Botanica Marina 49 (2006): 275–282 © 2006 by Walter de Gruyter • Berlin • New York. DOI 10.1515/BOT.2006.035

The use of chlorophyll fluorescence for monitoring photosynthetic condition of two tank-cultivated red macroalgae using fishpond effluents

Félix L. Figueroa[1,*], Rui Santos[2], Rafael Conde-Álvarez[1], Leonardo Mata[2], Juan Luis Gómez Pinchetti[3], Joana Matos[4], Pirjo Huovinen[5], Andreas Schuenhoff[2] and João Silva[2]

[1] Departamento de Ecología, Facultad de Ciencias, Universidad de Málaga, Campus Universitario de Teatinos s/n, 29071 Málaga, Spain, e-mail: Felix_lopez@uma.es

[2] Algae-Marine Plant Ecology Research Group, Centre of Marine Sciences of Algarve, University of Algarve, Gambelas, 8005-139 Faro, Portugal

[3] Centro de Algología Aplicada, Universidad de Las Palmas de G.C., Muelle de Taliarte s/n, 35214 -Telde, Las Palmas, Canary Islands, Spain

[4] Instituto Botanico, Universidade do Porto, Rua do Campo Alegre 1191, 41150 Porto, Portugal

[5] Centro de Investigación y Desarrollo de Recursos y Ambientes Costeros "i-mar", Universidad de Los Lagos, Camino a Chinquihue, km. 6 (Casilla 557), Puerto Montt, Chile

* Corresponding author

Abstract

In vivo chlorophyll fluorescence measured by pulse amplitude modulated (PAM) fluorometry was used as an indicator of photosynthetic activity in tank-cultivated red algae [*Gracilaria cornea* and *Asparagopsis armata* (tetrasporophyte phase, previously known as *Falkenbergia rufolanosa*)] using effluent seawater from sea bream fishponds *(Sparus aurata*). Optimal quantum yield of indoor-grown *G. cornea* did not change during the period analysed (2 months). In contrast, optimal quantum yield decreased drastically in algae transferred from indoor to outdoor conditions. Maximal electron transport rate increased and photosynthetic pigments decreased in algae growing outdoors, indicating an acclimation to high irradiance. After 16 days, chlorophyll fluorescence decreased drastically and recovered partially when algae were transferred again from outdoor to indoor conditions. *Falkenbergia rufolanosa* was grown at three different biomass densities (4, 6 and 8 g l^{-1}). The effects of three consecutive air compressor failure events on the photosynthetic activity were followed. An apparent degradation of the physiological conditions of the algae (resulting in their bleaching) was observed, concomitant with a strong decrease in the optimal quantum yield. This decrease was higher at algal biomass densities of 4 g l^{-1} than at 6 or 8 g l^{-1}. Three days after transfer to optimal conditions, i.e., normal aeration conditions, optimal quantum yield reached values around 0.50–0.55, close to the values before stress conditions. The results revealed that the use of chlorophyll fluorescence is a powerful means to rapidly detect different stress situations in integrated cultivation of seaweeds using fishpond effluents. In particular, the optimal quantum yield of algae can be monitored regularly as an early warning of the physiological stress in cultures.

Keywords: *Falkenbergia rufolanosa*; fishpond effluents; *Gracilaria cornea*; *in vivo* chlorophyll fluorescence; physiological stress.

Introduction

In order to quickly and easily assess physiological conditions in integrated cultures of seaweeds using fish farm effluents, it is necessary to find processes that are rapidly affected by various stress conditions. Photosynthetic activity is one of the parameters most extensively monitored in plant stress research (Schreiber et al. 1986), since photosynthetic rate is affected by temperature, high irradiance (of PAR), ultraviolet radiation, drought, salinity, etc. (Franklin and Forster 1997, Häder and Figueroa 1997). Photosynthesis can be estimated by gas-evolution measurements (O_2 evolution and CO_2 fixation). Although these methods are widely used in photosynthesis research, they are tedious and slow. Moreover, the equipment used is mostly restricted to laboratory studies. *In vivo* chlorophyll fluorescence is an alternative non-intrusive technique for the fast determination of photosynthetic activity (Schreiber et al. 1986). Pulse amplitude modulated (PAM) chlorophyll fluorescence associated with PSII was developed primarily to assess stress-dependent changes in photosynthesis of higher plants (Schreiber et al. 1986), but it is now being used extensively in cyanobacteria, micro- and macroalgae (Büchel and Wilhelm 1993, Schreiber et al. 1995, Flameling and Kromkamp 1998, Figueroa and Gómez 2001, Villafañe et al. 2003).

