{-2} in the PAR region, 50 W m^{-2} in the UV-A and about 1.6 W m^{-2} in the UV-B region (data not shown).

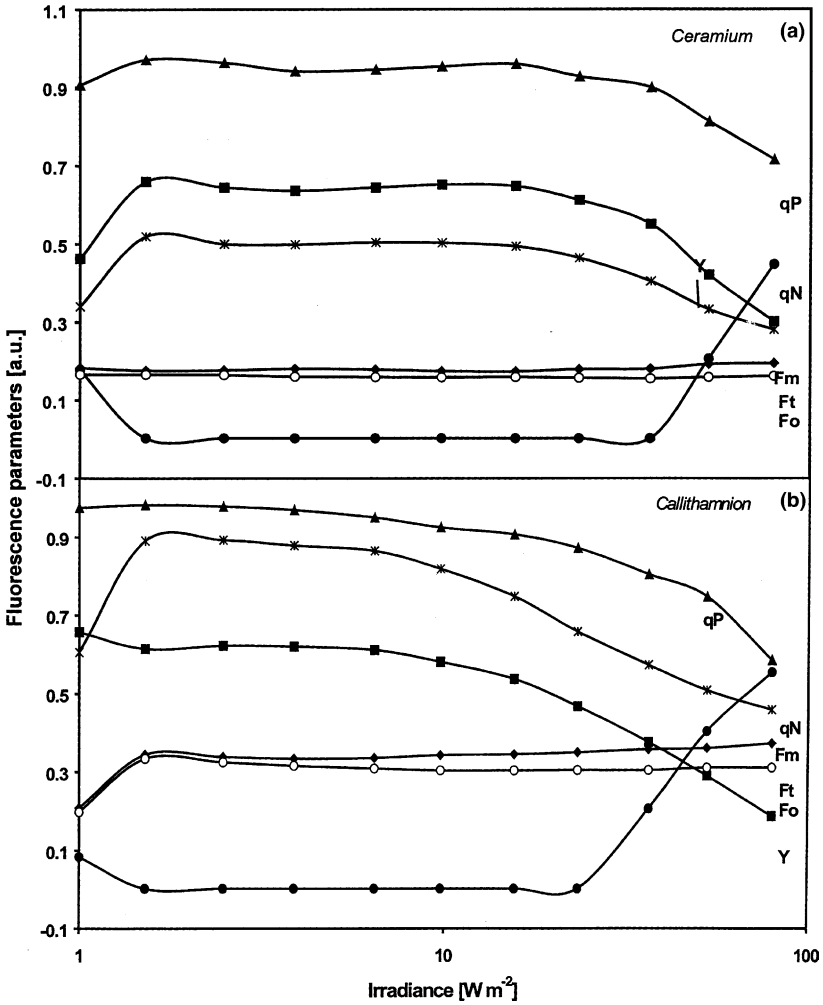

Fig. 1. Fluorescence parameters measured in *Ceramium* sp. (a) and *Callithamnion gaudichaudii* (b) as a function of the fluence rate of the actinic red light. Note that the *x* axis is a log scale.

