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## Effects of solar radiation on the Patagonian macroalga *Enteromorpha linza* (L.) J. Agardh — Chlorophyceae

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### Abstract

The photosynthetic performance of *Enteromorpha linza* (L.) J. Agardh — Chlorophyceae was determined with a portable PAM instrument in situ and under seminatural radiation conditions in Patagonia, Argentina. Solar radiation was measured in parallel with a three-channel radiometer, ELDONET (Real Time Computer, Möhrendorf, Germany), in three wavelength ranges, UV-B (280–315 nm), UV-A (315–400 nm), and PAR (400–700 nm). The effective photosynthetic quantum yield decreased after 15-min exposure to solar radiation when the thalli were kept in a fixed position but recovered in the subsequent shade conditions within several hours. A 30-min exposure of free floating thalli, however, caused less photoinhibition. The photosynthetic quantum yield of *E. linza* was also followed over whole days under clear sky, partly cloudy and rainy conditions in a large reservoir of water (free floating thalli) and in situ (thalli growing in rock pools). Most of the observed effect was due to visible radiation; however, the UV wavelength range, and especially UV-B, caused a significant reduction of the photosynthetic quantum yield. Fluence rate response curves indicated that the species is a typical shade plant which showed non-photochemical quenching at intermediate and higher irradiances. This is a surprising result since these algae are found in the upper eulittoral where they are exposed to high irradiances. Obviously they utilize light only during periods of low irradiances (morning, evening, high tide) while they shut down the electron transport chain during intensive exposure. Fast induction and relaxation kinetics have been measured in these algae for the first time and indicated a rapid adaptation of the photosynthetic capacity to the changing light conditions as well as a fast decrease of PS II fluorescence upon exposure to solar radiation. There was a strong bleaching of chlorophyll due to exposure to solar radiation but less drastic bleaching of carotenoids. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Chlorophyceae; *Enteromorpha*; PAM fluorescence; Photoinhibition; Photosynthetic yield; Solar ultraviolet radiation

### 1. Introduction

About half of the primary biomass production on our planet is based in aquatic ecosystems, equaling the combined productivity of all terrestrial ecosystems [1,2]. Most of the aquatic productivity is performed by phytoplankton [3,4], but macroalgae also play a significant role especially in coastal areas. Most macroalgae are sessile organisms and cannot adapt to the changing irradiation conditions by vertical movements in their habitat [5–7], so light is one of the most important factors determining the vertical distribution of macroalgae on the shore [8–12]. Macroalgae show a distinct pattern of vertical distribution in their

habitat; some inhabit the supralittoral (coast above high water mark) exposed only to the spray from the surf, whereas others populate the eulittoral (intertidal zone), which is characterized by the regular temporal change in the tides [13]. This zone is fairly extended on the Patagonian coast since the tidal change exceeds 2–3 m. Still other algae are never exposed to air since they are restricted to the sublittoral zone. The range in light exposure can be substantial, from over  $1000 \text{ W m}^{-2}$  (total solar radiation) at the surface to less than 0.01% of that which reaches the understory of a kelp habitat [14]. Macroalgae use the same mechanism of photoinhibition as higher plants to protect themselves by decreasing the photosynthetic electron transport during periods of excessive radiation [6]. But macroalgae differ in their ability to cope with enhanced UV radiation [11]. A broad survey was carried out to understand photosynthesis in aquatic ecosystems and the

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different adaptation strategies to solar radiation of ecologically important species of green, red and brown algae from the North Sea, Baltic Sea, Mediterranean, Atlantic, polar and tropical oceans [7,14–20]. However, although there are many descriptive studies on the macroalgae flora of Patagonia [21–23] there are no studies on the photosynthetic responses of these algae under natural conditions [24].

The aim of this paper is to evaluate the responses of a widespread green macroalga, *Enteromorpha linza* (L.) J. Agardh, to solar radiation under natural conditions at its growth site on the Patagonian coast, Argentina. While most of the studies indicated above investigated the short term photoinhibition and recovery of macroalgae to light stress, the present study extends the investigation to long-term (days) monitoring. Another important aspect is the analysis of fast induction and recovery kinetics to analyze the collaborative behavior of the redox components in the photosynthetic electron transport chain. To the best of our knowledge, such measurements have never been published before for macroalgae. These data reveal the capacity of the macroalgae to adapt to the fast changing light conditions in their habitat.

## 2. Materials and methods

### 2.1. Measurement of solar radiation

Solar radiation was measured using a filter radiometer (ELDONET, Real Time Computer, Möhrendorf, Germany) with three wavelength bands for UV-B, 280–315 nm; UV-A, 315–400 nm; and PAR, 400–700 nm [25]. The instrument, located on the roof of the Estación de Fotobiología Playa Unión [26], automatically records the irradiance in each channel as averages over 1-min intervals. All data are transferred to and are available on the central server of the ELDONET network (<http://www.ib.pci.cnr.it/eldonet/index.htm>) [27].

### 2.2. Plant material

Specimens of the macroalga *Enteromorpha linza* were harvested every morning from tidal pools high in the eulittoral on the east facing rocky shore south of Playa Unión (Playa Barrancas Blancas, 43° 19' S, 65° 3' W, Rawson, Patagonia, Argentina) during low tide. During the experimental period, March 2000, the water temperature was around 17°C and the salinity 34‰. After collection, the specimens were immediately transferred into custom-made UV transparent Plexiglas holders (GS 2458, Röhm and Haas, Darmstadt, Germany) with open sides to allow water to flow through the holders. Three different radiation treatments were implemented, at least four replicates in each treatment, with samples receiving: (a) full solar radiation, uncovered holders; (b) radiation above 320,

holders covered with UV cut-off filter foil (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany); and (c) 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. [4]. The sample holders with the specimens were kept in shallow water on site and shaded under black plastic foil for 30 min ( $<10 \text{ W m}^{-2}$ ). Then, the black plastic foil was removed and the specimens were exposed to solar radiation during local noon for 15 or 30 min. After exposure, the samples were transferred back into the shade to follow recovery of the photosynthetic yield. The photosynthetic parameters were determined by PAM fluorescence after the initial dark period, after the exposure time and at predefined times during the recovery period for up to 6 h. The distance of the fiber optic to the thallus was kept at 6 mm and the angle with the thallus surface at 60°.