Information related to *in situ* chlorophyll fluorescence measurements has increased considerably for macroalgae of different aquatic systems (Franklin and Forster 1997, Gómez and Figueroa 1998, Silva et al. 1998, Gómez et al. 2005). *In vivo* chlorophyll fluorescence has been also used as an indicator of photosynthesis in algae utilised in commercial aquaculture, i.e., microalgae such as *Dunaliella* species, *Phaeodactylum tricornutum* Bohlin, *Isochrysis galba* Parke, among others (see review of Flameling and Kromkamp 1998, Young and Beardall 2003), and for macroalgae under laboratory conditions [such as *Gelidium pulchellum* (Turner) Kützing (Gómez et al. 2001), *Gelidium sesquipedale* (Clem.) Born. *et* Thur (Gómez and Figueroa 1998) and *Ecklonia cava* Kjellman (Altamirano et al. 2004)], under outdoor conditions in tank-grown algae

(Aguirre-von Wobeser et al. 2000, Cabello-Pasini et al. 2000), under semi-intensive culture systems [such as *Gracilaria chilensis* Bird, McLachlan *et* Oliveira], in estuarine systems (Gómez et al. 2005), and *Palmaria palmata* (Linnaeus) O. Kuntze in coastal areas (Hanelt and Nultsch 1995).

To our knowledge, only Mata et al. (2006) have reported *in situ* chlorophyll fluorescence measurements in integrated culture of macroalgae for biofiltration of fishpond effluents. In this work, *in vivo* chlorophyll fluorescence using PAM fluorometry was used to monitor the photosynthetic activity of the red alga *Gracilaria cornea* J. Ag., and of the tetrasporophyte of *Asparagopsis armata* Harvey (*Falkenbergia rufolanosa* phase) cultivated in fishpond effluents. In both cases, there was a drastic deterioration of the cultivated seaweeds coincident with the monitoring period, which provided a unique opportunity to investigate the potential of chlorophyll fluorescence as a monitor of the photosynthetic condition of the seaweeds during the equipment failure events, and during the following recovery.

Materials and methods

Culture conditions and causes of deterioration

The red alga *Gracilaria cornea* has been cultivated in a greenhouse at the Centro de Algología Aplicada (Taliarte, Gran Canaria, Canary Islands) for more than 10 years. In May 2001, *G. cornea* was transferred to outdoor conditions and grown in 750 l aerated semicircular glass-fibre tanks (surface 1.8 m^2; water depth 0.8 m), at a biomass density of 6 g l^{-1} (total biomass of 4.5 kg). Seawater effluent exchange rates (4 vol d^{-1}) were provided from tanks with *Sparus aurata* L. at an average concentration of 130 μM NH_4^+-N. The outdoor conditions resulted in visible deterioration of the thalli. In different experiments, NH_4^+ concentration inflow in the seaweed tanks ranged between 10 and 300 μM, resulting in biofiltration capacities (NUE) for different red and green seaweed species close to 80%, and NH_4^+-N uptake rates (NUR) ranging between 5 and 30 mmol m^{-2} h^{-1} during periods longer than 3 months for the species assayed, and no toxicity due to ammonium was observed (Gómez Pinchetti et al. unpublished data).

The red alga *Asparagopsis armata* (Harvey) (tetrasphorophyte phase: *Falkenbergia rufolanosa*) was grown at three different biomass densities, viz., 4, 6 and 8 g l^{-1} in duplicate 110 l cylindrical white polyethylene tanks (Allibert Buckhorn, Allibert Manutención S.A. Santa Perpetua de Mogoda, Barcelona, Spain) C1100; light transparency ~10% at 0.5 m depth) under full solar radiation in a fish (*Sparus aurata*) farm (Aquamarim) located in Olhão (southern Portugal). Fishpond effluents with an NH_4^+-N concentration of about 100 μM were supplied to the tanks at an exchange rate of 24 volume d^{-1}. The available ammonium concentration, 100 μM, was not toxic (Schuenhoff et al. 2006).

A failure of the air compressor that maintained the seaweeds in suspension inside the tanks occurred during the night, resulting in a visible deterioration of the thalli.

Light under field conditions

In the case of *Gracilaria cornea* cultures, the air and underwater levels of PAR (400–700 nm), UV-A (315–400 nm) and UV-B (280–315 nm) were determined using an Eldonet radiometer (Real Time computer, Erlangen, Germany). PAR irradiance was expressed as photon fluence rate, i.e., μmol m^{-2} s^{-1} and UV radiation was expressed in energy units, i.e., W m^{-2} according to the recommendations reported by Björn et al. (1996). In the case of *Falkenbergia rufolanosa* cultures, the daily level of PAR in the air was determined continuously using a Licor sensor (Li-190SA quantum sensor connected to a Li-1000 data logger; Li-Cor, Lincoln, USA). Underwater PAR, UV-A and UV-B irradiances were determined with a spectroradiometer (Licor-1800 UW, Li-Cor, Lincoln, USA).