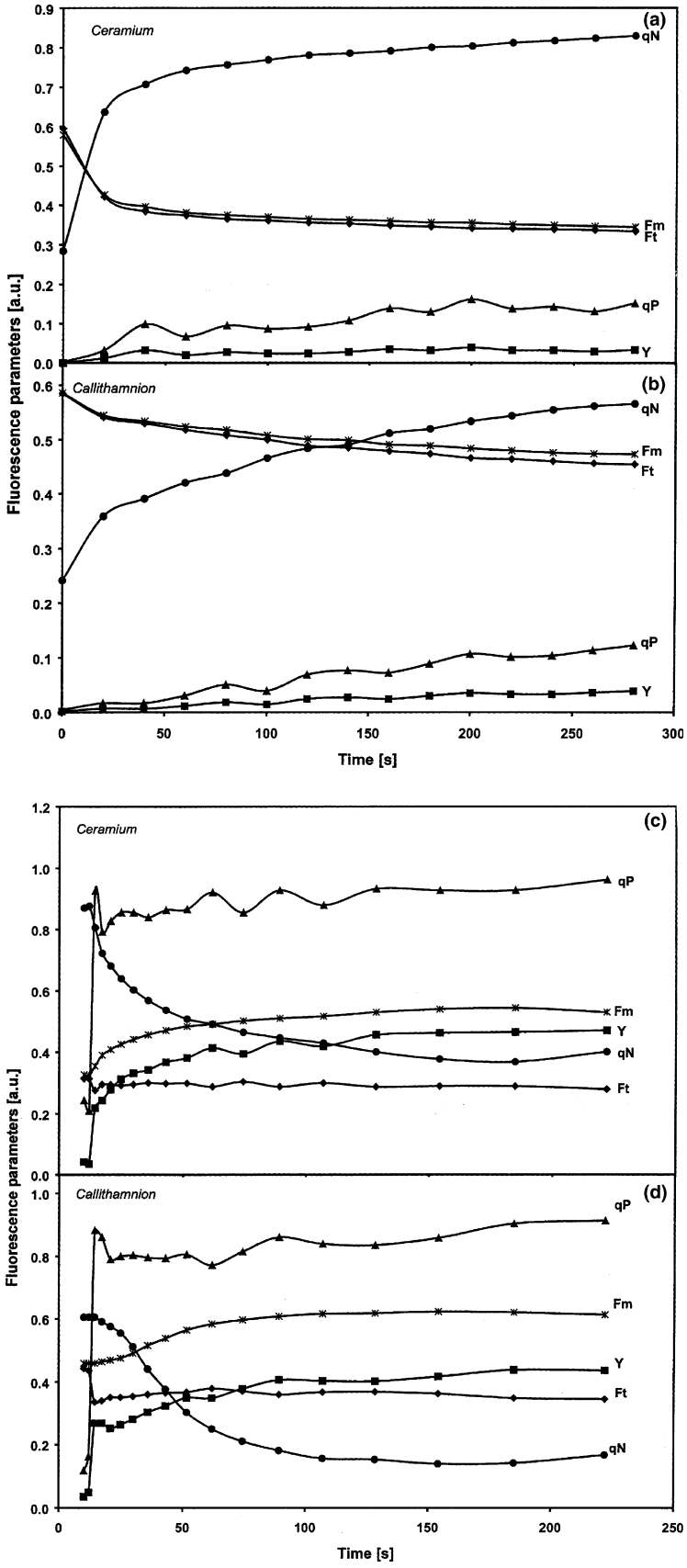

Fig. 2. Induction curves with quenching analysis in *Ceramium* sp. (a) and *Callithamnion gaudichaudii* (b) as well as relaxation kinetics measured immediately after induction in *Ceramium* sp. (c) and *Callithamnion gaudichaudii* (d).

The irradiance–response curves for photosynthetic parameters of the two species are shown in Fig. 1. F'_m, q_P and Y decreased with increasing irradiances after an initial increase in both species. F_t and F_0 remained constant. q_N showed an interesting phenomenon: even the preirradiation caused a measurable value in both species, which decayed at low irradiances but increased at higher irradiances. The photochemical quenching decreased to slightly lower values in *Callithamnion gaudichaudii* (Fig. 1(b)) than in *Ceramium* sp. (Fig. 1(a)) at high irradiances. F'_m, F_t and F'_0 were also considerably higher in *C. gaudichaudii* (Fig. 1(b)) than in *Ceramium* (Fig. 1(a)).

The induction curves with quenching analysis measured at 10 ms sampling rate (Fig. 2(a) and (b)) showed first a fast and then a steady decline in F_t and F_m for both species. The non-photochemical quenching followed a reverse pattern to the change in F_t and F_m, but with a steeper kinetics. q_N was higher in *Ceramium* than in *Callithamnion*. The built-in halogen lamp was used in order to induce a significant non-photochemical quenching. Y hardly increased above zero while q_P only slightly increased (Fig. 2(c) and (d)). F_t stayed fairly stable and Y increased to values of above 0.3 after the first few seconds. The non-photochemical quenching only gradually decreased to values below 0.4 (*Ceramium* sp.) and below 0.2 (*C. gaudichaudii*) with time up to 100–150 s and then remained constant. It is interesting to note that adaptation of the fluorescence parameters occurred on a very short time scale with the order of a few minutes.