Alternatively, during early morning, plants were harvested still attached to larger pebbles or stones and kept in a large volume of seawater in darkness during transport to the laboratory (~20 min) until exposure to solar radiation for one or several days. 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. Three radiation treatments were implemented, as mentioned above, by covering the flat containers with the appropriate filter foils. Photosynthetic parameters (see later) were determined at time zero and at times ranging from 30 min to 14 h. In these two sets of experiments described above, controls were implemented by subjecting the thalli to the same treatment except for solar radiation.

A third group of experiments was carried out at Bahía Bustamante (250 km south of Rawson) in a natural rock pool on site during low tide without removing the thalli from their growth site. At this site, photosynthetic parameters were measured every hour for 5 h centered around local noon.

### 2.3. Measurements of PAM fluorescence

A portable pulse amplitude modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany) was used to determine the in vivo chlorophyll fluorescence non-invasively [28]. This instrument determines fluorescence signals from chlorophyll *a* in photosystem II (PS II) using different experimental protocols. The effective photosynthetic quantum yield (*Y*) was calculated by the equations of Genty et al. [29] and Weis and Berry [30], as:

$$Y = (F_m' - F_t) / F_m' = F_v' / F_m'$$

where  $F_m'$  is the maximal fluorescence induced by a saturating pulse and  $F_t$  the current steady state fluorescence induced by a weak red light in a light adapted plant (ambient radiation). The optimal quantum yield was measured in dark adapted plants; in this case  $F_m'$  is substituted

by  $F_m$ , the maximal fluorescence induced by a saturating pulse, and  $F_t$  by  $F_o$ , the ground fluorescence induced by a weak red background light.

Based on the measured PAM signals fluorescence quenching analysis was performed. This analysis assumes that two parallel processes, photochemical and non-photochemical quenching ( $qP$  and  $qN$ ), reduce the maximal fluorescence yield  $F_m$ . Photochemical quenching, due to the utilization of the radiation energy in the photosynthetic apparatus, was determined as:

$$qP = (F_m' - F_t)/(F_m' - F_o')$$

Non-photochemical quenching, believed to be due to an increasing pH gradient across the thylakoid membrane [30,31], was calculated as:

$$qN = 1 - (F_m' - F_o')/(F_m - F_o)$$

The PAM instrument also allows to run pre-programmed experiments. Using one of these runs, induction curves were determined with on-line quenching analysis at 10 ms per point sampling rate. At the beginning of this experimental sequence the measuring light was switched on and a saturation pulse applied every 20 s for  $F_o$  and  $F_m$  determination followed by the kinetic recording of the fluorescence yield. After every saturating pulse the quantum yield,  $qN$  and  $qP$  were calculated. The same experimental protocol was run at 30 ms per data point. In this case the saturating pulses were not elicited at constant time intervals, but 10 pulses were given at 20-s intervals, five pulses at 40-s intervals and finally six pulses at 80-s intervals. The saturation light pulses were 1.2 s rather than 0.8 s in the first case.

Immediately following the induction curve, the relaxation kinetics of  $qN$  was determined in a different pre-defined experimental sequence. For this purpose it was necessary that the specimen has reached a steady state fluorescence and developed non-photochemical fluorescence quenching. The thalli was exposed to the actinic light, and  $F_o$  and  $F_m$  were determined at a rate of 30 ms per data point. 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  $t = 10 \times 1.2^{n-1}$ . Instead of the built-in red light emitting diode (LED) the halogen lamp was used in order to induce strong  $qN$ .

Rapid induction kinetics were measured at a sampling rate of 1000  $\mu$ s per data point. After the measurement light was switched on,  $F_o$  was determined before the actinic light (modulated at 20 kHz) pulsed on for 2 s. The recording continued for another 2 s after the end of the actinic light pulse to monitor the relaxation kinetics. The reoxidation of the different acceptor pools in PS II was

accelerated by simultaneously switching on a far red background light. A similar experimental sequence was determined at 300  $\mu$ s per data point, however in this case the dark relaxation was not recorded.

A final sequence was designed to determine the irradiance dependence of important fluorescence parameters as well as photochemical and non-photochemical quenching. Before the experimental sequence,  $F_o$  and  $F_m$  were determined in dark-adapted thalli followed by a 10-min irradiation period at an intermediate irradiance of 23  $W m^{-2}$  to light adapt the sample and to activate the Calvin cycle enzymes. Then the actinic light from a red LED was stepped up every 6.5 min from its lowest level to the maximum.  $F_o'$  was determined at each irradiance level and the electron transport chain was oxidized before each measurement by applying a proceeding far red pulse.

#### 2.4. Chlorophyll determinations and absorption spectra

The chlorophyll concentration was determined in thalli exposed to a full day of solar radiation as well as in control thalli kept in dim light. Same amounts of fresh weight were placed in centrifuge tubes and extracted in 10 ml of absolute methanol for at least 1 h at 4°C. After the extraction period, the tubes were centrifuged for 10 min at 1000 rev./min and the fluorescence of the methanolic extract was measured in a Turner Designs fluorometer (TD 700) before and after acidification; the concentration of chlorophyll *a* was calculated from these readings. The fluorometer was calibrated using pure chlorophyll *a* from *Anacystis nidulans* (Sigma C 6144). After extraction and centrifugation the thalli were dried at 40°C for 24 h and the dry weight determined. The absorption spectra of the algae were also determined from the methanolic extract (before acidification) from 250 to 750 nm [32] using a UV-visible spectrophotometer (Hewlett-Packard 8453E).

#### 2.5. Statistics

Mean values and standard deviation were determined from a minimum of eight independent PAM measurements on different thalli. All experimental runs were repeated several times on different days. Student's *t*-tests were calculated to determine whether the different irradiance treatments caused statistically significant differences [33].