The diffuse vertical attenuation coefficient of downward PAR ($K_{d,PAR}$) was determined using the following formula (Kirk 1994):

$$K_{d,PAR}=\ln [Ed_{(z_2)}/Ed_{(z_1)}]\times(z_1-z_2)-1 \quad (1)$$

where $Ed_{(z_1)}$ and $Ed_{(z_2)}$ are the levels of PAR at depths z_1 and z_2. Logarithmic dependencies of light attenuation by water depth were calculated with non-linear regression over a depth profile in the tanks.

In both sampling sites, the solar light field was not affected by clouds for the whole sampling period. The radiometers and Li-1800 UW spectroradiometer were calibrated by the supply companies one year before the experiments.

Photosynthetic measurements

In vivo chlorophyll fluorescence of PSII was measured with a portable pulse modulation fluorometer (PAM-2000, Waltz, Effeltrich, Germany). The optimal quantum yield, F_v/F_m (Schreiber et al. 1986), was determined after preincubation in darkness, as previously reported (Hanelt et al. 1997, Figueroa et al. 2003). The effective quantum yield ($\Delta F/F'_m$) was calculated according to Genty et al. (1989). Calculations of electron transport rate (ETR) were then made by multiplying the effective quantum yield by the irradiance of the halogen lamp (E), absorptance (A) and the factor 0.5, which comes from the quanta/electron ratio (one electron requires absorption of two quanta as two photosystems are involved). This factor accounts for the presence of two photosystems, assuming equal involvement in linear electron flow (Schreiber et al. 1995).

$$ETR=\Delta F/F'_m\times E\times A\times 0.5 \ (\mu mol \text{ electrons } m^{-2} \ s^{-1}) \quad (2)$$

The absorptance (A) was determined according to Beer et al. (2000) as $A=1-E_t/E_o$, where E_o, the incident irradiance, was determined with a cosine corrected quantum sensor (Licor-192 SB, Li-Cor, Lincoln, USA), and E_t is the transmitted irradiance of the algal thallus. E_t was determined by placing pieces of the algae upon the light sensor.

To estimate parameters from the ETR curves analogous to those estimated from the photosynthesis vs. irra-

diance curves, a modification of the non-linear function of Jassby and Platt (1976) was employed:

$$ETR = ETR_{max} \tanh (ETR_{IS} E/ETR_{max}) \quad (3)$$

where ETR is the relative electron transport rate mentioned above, ETR_{max} is the saturated ETR, tanh is the hyperbolic tangent function, ETR_{IS} is the efficiency of the electron transport (initial slope of the ETR vs. irradiance curves) and E is the incident irradiance. The saturation irradiance for the electron transport (E_k) was also calculated as the intercept between ETR_{max} and the ETR_{IS} values.

For both *Gracilaria cornea* and *Falkenbergia rufolanosa*, the photosynthetic performances were monitored through the optimal quantum yield (F_v/F_m). In the case of *G. cornea*, the deterioration was visible after 15 days growing in outdoor conditions. At this time, a portion of the biomass was transferred indoors once more to assess recovery. The photosynthetic recovery of *G. cornea* was monitored by comparing the F_v/F_m in seaweeds maintained in outdoor tanks with that of seaweeds transferred from outdoor to indoor tanks and with that of seaweeds that were permanently indoors. *In situ* ETR curves were plotted to assess the effects on photosynthetic performances of transferring *G. cornea* from indoor to outdoor conditions. ETR vs. E curves were plotted for indoor algae, and subsequently 14 and 28 days after transfer to outdoors.

In the morning after the air compressor failure in the *Falkenbergia rufolanosa* cultivation, two sets of tanks with three stocking densities in each were prepared. One was maintained under full solar radiation, while the other was covered with neutral filters (grey nets) which filtered out about 70% of incident irradiance. The recovery of *F. rufolanosa* was monitored by comparing the F_v/F_m values at noon, 1 and 3 days after the air compressor failure. The effects of density and shading on the photosynthetic recovery of seaweeds were assessed using rapid-light curves (RLCs) obtained with a Diving-PAM, (Walz, Germany) through the day. RLCs were obtained from 5 replicates for each tank.

Chlorophyll and phycobiliprotein content

Samples of *Gracilaria cornea* for chlorophyll determination were taken at the same time as those used for photosynthetic measurements, and then stored in liquid nitrogen until analysis. Chlorophyll *a* (Chl *a*) was extracted following the methodology of Inskeep and Bloom (1985). Samples (1 mg fresh weight) of the apical portions of thalli were thawed at room temperature and incubated in 2.5 ml N,N-dimethylformamide (DMF) for 24 h at 4°C, in darkness. The chlorophyll *a* concentrations were determined in a Beckman DU-7 (Beckman España, Barcelona, Spain) spectrophotometer. Phycoerythrin and phycocyanin pigments were extracted from samples of 30–40 mg using 2.5 ml of 0.1 M phosphate buffer (pH=6.8) during 24 h at 4°C, in darkness. The extracts were centrifuged at 5000 *g* for 10 min and the supernatant was used for phycobiliprotein determination using the equations of Beer and Eshel (1985).