The fast induction kinetics at 1 ms sampling rate revealed the likely involvement of several components of PS II (Fig. 3(a) and (b)). In *Ceramium* sp. (Fig. 3(a)) there might be at least two distinct components on the rising edge which lead to a transient fluorescent maximum. *C. gaudichaudii* appeared to have two components, which were less easily differentiated and appeared to have a steady state fluorescence level (Fig. 3(b)). After the actinic pulse the fluorescence decay also suggested that these might be at least two components. The two components in the fluorescence rise were better separated when a sampling rate of 300 μs was chosen and the data were plotted logarithmically (data not shown). The induction kinetics was measured before and at regular time intervals during exposure to solar radiation under

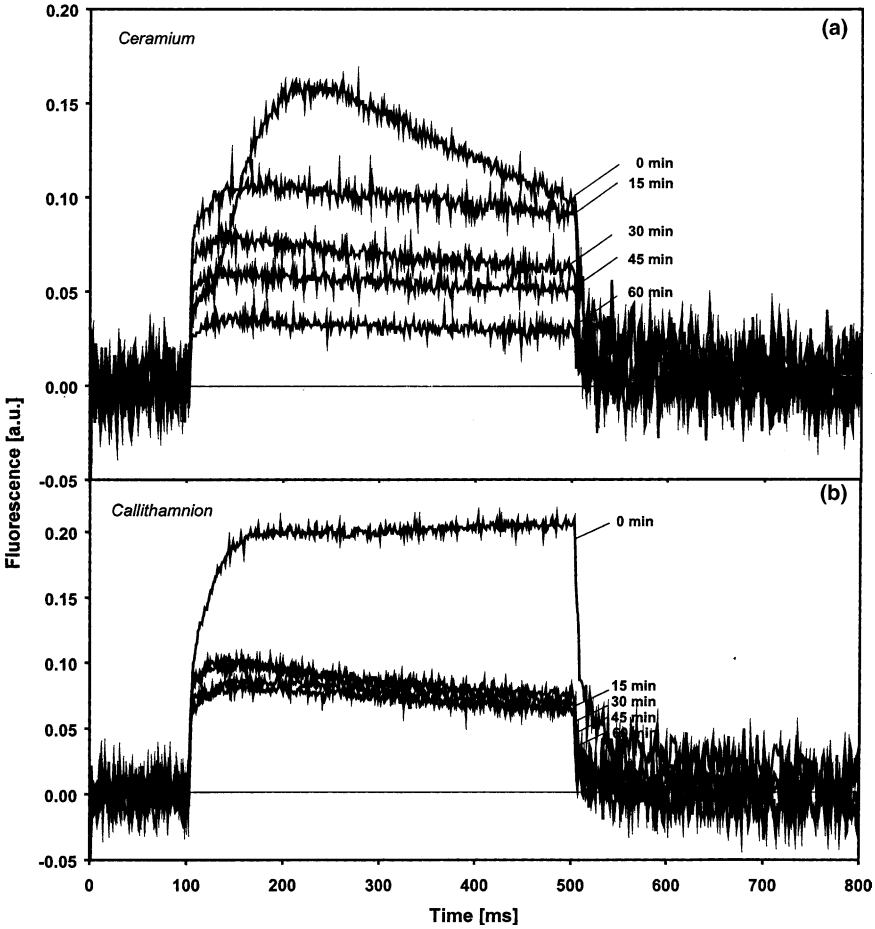

Fig. 3. Rapid induction and relaxation kinetics at 1000 μs/data point in *Ceramium* sp. (a) and *Callithamnion gaudichaudii* (b).