### 3. Results

The variation of incident solar radiation for a representative day (17 March 2000), for UV-B, UV-A and PAR is shown in Fig. 1. The sky was clear throughout the day with maximal irradiances of about 380  $W m^{-2}$  in the PAR region, 50  $W m^{-2}$  in the UV-A and about 1.0  $W m^{-2}$  in the UV-B region. Daily doses of solar radiation during March 2000 varied from 5 to 27  $kJ m^{-2}$  for UV-B and

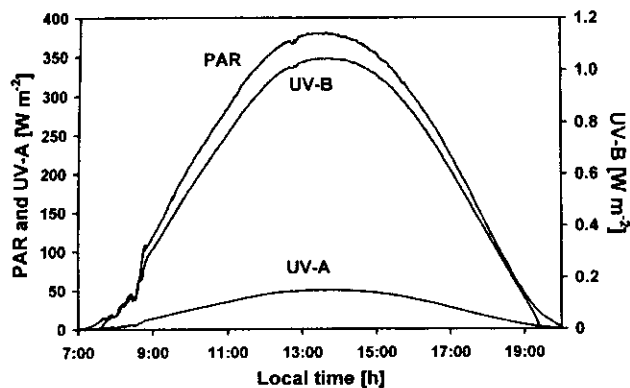


Fig. 1. Surface irradiance in Playa Unión (43° 18' S; 65° 03' W). Rawson, Argentina, in  $W m^{-2}$  for a representative day (17 March 2000) during the study period. Measurements were done with an ELDONET radiometer in the PAR (400–700 nm), UV-A (315–400 nm) and UV-B (280–315 nm) wavelength ranges.

from 400 to 1400  $kJ m^{-2}$  for UV-A and from 2800 to 10,600  $kJ m^{-2}$  for PAR.

The irradiance–response curve for photosynthetic parameters of *E. linza* is shown in Fig. 2.  $Fm'$  had a slow but steady decline with irradiance up to about 10  $W m^{-2}$ , and a steeper decrease thereafter. The photochemical quenching started at a value close to 1 and decreased down to 0.5 at about 90  $W m^{-2}$ . Likewise, the yield decreased from an initial value of 0.7 to about 0.2.  $Fo'$  and  $Ft$  did not vary significantly while the non-photochemical quenching increased from 0 to above 0.8 as mirror image of  $Fm'$  indicating a high degree of photoinhibition, especially over 10  $W m^{-2}$ .

The induction curve with quenching analysis measured at 10 ms sampling rate (Fig. 3) showed a fast drop of  $Ft$  and  $Fm'$  after the first saturating pulse followed by a rise to an intermediate maximum and subsequently both declined monotonously to a lower value (Fig. 3A). The non-photochemical quenching followed antagonistically to

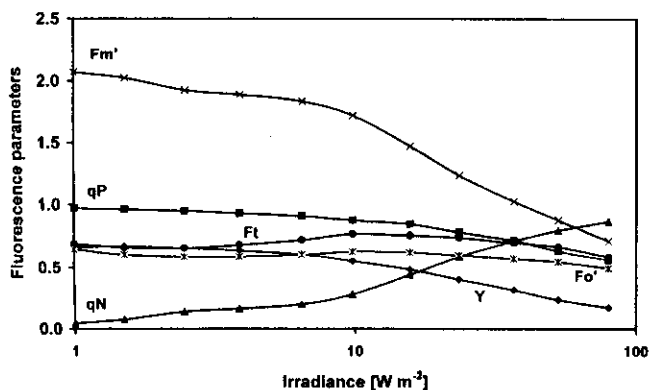


Fig. 2. Fluorescence parameters measured in *E. linza* as a function of the fluence rate of the actinic red light. Note that the x-axis is a log scale. Diamonds, photosynthetic quantum yield; closed circles,  $Ft$ ; squares, photochemical quenching; triangles, non-photochemical quenching; crosses,  $Fm'$  and asterisks,  $Fo'$ .

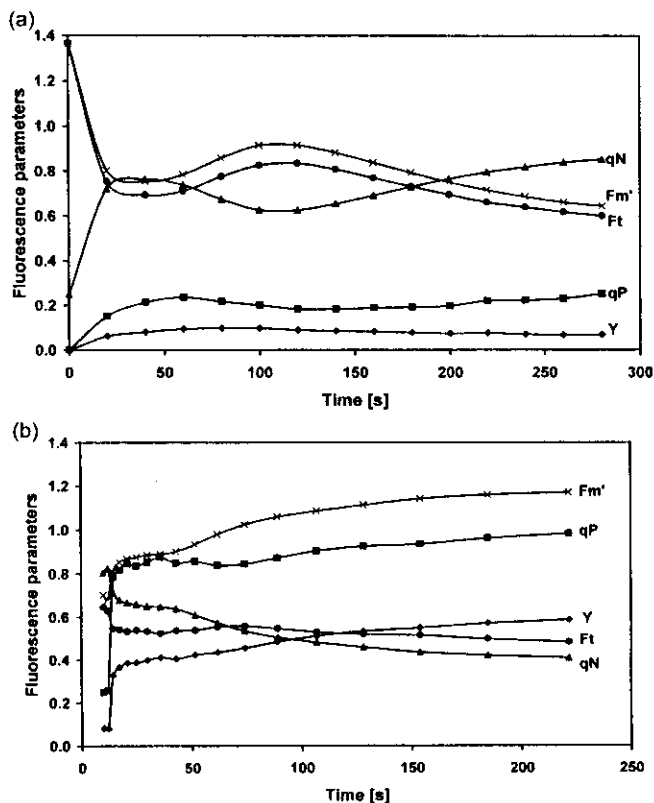


Fig. 3. (A) Induction curve with quenching analysis and (B) relaxation kinetics of *E. linza* at 30 ms per data point. Diamonds, photosynthetic quantum yield; triangles,  $qN$ ; crosses,  $Fm'$ ; squares,  $qP$ ; closed circles,  $Ft$ .

this pattern. It is interesting to note that adaptation of the fluorescence parameters occurred in such a short time. The yield stayed at a low level (close to 0.1), and the photochemical quenching rose only slightly over the recording period. The built-in halogen lamp was used in order to induce a significant non-photochemical quenching up to 0.85. Immediately after this, the relaxation kinetics of  $qN$  was determined at 30 ms per data point (Fig. 3B). The non-photochemical quenching and  $Ft$  gradually declined over the recording period, to a value of 0.4. In contrast,  $Fm'$  rose antagonistically and both the yield and  $qP$  increased after the first two saturating pulses and continued to rise at a lower rate thereafter, reaching a maximum yield of almost 0.6. Like the induction kinetics, the relaxation kinetics indicated a very fast adaptation of the photosynthetic apparatus to changing light conditions.