Statistics

Differences between light treatments (F_{irrad}) and time of day (F_{hour}) were assessed using two-way ANOVA (model I). When a significant effect was detected, one-way ANOVA and LSD Fisher test were used for comparisons of means, according to Sokal and Rohlf (1995). In order to achieve normality and to avoid correlation between means and variances, data were arcsine transformed. Normality and homogeneity of variances in the data before analysis were examined using Barlett and Chi-square tests, respectively.

Results

The light field in the greenhouse (indoor) for *Gracilaria cornea* cultures was very different from outdoor conditions. Indoor maximal averaged PAR was about 14% of the maximal PAR reached in outdoor conditions (Table 1). The plastic cover of the greenhouse completely screened out UV radiation. In the outdoor tanks, at 10 cm depth, PAR decreased by about 10%, UV-A by about 31% and UV-B by about 78%. The decrease in PAR irra-

Table 1 Maximal level of PAR (400–700 nm) expressed in μmol m^{-2} s^{-1}, UV-A (315–400 nm) and UV-B (280–315 nm), expressed in W m^{-2} and attenuation coefficient of PAR ($K_{d,PAR}$), expressed in m^{-1}, at noon in the tanks containing *Gracilaria cornea* at Taliarte (Gran Canaria, Spain) from 12 May 2001 to 3 July 2001, and in tanks containing *Falkenbergia rufolanosa* at Olhão (Southern Portugal) from 17 to 22 June 2002.

Algae	E_o (air)			$E_{UW\ (0.10\ m)}$			$K_{d,PAR}$ (m^{-1})
	PAR (μmol m^{-2} s^{-1})	UVA (Wm^{-2})	UVB (Wm^{-2})	PAR (μmol m^{-2} s^{-1})	UVA (Wm^{-2})	UVB (Wm^{-2})	
G. cornea							
Outdoors	1858±100	24±1.6	1.8±0.09	1672±79.2	16.5±1.2	0.4±0.02	0.99±0.05
Indoors	264±15.4	0	0	224±16.7	0	0	1.58±0.08
F. rufolanosa							
4 g l^{-1}	2112±44	41±2.0	2.8±0.1	264±15.4	2.3±0.08	0	20.89±1.2
6 g l^{-1}	2112±44	41±2.0	2.8±0.1	220±16.7	1.6±0.06	0	22.72±1.3
8 g l^{-1}	2112±44	41±2.0	2.8±0.1	110±5.7	0.3±0.01	0	29.65±1.9

In the case of *G. cornea*, the irradiance is presented in the air (E_o) and underwater at 0.1 m depth ($E_{UW\ (0.10\ m)}$) in the tanks where the algae were growing, both for outdoor and indoor conditions. For *F. rufolanosa*, the irradiances in the air (E_o) and underwater (at 0.10 m depth) in the tanks under different algal biomass densities (4, 6 and 8 g l^{-1}) are given as means±SD.

diance at 10 cm depth in the indoor tanks was about 15%. The attenuation coefficient was higher in the indoor than that in the outdoor tanks (Table 1).

In the case of *Falkenbergia rufolanosa*, the maximal averaged irradiance decreased drastically in the tanks (at 10 cm depth) due to the high algal biomass density. The decrease of PAR at 10 cm depth was about 87.5% at an algal density of 4 g l^{-1}, 89.5% at 6 g l^{-1} and 95% at 8 g l^{-1} (Table 1). The attenuation of UV-A radiation was 94% at 4 g l^{-1}, 96% at 6 g l^{-1} and 99% at 8 g l^{-1}. UV-B radiation at noon was fully attenuated at 10 cm depth, while there was complete darkness at 25 cm depth. The irradiance in the tanks covered by neutral filters was only about 30% of incident solar irradiance (data not shown). The vertical attenuation coefficient of PAR was much higher in tanks with *F. rufolanosa* than those with *G. cornea.*

F_v/F_m values of *Gracilaria cornea* growing indoors remained stable (about 0.65) during the study period (Figure 1). By contrast, F_v/F_m of algae transferred to outdoor conditions decreased sharply the following day to 0.2, recovering to around 80% of the initial values after 6 days. However, after 13 days, F_v/F_m values decreased consistently, coincident with the deterioration of thalli that started to fragment. At this stage, some biomass was transferred indoors, while the rest was maintained outdoors. Algae transferred to indoor conditions showed a gradual recovery within 4 days of F_v/F_m values, which were similar to those of the control. On the other hand, the F_v/F_m of algae growing outdoors decreased gradually to values close to 0.3 within 4 days, maintaining these values until the end of the experiment (Figure 1). Optimal quantum yields due to the light treatments and time were significantly different (ANOVA, $p<0.01$, $F_{(2,186)}=1642.5$, and $F_{(30,72)}=20.2$, respectively). These algae remained fragmented and bleached for the whole period. No recovery was observed at this stage upon transfer to indoor conditions (data not shown).