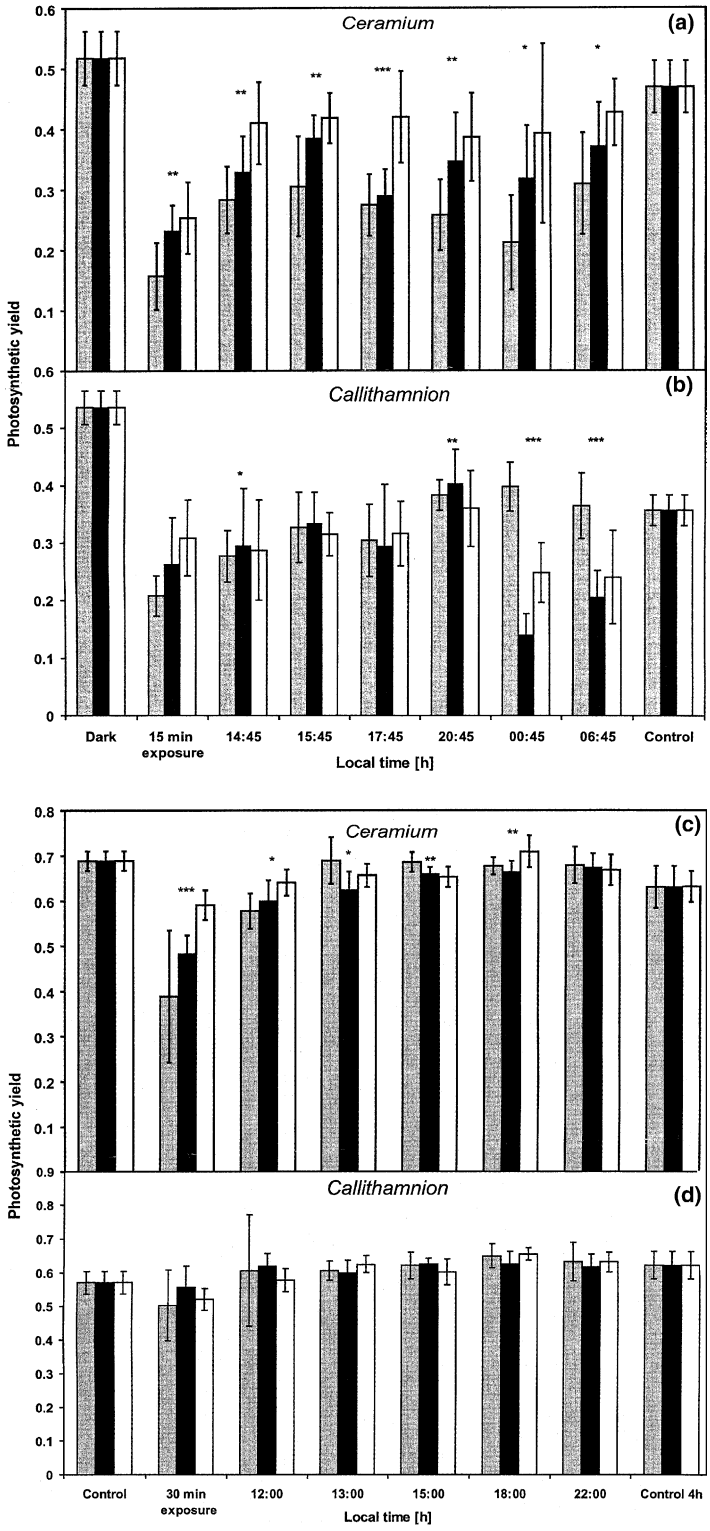

Fig. 4. Effective photosynthetic quantum yield in *Ceramium* sp. (a, c) and *Callithamnion gaudichaudii* (b, d) measured after 30 min dark adaptation, 15 min exposure kept in a fixed position (a, b) or 30 min exposure free floating in a large volume of water (c, d) and after increasing recovery times in the shade calculated as $(F'_m - F_t)/F'_m$. Gray bars, specimens exposed to unfiltered solar radiation. Black bars, specimens exposed to UV-A + PAR. White bars, specimens exposed to PAR only. For each data point n was equal to 8 (the lines on top of the bars indicate one SD). The values between the three light treatments are statistically significantly different from each other with $^{***}P < 0.001$, $^{**}P < 0.01$ or $^{*}P < 0.1$, respectively. Maximal irradiances for the experiment in (a) and (b) were, PAR 349 W m^{-2}, UV-A 44.15 W m^{-2} and UV-B 1.34 W m^{-2}. The total doses for this day were, PAR 8.18 MJ m^{-2}, UV-A 1.04 MJ m^{-2} and UV-B 26.8 kJ m^{-2}. Maximal irradiances for the experiment in (c) and (d) were, PAR 371 W m^{-2}, UV-A 44.7 W m^{-2} and UV-B 1.10 W m^{-2}. The total doses for this day were, PAR 4.80 MJ m^{-2}, UV-A 0.65 MJ m^{-2} and UV-B 14.63 kJ m^{-2}.

clear skies. Even after 15 min the fluorescence level had dropped dramatically. The first component decreased less strongly and thus became more obvious. After 60 min the maximal fluorescence yield had dropped to a very low residual value.