When specimens of *E. linza* were exposed to 15 min of unfiltered solar radiation in the Plexiglas holders (i.e. thalli in one position) floating in a larger volume of seawater, the effective photosynthetic quantum yield decreased from 0.7 (value right after the dark period) to about 0.2 (Fig. 4A). Within 30 min in the shade, the quantum yield recovered to 0.45 and slightly increased during the rest of the recovery period of 6 h, when it reached a value of 0.55 (gray bars). When the UV-B was removed with a cut-off

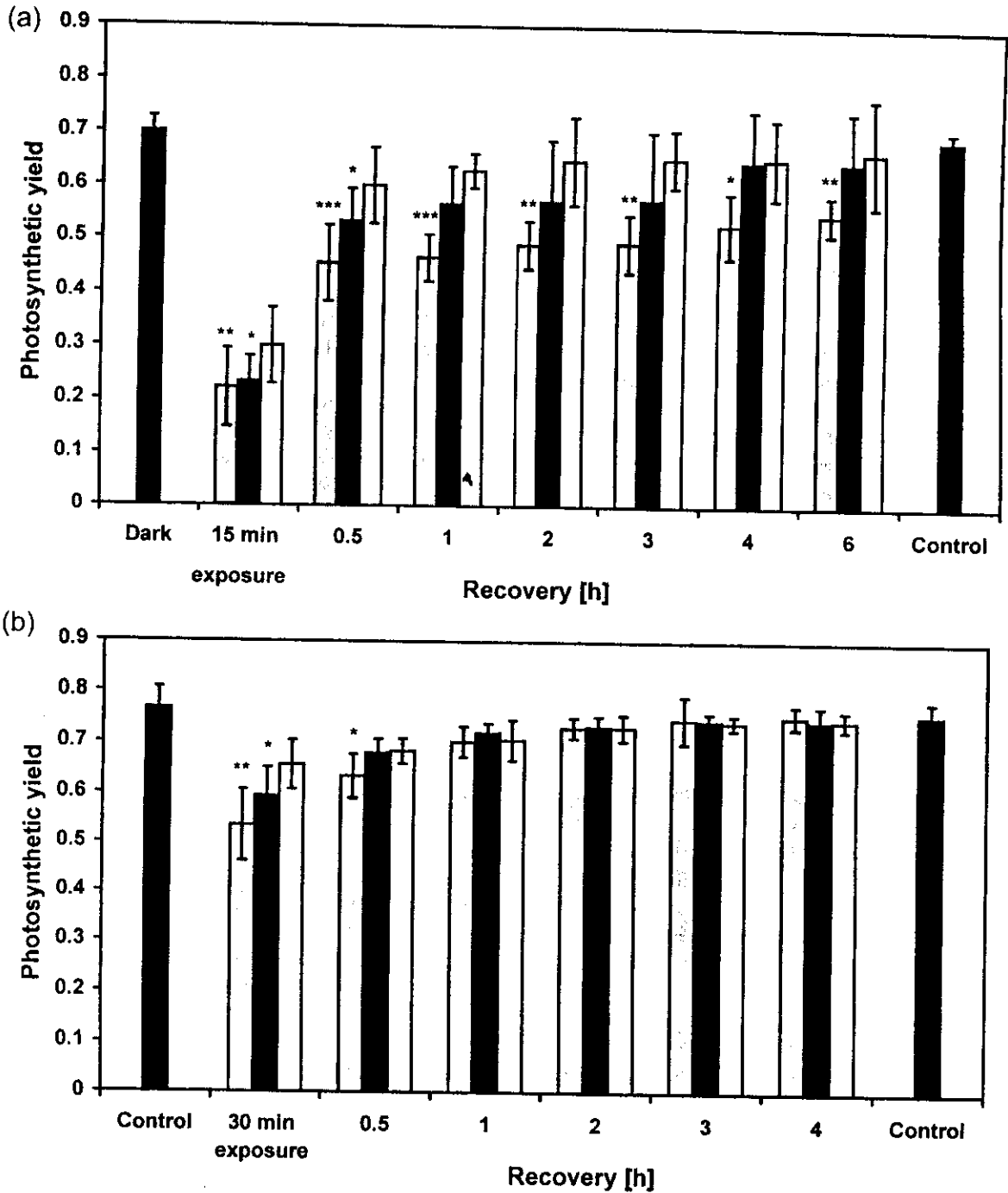


Fig. 4. Effective photosynthetic quantum yield of *E. linza* measured after 30 min dark adaptation, 15 min exposure confined in Plexiglas holders (A) or 30 min exposure free floating in a large volume of water (B) and after increasing recovery times in the shade calculated as  $(Fm' - Ft)/Fm'$ . Black bars, specimens exposed to UV-A + PAR. White bars, specimens exposed to PAR only. For each data point  $n$  was equal to 8 (thin lines indicate 1 S.D.). The values for unfiltered solar radiation and under the 320 nm cut-off filter treatments are significantly different from the PAR-only values (395 nm cut-off) in each set with \*\*\* $P < 0.001$ , \*\* $P < 0.01$  or \* $P < 0.1$ , respectively, as indicated by Student's  $t$ -test. Maximal irradiances for the experiment in Fig. 4A were: PAR  $349 \text{ W m}^{-2}$ , UV-A  $44.15 \text{ W m}^{-2}$  and UV-B  $1.34 \text{ W m}^{-2}$ . The total doses for this day were: PAR  $8.18 \text{ MJ m}^{-2}$ , UV-A  $1.04 \text{ MJ m}^{-2}$  and UV-B  $26.8 \text{ kJ m}^{-2}$ . Maximal irradiances for the experiment in Fig. 4B were: PAR  $371 \text{ W m}^{-2}$ , UV-A  $44.7 \text{ W m}^{-2}$  and UV-B  $1.10 \text{ W m}^{-2}$ . The total doses for this day were: PAR  $4.80 \text{ MJ m}^{-2}$ , UV-A  $0.65 \text{ MJ m}^{-2}$  and UV-B  $14.63 \text{ kJ m}^{-2}$ .

filter foil, the yield measured after the exposure period did not differ significantly from the unfiltered value, however, during the recovery period the thalli seemed to recover quickly and the yield measured at various times was

significantly higher. Excluding the entire UVR (white bars) resulted in higher yield not only after the exposure time, but also during recovery. The thalli exposed only to PAR, recovered almost completely after 1–2 h of recovery, those

exposed to UV-A + PAR after 4 h whereas those exposed to unfiltered radiation did not recover completely even after 6 h of the stress. Control specimens were treated the same way as the exposed samples except for the exposure period. These plants showed a similar yield (0.7) as the dark adapted plants before exposure indicating that the experimental conditions did not affect the yield parameter, thus it is safe to conclude that the yield is controlled only by the exposure to solar radiation. Similar results were obtained when the experiment was repeated on site with the experimental containers floating in shallow water (data not shown).