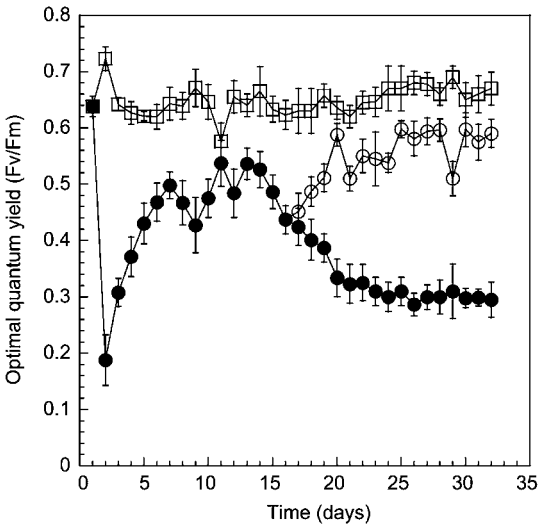

Figure 1 Optimal quantum yield (Fv/Fm) determined at 15:00 for the red alga *Gracilaria cornea* grown indoors (open squares) and transferred from indoor to outdoor (closed circles) conditions.
Open circles show the F_v/F_m recovery after transfer back to indoor conditions (on the 16th day).

The efficiency of *Gracilaria cornea* ETR (initial slope of ETR vs. irradiance) decreased when algae were transferred from indoor (0.29 μmol electrons/μmol photons) to outdoor conditions (0.16 μmol electrons/μmol photons), and continued to decrease to values of 0.06 μmol electrons/μmol photons after 4 weeks (Figure 2). Thus, after 4 weeks growing in outdoor conditions, when algae became fragmented, the efficiency of ETR decreased about 5-fold. Maximal ETR after two weeks outdoors was 357 μmol electrons m^{-2} s^{-1}, whereas after 4 weeks it was only 60 μmol electrons m^{-2} s^{-1} (Figure 2b,c).