Both species showed an optimal quantum yield of above 0.5 after dark adaptation (Fig. 4(a) and (b)). When exposed to 15 min of unfiltered solar radiation in a fixed position (in the Plexiglas holder floating in a larger volume of seawater), the effective photosynthetic quantum yield decreased significantly. In dim light (<10 W m^2) the yield increased but never reached the initial level even after a night period. In *Ceramium* sp. the degree of photoinhibition was even more pronounced than in *C. gaudichaudii*. While most of the photoinhibition is due to the white light component (open bars) a significant increase of the inhibition by UV, especially UV-B (gray bars), was observed with a limited recovery, specifically in *Ceramium* sp. This is statistically significant for the exposure time and the recovery as indicated in the figures. Control specimens were treated the same way for both species as were the exposed samples except for the irradiation treatments. These specimens showed an only slightly depressed yield compared to the dark-adapted plants before exposure indicating that the experimental conditions did not affect the organisms.

In the experiment described above the specimens were fixed in one position so that the same surface was always exposed. The experiment was repeated with specimens floating freely in a larger volume of water. Since there was no significant photoinhibition after 15 min (data not shown) the exposure time was increased to 30 min (Fig. 4(c) and (d)). Even after this longer exposure time the inhibition was less dramatic than that after 15 min. Again, *Ceramium* sp. was more affected by the exposure than *C. gaudichaudii* which showed hardly any response. In *Ceramium* sp. a statistically significant greater photoinhibition was detected during exposure and at some points during recovery when the UV component was added (Fig. 4(c)); this was not observed in *C. gaudichaudii* (Fig. 4(d)). Both species showed a fast recovery of the photosynthetic yield reaching the dark value.

Free floating specimens were exposed continuously to solar radiation over the day starting at 11 h local time after dark adaptation. Significant photoinhibition was observed during a clear day with only scattered clouds (Fig. 5(a) and (b)) and only recovered after the following night. Photoinhibition was much less pronounced on a rainy day and it was apparent only during some bright periods (Fig. 5(c) and (d)). In both species the UV effect was pronounced, and statistically significant especially in *Ceramium*, even on the cloudy day (Fig. 5(c)).

Photoinhibition in *Ceramium* sp. was also determined in a natural environment in a rock pool at Bahía Bustamante where *C. gaudichaudii* was not found. The habitat was accessible only during low tide and fully