In the procedure described above (Fig. 4A) the experimental conditions confined the thalli in one position so that the same surface was always exposed. The experiment was repeated with plants floating freely in a larger volume of water exposed for the same period of time. Interestingly there was hardly any photoinhibition after the same exposure time (15 min; data not shown). Therefore the exposure time was increased to 30 min (Fig. 4B). Under these conditions, the inhibition due to UV-B was less dramatic (0.53) even though the thalli were exposed for 30 min; the initial yield values were 0.77. After the 30-min exposure period there were significant differences among the three radiation treatments. However, the recovery was fast and the differences between the treatments were no longer significant after 1 h of recovery (Fig. 4B).

When free floating thalli were exposed continuously to solar radiation over the day (starting at 11 h local time) a significant photoinhibition was measured around the local noon hours (i.e. 13:00–14:00 h) during a day with only scattered clouds (Fig. 5A). In this experiment, the yield in the samples that received all radiation dropped significantly (below 0.5) after 1 h of exposure and remained low for about 2 h more; the inhibitions due to UV-A and PAR were less dramatic, and significantly different from the unfiltered radiation treatment during this 3-h period. The algae started to recover in the early afternoon (15:30 h) and there were no longer differences among the three treatments. There was hardly any photoinhibition and the differences less pronounced on a rainy day (Fig. 5B).

Photoinhibition was also determined in a natural environment in a rock pool at Bahía Bustamante. The site was accessible only during low tide and completely covered during high tide. On a clear day, the first measurement shortly after the retreat of the water at 11:00 h local time showed a yield around 0.65 (Fig. 6). Measurements were continued on an hourly basis and showed a steep decline in the yield to 0.15 at 15:00 h. Similar patterns of the yield were seen on subsequent days.

The fast induction kinetics at 1000  $\mu\text{s}$  per data point shows the involvement of several components of the PS II redox system (Fig. 7). After determination of  $F_0$  the actinic halogen light is switched on for 2 s. There are at least two distinct rise components which lead to a transient fluorescent maximum with a subsequent drop to a lower

level steady state. Also the decay shows a biphasic behavior. The two components in the fluorescence rise are even better separated at a higher sampling rate of 300  $\mu\text{s}$  per data point and a logarithmic plot to blow up the initial phase (Fig. 8). The recording was repeated after 15, 30, 45 and 60 min of exposure to solar radiation under a clear sky. Even after 15 min the fluorescence level had dropped dramatically and the first component became more obvious. This trend continued for the rest of the exposure time; after 60 min of exposure the maximal fluorescence yield had dropped to 16% of the initial value.

Absorption spectra of methanolic extracts from samples exposed for 8 h centered around local noon during a clear day indicate a massive bleaching of all photosynthetic pigments as compared to the control kept in darkness (Fig. 9). In the bleached sample there was a change in the ratio of the peak at 440 nm to that at 470 nm as compared to the dark control, indicating that chlorophyll *a* was more drastically bleached than the carotenoids.

#### 4. Discussion

Photosynthetic organisms respond to high levels of solar radiation by various degrees of photoinhibition, a common phenomenon in higher plants and algae [31,34–37], or even irreversible photodamage. Chronic photoinhibition is due to photodegradation of the D1 protein in photosystem II (PS II) causing a decrease in the photosynthetic electron transport [7,38–40]. These adaptive processes in algae are summarized in recent reviews [6,7].

Many organisms show a typical midday depression in the photosynthetic yield between noon and the early afternoon hours. This diurnal pattern is believed to be mainly due to dynamic photoinhibition [41–43]. In marine algae this pattern is complicated by the tidal rhythm, so that the highest irradiance stress occurs when low tide coincides with high solar angles [43–45]. Thus, macroalgae are subjected to a complicated pattern of irradiances controlled by the daily cycle, the tidal rhythm which varies from day to day, and the changing cloud cover. Judging from the drastically changing exposure conditions in the habitat the algae need to accomplish positive net photosynthesis at low and intermediate irradiances. In contrast, during excessive irradiation they need effective preventive mechanisms to protect themselves from photooxidative damage. In higher plants and macroalgae the 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 [46]. The fast induction and relaxation kinetics demonstrate impressively how fast the photosynthetic apparatus can adapt to the ambient light conditions. To the best of our knowledge this is the first time that such fast kinetic studies were performed in macroalgae.