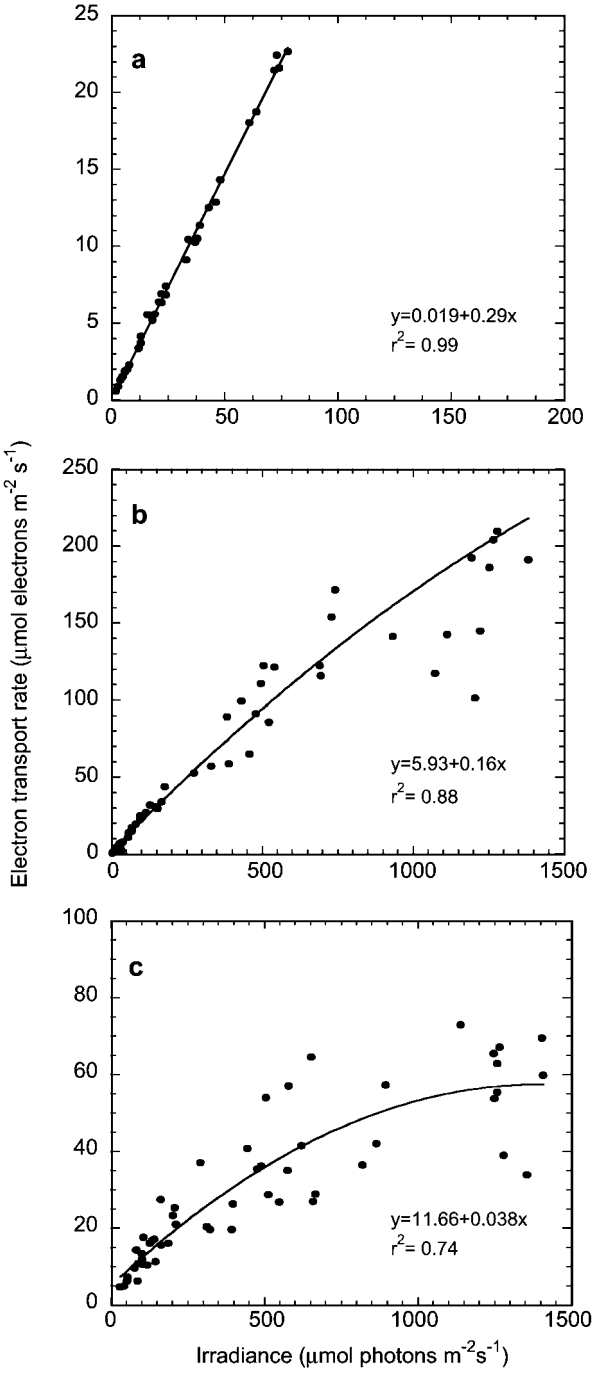

Figure 2 *In situ* electron transport rate (ETR) expressed as μmol electrons m^{-2} s^{-1} in thalli of *Gracilaria cornea* grown indoors (a) and 2 (b) and 4 (c) weeks after transfer from indoor to outdoor (full solar radiation) conditions.

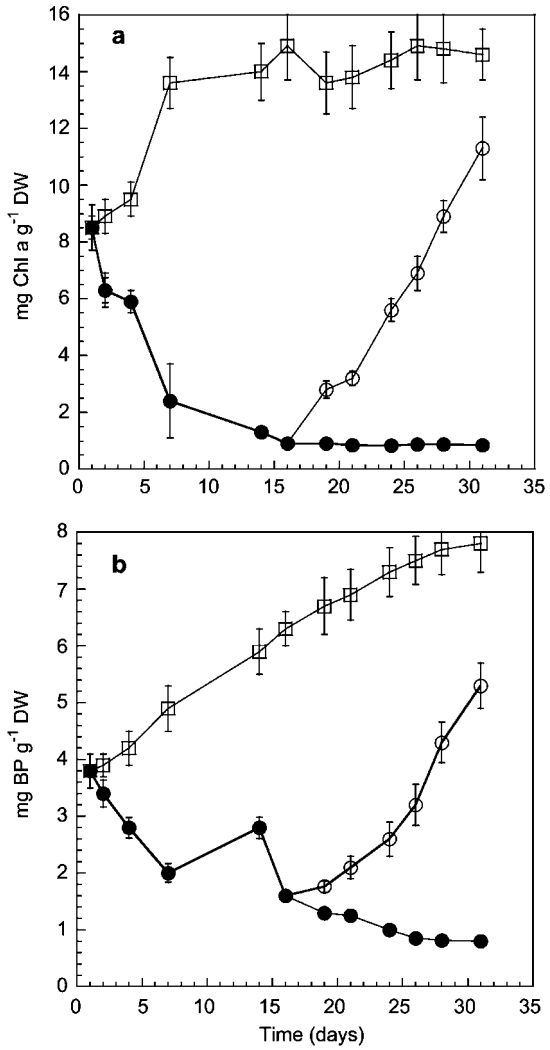

Figure 3 Chlorophyll (Chl *a*) (a) and biliproteins: phycoerythrin plus phycocyanin (b), expressed in mg g^{-1} DW in the red alga *Gracilaria cornea* grown indoors (open squares), and when transferred from indoor to outdoor (full circles) conditions.
Open circles show pigment recovery after transfer back to indoor conditions (on the 16th day).

Chlorophyll and biliprotein (phycoerythrin plus phycocyanin) concentrations were significantly ($p<0.01$, ANOVA, $F_{(2,72)}=2231.2$, and $F_{(2,72)}=2826.6$, respectively) higher in algae growing indoors compared to algae transferred from indoor to outdoor conditions (Figure 3). In addition, the effect of time was also significantly different for chlorophyll ($p<0.01$, ANOVA, $F_{(11,72)}=2231.2$) and biliproteins ($p<0.01$, ANOVA, $F_{(2,72)}=2836.6$). About 4 weeks after the transfer, chlorophyll and biliprotein concentrations became, respectively, 14- and 9-fold lower than those in algae growing indoors. After 16 days in outdoor conditions, a portion of the biomass was transferred indoors again (Figure 3), after which, the content of both chlorophyll and biliproteins increased. Fifteen days after transfer indoors, the content of chlorophyll was about 85% of the chlorophyll content of algae growing continuously indoors, whereas the biliprotein content reached only 66% of the values of the algae growing continuously indoors.

The deterioration of *Falkenbergia rufolanosa* cultivation conditions was evident in the strong decrease in the daily average of optimal quantum yield (F_v/F_m), particularly at the lowest cultivation density of 4 g l^{-1} (Figure 4). This decrease was significantly ($p<0.01$, ANOVA, $F_{(2,30)}=49.1$) lower within the tanks covered with neutral filters than within the tanks exposed to full solar radiation (Figure 4). F_v/F_m also varied significantly through the incubation time ($p<0.01$, ANOVA, $F_{(4,30)}=21.9$). The optimal quantum yield was lower at the lowest algal densities (4 g l^{-1}) than that at 6 and 8 g l^{-1}. Three days later, F_v/F_m recovered to values similar to those observed under pre-failure conditions.