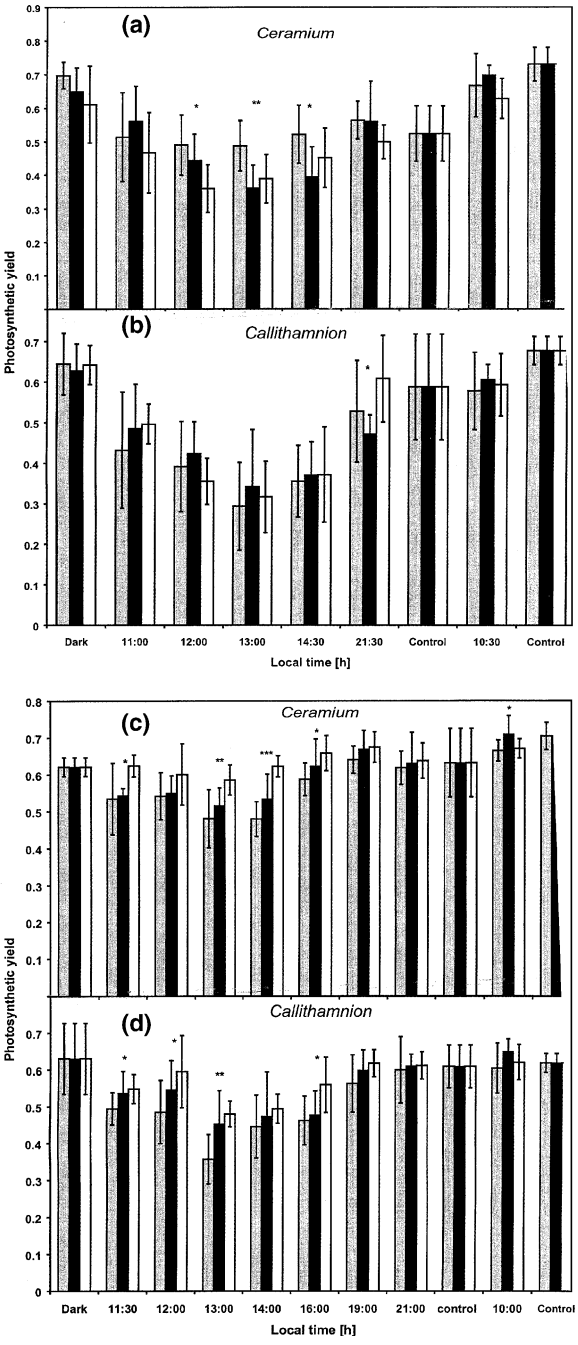

Fig. 5. Effective photosynthetic quantum yield of *Ceramium* sp. (a, c) and *Callithamnion gaudichaudii* (b, d) exposed free floating in open vessels on an almost cloudless day (a, b) and a rainy day (c, d), respectively. Gray bars, specimens exposed to unfiltered solar radiation. Black bars, specimens exposed to UV-A + PAR. White bars, specimens exposed to PAR only. For each data point n was equal to 8 (the lines on top of the bars indicate one SD). The values for the different light treatments are statistically significantly different from each other (395 nm cut-off) in each set with $^{***}P < 0.001$, $^{**}P < 0.01$ or $^{*}P < 0.1$, respectively. Maximal irradiances for the experiment in (a) and (b) were, PAR 328 W m^{-2}, UV-A 43.5 W m^{-2} and UV-B 1.13 W m^{-2}. The total doses for this day were, PAR 6.99 MJ m^{-2}, UV-A 0.95 MJ m^{-2} and UV-B 21.43 kJ m^{-2}. Maximal irradiances for the experiment in (c) and (d) were, PAR 348 W m^{-2}, UV-A 41.3 W m^{-2} and UV-B 0.94 W m^{-2}. The total doses for this day were, PAR 4.40 MJ m^{-2}, UV-A 0.60 MJ m^{-2} and UV-B 12.04 kJ m^{-2}.

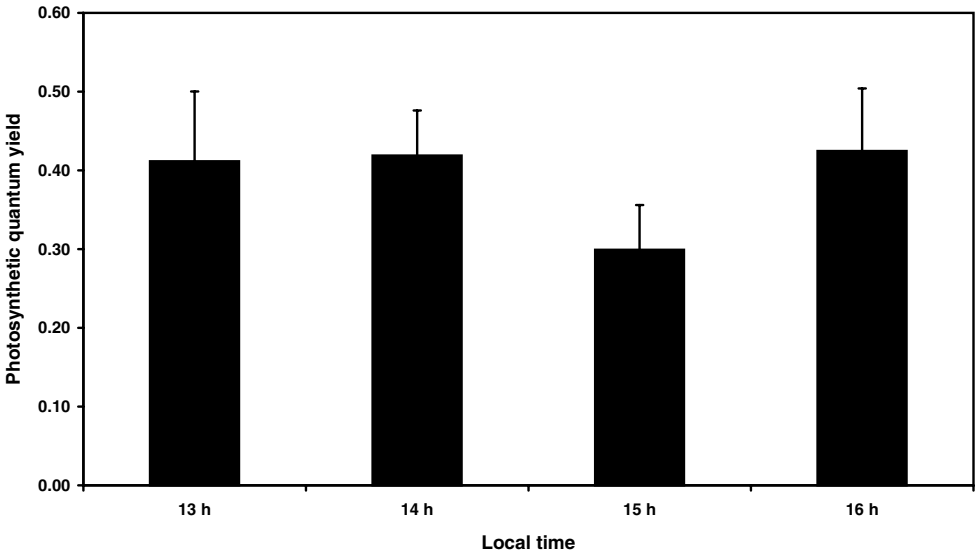

Fig. 6. Effective photosynthetic quantum yield of *Ceramium* sp. in its natural environment in a rock pool in Bahía Bustamante measured at hourly intervals during low tide.