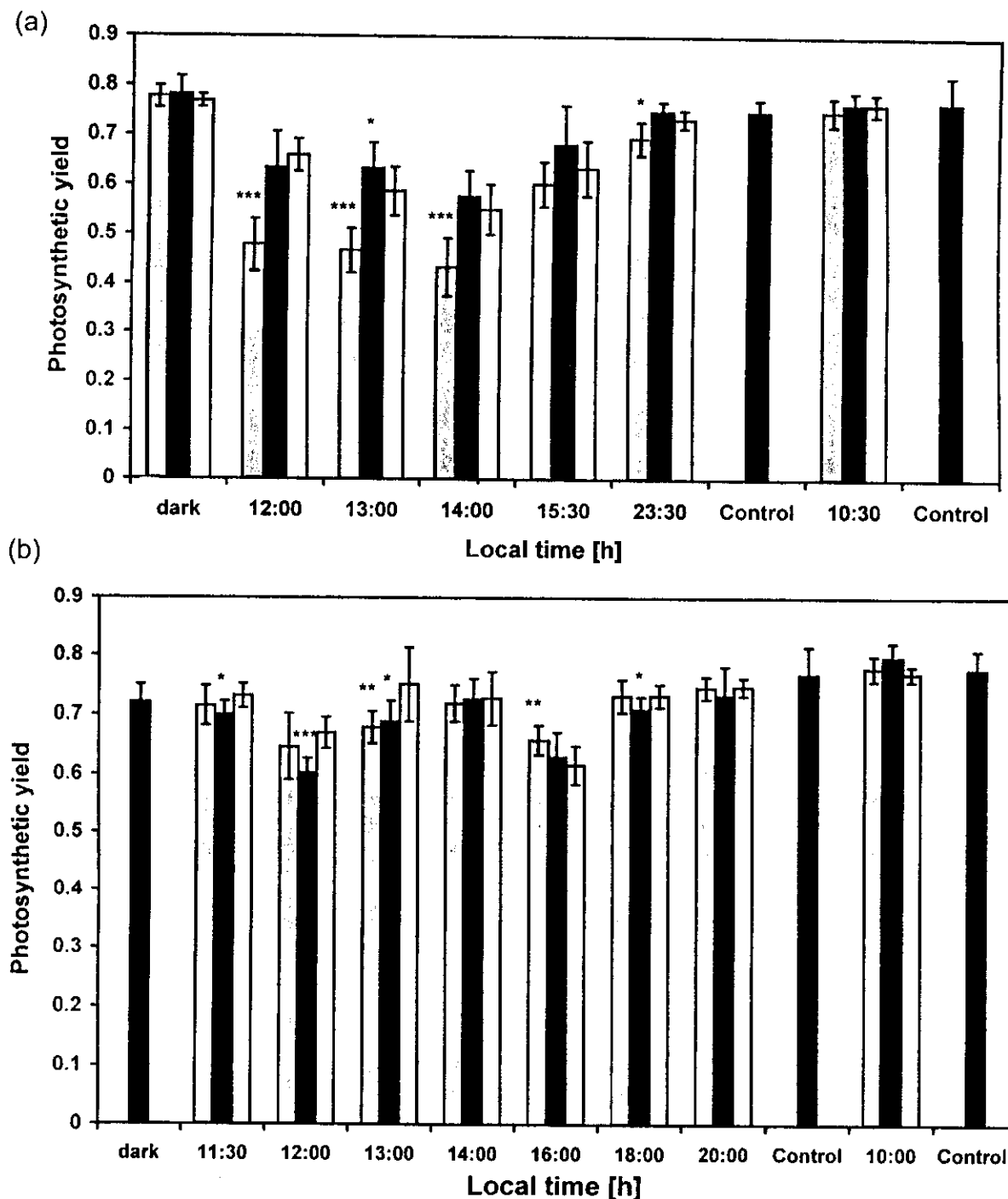


Fig. 5. Effective photosynthetic quantum yield of *E. linza* exposed free floating in open vessels on an almost cloudless day (A) and a rainy day (B). Gray bars, specimens exposed to unfiltered solar radiation. Black bars, specimens exposed to UV-A + PAR. White bars, specimens exposed to PAR only. For each data point  $n$  was equal to 8 (the lines on top of the bars indicate 1 S.D.). The values for unfiltered solar radiation and under the 320 nm cut-off filter treatments are statistically significantly different from the PAR-only values (395 nm cut-off) in each set with \*\*\* $P < 0.001$ , \*\* $P < 0.01$  or \* $P < 0.1$ , respectively, as indicated by the Student's *t*-test. Maximal irradiances for the experiment in (A) were: PAR  $328 \text{ W m}^{-2}$ , UV-A  $43.5 \text{ W m}^{-2}$  and UV-B  $1.13 \text{ W m}^{-2}$ . The total doses for this day were: PAR  $6.99 \text{ MJ m}^{-2}$ , UV-A  $0.95 \text{ MJ m}^{-2}$  and UV-B  $21.43 \text{ kJ m}^{-2}$ . Maximal irradiances for the experiment in (B) were: PAR  $348 \text{ W m}^{-2}$ , UV-A  $41.3 \text{ W m}^{-2}$  and UV-B  $0.94 \text{ W m}^{-2}$ . The total doses for this day were: PAR  $4.40 \text{ MJ m}^{-2}$ , UV-A  $0.60 \text{ MJ m}^{-2}$  and UV-B  $12.04 \text{ kJ m}^{-2}$ .

Photoinhibition is not only found in macroalgae from the tropics and the temperate zone [11,47,48] but also in Arctic and Antarctic macroalgae [49–52]. Most of the

photoinhibition is due to PAR. However, a significant fraction of photoinhibition is caused by UV-B (and less so by UV-A) radiation in the top few meters of the water

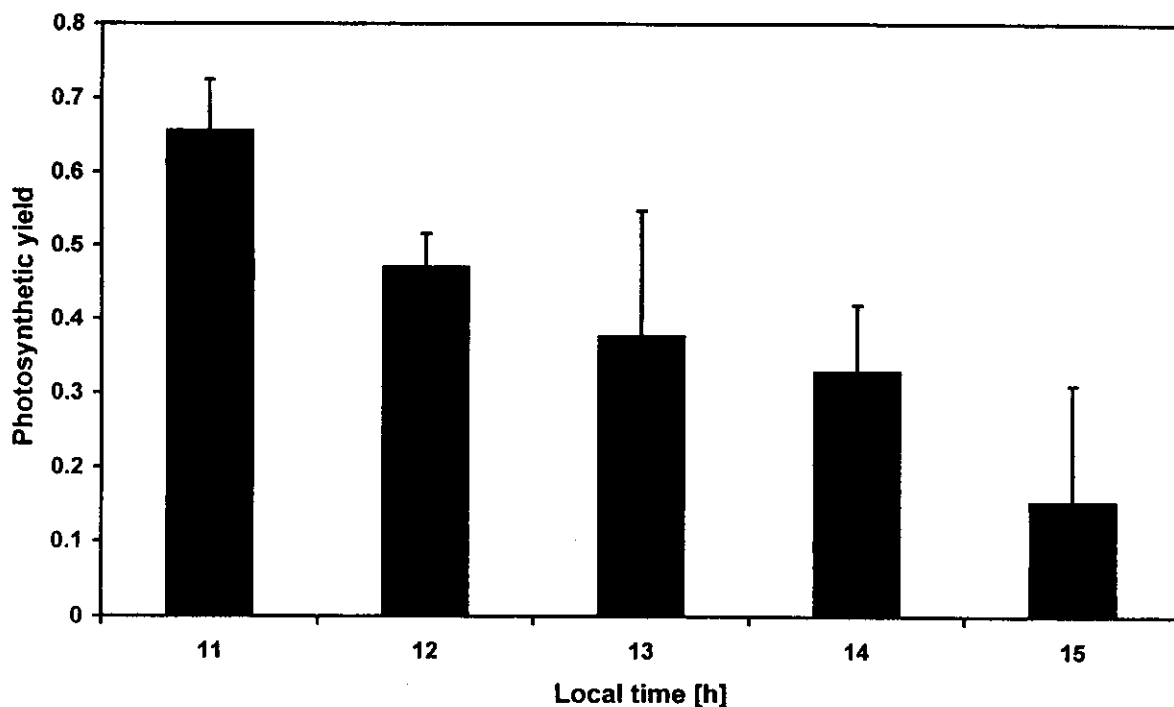


Fig. 6. Effective photosynthetic quantum yield of *E. linza* in its natural environment in a rock pool in Bahía Bustamante measured at hourly intervals during low tide.

column. Similar results were found in macroalgae from the northern hemisphere at similar latitudes [11,53,54], although the share in the quantum energy of UV-B reaching the Earth's surface is less than 1% of the total radiation. This unproportionally high photoinhibition by solar UV

has been demonstrated in a number of marine algal [7,35] and phytoplankton species in the North Atlantic and Mediterranean Sea [4,55].