The electron transport rate of *Falkenbergia rufolanosa* at 10:00 in the morning was lower than in the other time periods (11:30, 15:30, 17:30 and 19:00) under all density and shading conditions (Figure 5). Maximal ETR of *F. rufolanosa* showed a fast recovery between 10:00 and 11:30 in the morning under all experimental conditions, reflecting the recovery of the photosynthetic capacity after the stress event. At this hour, there was a decrease of maximal ETR at irradiances higher than 400 μmol photons m^{-2} s^{-1}, particularly at lower algal densities, suggesting the existence of photoinhibition. At a biomass density of 8 g l^{-1}, ETR did not decrease with the irradiance. In general, there was no effect of shading on either the shape of ETR/E curves or on recovery.

Discussion

The results presented in this study reveal that the use of chlorophyll fluorescence is a powerful way to detect rapidly different stress situations in integrated cultivation of red macroalgae using fishpond effluents. In particular, the

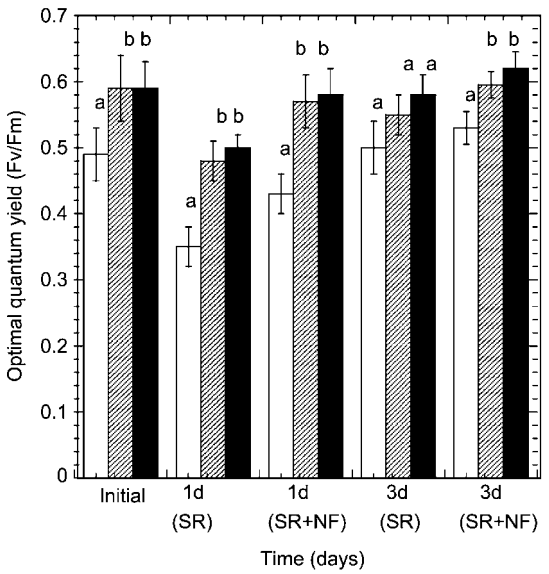

Figure 4 Mean daily optimal quantum yield (Fv/Fm) of *Falkenbergia rufolanosa* before compressor failure (initial time) and after one (1d) and three days (3d).
The algae were then exposed to full solar radiation (SR) or shaded by neutral filters (SR+F), which removed about 70% of incident light. Three different algal biomass densities were tested: 4 g l^{-1} (open bars), 6 g l^{-1} (hatched bars) and 8 g l^{-1} (black bars). The results are expressed as means±SD, similar letters connecting homogeneous groups.