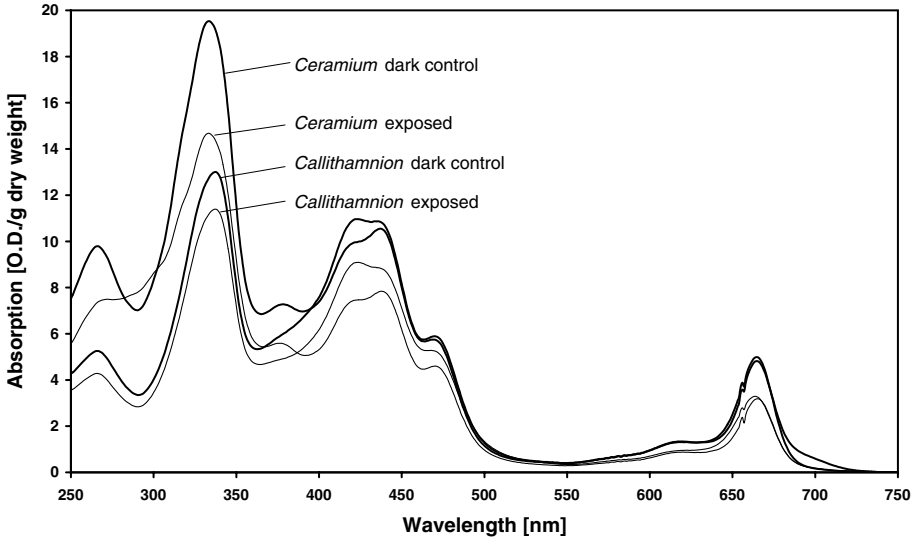

Fig. 7. Absorption spectra of methanolic extracts of *Ceramium* sp. and *Callithamnion gaudichaudii* thalli kept under shaded conditions (upper curve) and thalli exposed to solar radiation for 8 h centered around local noon on a clear day.

covered during high tide. On a clear day, the first measurement made 1 h after the retreat of the water at 12 h local time showed a yield of 0.43 (Fig. 6). Measurements were continued on an hourly basis and showed a decrease in the early afternoon (15:00 h). One hour later the yield increased again. Similar patterns of the yield were seen on subsequent days (data not shown).

Absorption spectra of methanolic extracts are shown in Fig. 7. *Ceramium* sp. had slightly higher concentrations of all photosynthetic pigments than *C. gaudichaudii*, and in both species there was a considerable lower absorption in the light-exposed samples than the ones kept in the shade. Three absorption spectra were measured from independent samples with essentially the same result. No specific bleaching of any of the major photosynthetic pigments, all being affected to a similar extent. The methanolic extracts of both strains were observed with a strong absorption in the UV region.

4. Discussion

Photoinhibition is a common defense mechanism of photosynthetic organisms to excessive solar radiation; this behavior is found in both higher plants and algae [5,8,50–52]. Chronic photoinhibition results from pho-

todegradation of the D1 protein in photosystem II (PS II) which causes a decrease in the photosynthetic electron transport [53–55]. Not only higher plants but also macroalgae were found to show a typical midday reduction in the photosynthetic yield during the hours of highest radiation. This daily variation is thought to be caused by dynamic photoinhibition [56–58]. Most of the photoinhibition is due to the visible radiation (PAR) but a considerable percentage of yield reduction is caused by UV-B (and less so by UV-A) radiation in the top few meters of the water column and during exposure to the air, although the share in the quantum energy of UV-B reaching the Earth's surface is small. This unproportionally high photoinhibition by solar UV has been demonstrated in a number of marine algal [5,59] and also phytoplankton species in the North Atlantic and Mediterranean Sea [45,60]. These results are supported by findings in macroalgae from the Northern Hemisphere at similar latitudes [14,23,30]. The effective quantum yield appears to be a valid indicator of the photosynthetic efficiency in these rhodophytes. The additive effect of solar UVR (280–400 nm) is apparent not only during exposure but also during recovery, indicating that dynamic photoinhibition is the photoprotective mechanism. The degree of photoinhibition is similar to other green and surface algae studied in the eulittoral [9,61–63].

In many marine macroalgae in the intertidal zone this rhythm is modulated by the tides. Due to the high concentration of dissolved organic matter (DOM) in addition to particulate organic and inorganic substances light penetration is limited in coastal areas. Therefore they receive considerably less solar visible radiation under a few meters of water and even more so for solar UV. Thus, the organisms encounter the highest light stress when low tides co-occur with high solar angles [58,63,64]. Since the tides do not follow the daily cycle, macroalgae experience a complicated pattern of irradiances controlled by the superimposition of the daily cycle and the tidal rhythm. In addition they are subjected to a changing cloud cover. The resulting radiation patterns can vary on a fast scale (minutes); therefore it is not surprising that macroalgae have adjusted their behavior to this phenomenon by fast adaptation mechanisms in order to accomplish positive net photosynthesis at low and intermediate irradiances. In fact, most macroalgae can be considered as shade plants even when they grow in the intertidal zone with high irradiances.