The fluence rate–response curve indicates that Patagonian specimens of *E. linza* possesses an effective adapta-

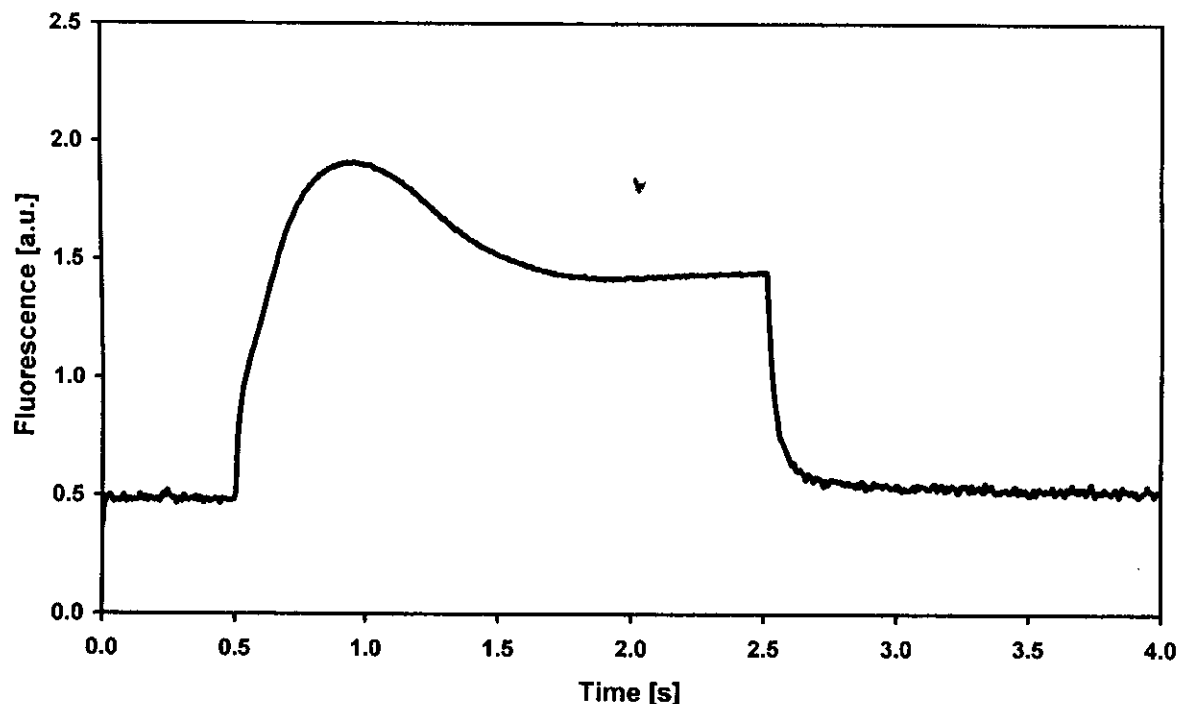


Fig. 7. Rapid induction and relaxation kinetics at 1000  $\mu\text{s}/\text{data point}$  in *E. linza*.

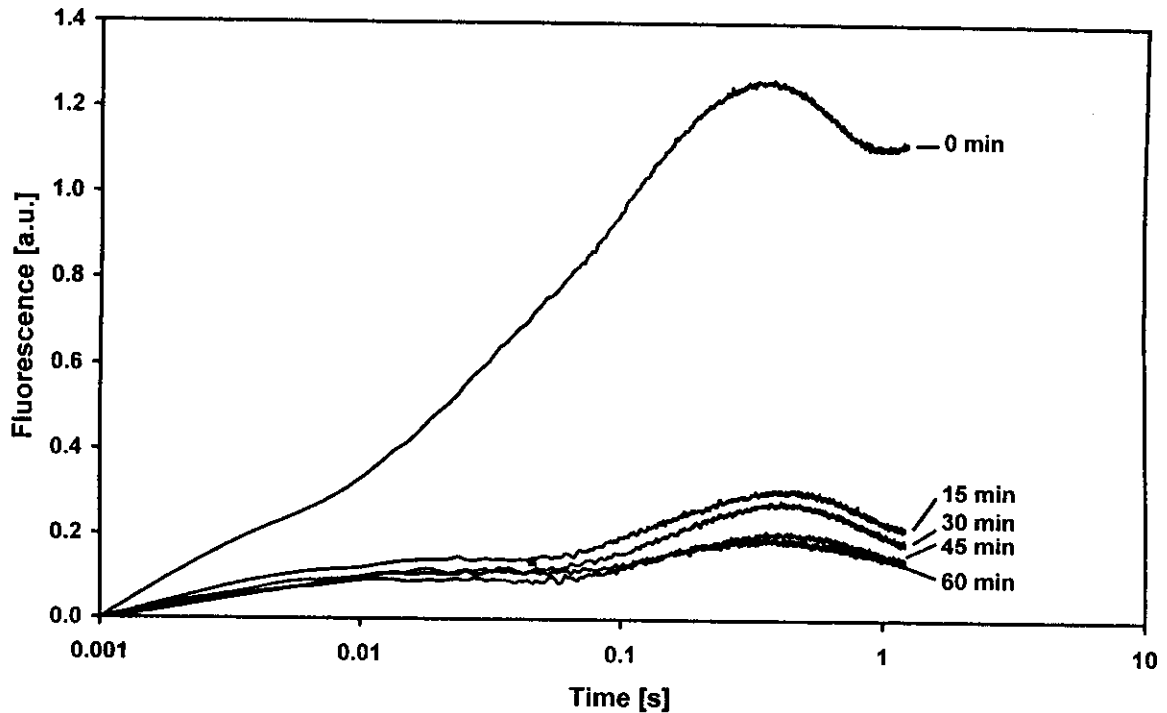


Fig. 8. Rapid induction and relaxation kinetics at  $300 \mu\text{s}/\text{data point}$  in *E. linza* plotted logarithmically. The recording was repeated after 15, 30, 45 and 60 min of exposure to solar radiation under a clear sky.

tion mechanism to adjust to the prevailing light conditions of high solar radiation in conjunction with very clear skies during spring–summer. Judging from the irradiances at which non-photochemical quenching commences and photochemical quenching declines (Fig. 2), this alga is a

typical shade plant adapted to effectively harvest and utilize light energy at low fluence rates. This implies that the algae need effective protective mechanisms for high light conditions. The induction and relaxation kinetics of  $qN$  (Fig. 3A and B) indicate that the algae can adapt to

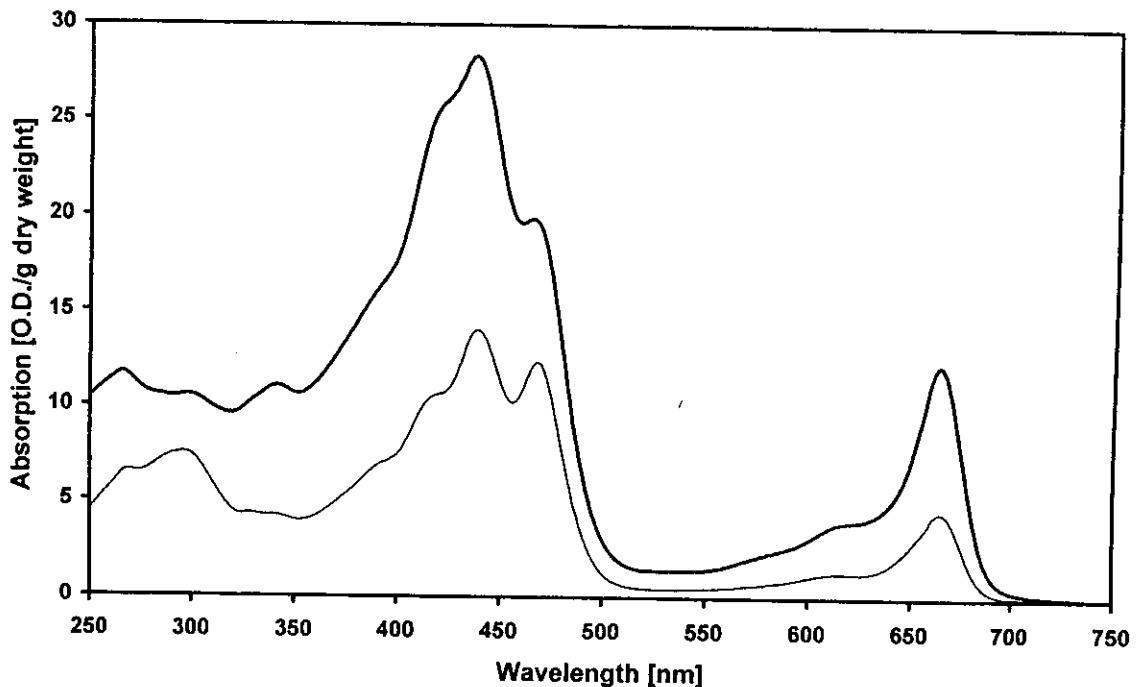


Fig. 9. Absorption spectra of methanolic extracts of *E. linza* thalli kept under shaded conditions (upper curve) and thalli exposed to solar radiation for 8 h centered around local noon on a clear day.

changing light conditions within minutes. The biphasic increase in fluorescence during the fast induction kinetics (Fig. 7) can be attributed to a reduction of  $Q_A$  and  $Q_B$  followed by a subsequent and slower reduction of the plastoquinone pool. The slow decrease to lower fluorescence values indicate that it takes over 1 s until the photosynthetic electron transport chain operates smoothly. During exposure to unfiltered solar radiation the fluorescence signal decreases within 15 min to less than 20% of its initial value (Fig. 8). Similar results were found in macroalgae at similar latitudes on the Northern Hemisphere [7,53,56]. The relaxation kinetics of  $qN$  provide information on the different parts of non-photochemical quenching induced by high irradiance.

*E. linza* showed a high photosynthetic quantum yield after dark adaptation or in its natural habitat at the end of the night (Figs. 4–6). This observation corresponds with results found in a number of eulittoral Mediterranean and Atlantic species [7]. Surface-adapted algae recover much faster from exposure to unfiltered solar radiation than algae adapted to deeper water [56,57]. Ecologically more important is the finding that the algae suffered photoinhibition in semi-natural conditions when exposed free-floating or at their natural growth site during low tide (Figs. 4B, 5A, B and 6). Therefore, it may be necessary to reconsider our conception about the timing of maximal photosynthesis in supralittoral and subtidal macroalgae. Obviously the algae show 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 factors thus result in a complicated pattern of the photosynthetic yield. It will be interesting in the future to determine if this behavior is endogenously regulated. A remarkable observation is the fact that solar UV-B had a significant share in photoinhibition (Figs. 4A and 5A) under natural conditions even though the incident energy represents only a small fraction in solar radiation.

Obviously the effective quantum yield is a valid indicator of the photosynthetic efficiency in this green alga showing statistically significant differences between the different light treatments. This is not only visible during exposure but also during recovery, indicating that dynamic photoinhibition is a photoprotective mechanism. Quantitatively speaking, the degree of photoinhibition is similar to other green and surface algae studied in the eulittoral [6,7,20,44]. Another interesting point is that the photosynthetic pigments are bleached by natural radiation over the day and need to be resynthesized at night. The chlorophylls are more rapidly bleached than the protective carotenoids.

In conclusion, the present studies indicate that supralittoral and eulittoral macroalgae do not simply follow the daily pattern of solar radiation in their photosynthetic capacity but adapt to the rapidly changing light conditions in their natural habitat modified by the tidal rhythm and the changing cloud cover. These organisms have obviously

developed several effective mechanisms to counter excessive irradiation and rapidly optimize their photosynthetic apparatus to the environmental conditions.

## 5. Abbreviations

<i>F<sub>o</sub></i>	initial chlorophyll fluorescence in a dark-adapted plant, all reaction centers are open
<i>F<sub>m</sub></i>	maximal fluorescence in a dark-adapted plant, all reaction centers are closed
<i>F<sub>v</sub></i>	variable fluorescence = $F_m - F_o$
<i>F<sub>o'</sub></i> , <i>F<sub>m'</sub></i> and <i>F<sub>v'</sub></i>	the same for the light-adapted state
<i>F<sub>t</sub></i>	current fluorescence of a light-adapted plant
LHC	light harvesting complex
PAM	pulse amplitude modulated fluorometer
PAR	photosynthetic active radiation
PS II	photosystem II
<i>qE</i>	energy quenching
<i>qN</i>	non-photochemical quenching
<i>qP</i>	photochemical quenching

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