These characteristics must require efficient mitigation mechanisms to protect the organisms from excessive radiation and photooxidative damage by high light. These regulatory mechanisms designed to ameliorate light stress include adjustment of the antenna size, thermal dissipation of excess excitation energy, antioxidant systems and the fast repair of photooxidative damage [65]. The rapid induction and relaxation kinetics show convincingly how fast the photosynthetic apparatus can respond to the ambient light conditions. To the best of our knowledge this is the first report that such fast kinetics has been found in these macroalgae.

The fast induction and relaxation kinetics are valuable methods to identify the relevant redox components in the photosynthetic electron transport chain. The biphasic increase in fluorescence during the fast induction kinetics indicates the reduction of Q_A and Q_B followed by a subsequent and slower reduction of the plastoquinone pool. During exposure to unfiltered solar radiation the fluorescence signal decreases rapidly, indicating the fast adaptation of the electron transport chain to the solar radiation stress. The dose–response curves and the slower induction and relaxation kinetics allow us an insight into the quenching parameters and thus the allocation of excitation energy to the photosynthetic process or to the different components of non-photochemical quenching induced by high irradiance.

Both species showed a moderate photosynthetic quantum yield after dark adaptation or in its natural habitat at the end of the night in contrast to the lowered quantum yield in Phaeophytes or Chlorophytes in the same geographic region [31,32,66]. This observation corresponds with those results found in a number of eulittoral Mediterranean and Atlantic species [7].

Surface-growing algae recover faster from excessive solar radiation than algae adapted to deeper water [29,67]. One of the novel findings in this study is that the algae suffered photoinhibition in semi-natural conditions when exposed in free-floating forms or at their natural site growth during low tide. These and similar findings in other species make it necessary to modify our conception of the physiological activity in supralittoral and subtidal macroalgae. The algae operate with an optimal photosynthetic quantum yield either early in the morning and evening hours during low tide or when high tides coincide with high solar angles. These independent external factors result in a complicated pattern of the photosynthetic yield. In the future it will be interesting to determine if this behavior is controlled by an endogenous rhythm.

Another interesting point is that the photosynthetic pigments are bleached by natural radiation during the day and need to be resynthesized at night. A more detailed study is being carried out to understand this phenomenon on shorter time scales. The chlorophylls are bleached at a comparable rate as the photoprotective carotenoids. *Ceramium* sp. has a higher concentration of mycosporine-like amino acids, absorbing in the UV-A region, than *C. gaudichaudii*. However it seems to be more sensitive to solar radiation and solar UV than the other species. This may be due to the ecological situation

during low tide: *Ceramium* sp. was never found falling dry but floating in rock pools. In contrast, *C. gaudichaudii* fell dry and the trichomes attached to each other forming a dense thallus protecting the inner parts from excessive radiation due to high absorption in the outer layers.

5. Conclusions

The present studies indicate that the photosynthetic capacity in intertidal macroalgae is not simply entrained by the daily pattern of solar radiation. Rather they adapt to the rapidly changing light conditions in their natural habitat modified by the tidal rhythm and the changing cloud cover. These macroalgae have developed several effective mechanisms to ameliorate excessive light stress and rapidly optimize their photosynthetic performance to the environmental conditions.

6. Nomenclature

F_0	initial chlorophyll fluorescence in a dark-adapted plant, all reaction centers are open
F_m	maximal fluorescence in a dark-adapted plant, all reaction centers are closed
F_v	variable fluorescence $= F_m - F_0$
F_0', F_m' and F_v'	the same for the light-adapted state
F_t	current fluorescence of a light-adapted plant
PAM	pulse amplitude modulated fluorometer
PAR	photosynthetic active radiation
PS II	photosystem II
q_N	non-photochemical quenching
q_P	photochemical quenching
UV-A	ultraviolet radiation 315–400 nm
UV-B	ultraviolet radiation 280–315 nm
UVR	ultraviolet radiation

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