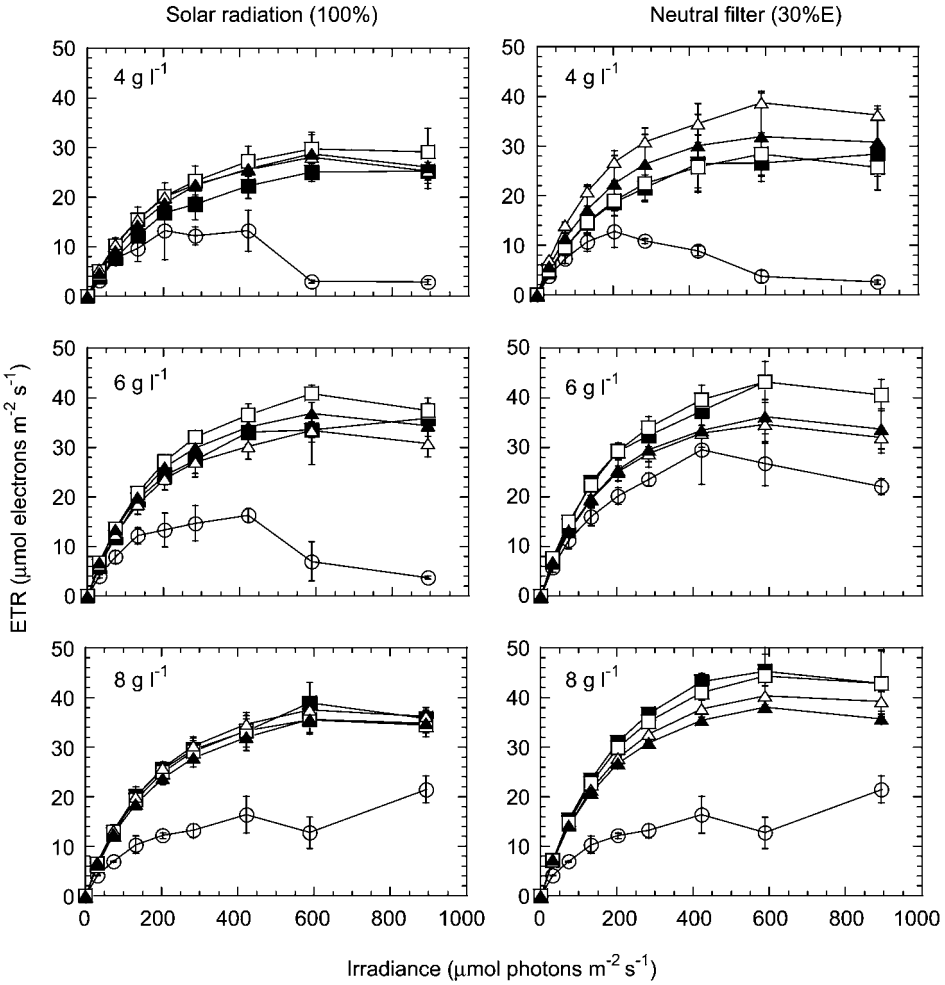

Figure 5 Electron transport rate (ETR) expressed in µmol electrons m^{-2} s^{-1} under different irradiances provided by the halogen lamp of Diving-PAM, on 18 June 2002, immediately following the last night without aeration: 10:00 (open circles), 11:30 (closed squares), 15:30 (open squares), 17:30 (open triangles) and 19:00 (closed triangles).

optimal quantum yield (F_v/F_m), which is easily and rapidly obtained, can be regularly monitored as an early warning of physiological stress. Both the transfer of *Gracilaria cornea* from indoor to outdoor tanks and the aeration failure in the *Falkenbergia rufolanosa* cultivation system caused very rapid decreases of F_v/F_m. In both cases, recovery of the photosynthetic condition of the cultures was also clearly revealed by the F_v/F_m measurements.

In the case of *Gracilaria cornea*, the decrease of F_v/F_m in the algae transferred to full solar radiation indicated that photoinhibition occurred. In fact, this clone of *G. cornea* had been cultivated in greenhouse conditions for about 10 years under irradiances 7 times lower than those under outdoor conditions (Table 1). The lowest F_v/F_m value observed one day after the transfer indicates a strong instantaneous photoinhibition. *G. cornea* was able to acclimate to full solar radiation, as shown by the gradual increase in F_v/F_m through the first two weeks. However, this was a temporary phase as a new sharp decrease occurred afterwards. This was coincident with the degradation and fragmentation of the seaweed thalli, suggesting that necrosis was caused by further physiological degradation. The physiological degradation must have involved rupture of the reaction centres of photosystem II, as indicated by the continuous decline in the Chl *a* content (Figure 3), which started as soon as the algae were transferred outdoors. Thallus degradation under outdoor conditions was coincident with the decrease in levels of photosynthetic pigments.

Transfers of algae from indoor conditions or laboratory cultures to outdoors, or upscaling of outdoor cultures are common problem areas in seaweed aquaculture. Sudden transfer of low irradiance-acclimated algae to high irradiances can be fatal, as was also shown for *Chaetoceros brevis* Schütt (Van de Poll et al. 2005) and for *Gracilaria chilensis* transferred from the benthos, i.e., shade-acclimated algae, to the surface of an estuarine system (Gómez et al. 2005). Thus, in order to avoid fatal photodegradation, sequential transfer to increasing irradiances is necessary. This can be done by using neutral filters or by maintaining the algae in high density cultures. The F_v/F_m ratio is a rapid and easy measure that can be used to monitor photosynthetic adaptation during the sequential transfer.

When *Gracilaria cornea* thalli were transferred back to indoor conditions, the concentration of photosynthetic pigments and the F_v/F_m increased to initial values. This recovery was observed only for algae transferred before F_v/F_m values reached about 0.3–0.4. Thus, in the culture system analysed, regular measurements of F_v/F_m could

be used as an early warning indicator of the critical values of 0.3–0.4, and consequent failure of the culture.

The F_v/F_m ratio was also useful in detecting *Falkenbergia rufolanosa* physiological stress caused by a period of three consecutive nights without aeration. For *F. rufolanosa*, a rapid recovery during the day following the last aeration failure was observed. The decrease of F_v/F_m was steeper and the recovery slower in tanks under full solar radiation than in tanks covered with neutral filters. This may be related to the irradiance stress in the cultures. It is likely that increasing the light attenuation coefficient of the water column, either by applying neutral filters or by increasing the biomass within the culture tanks, promotes self-shading and decreases photoinhibition levels. *F. rufolanosa* cultivated in higher densities of 6 and 8 g l^{-1}, had higher F_v/F_m (Figure 4) and ETR values (Figure 5) than those cultivated at 4 g l^{-1}.

Falkenbergia rufolanosa cultures are very resistant to high irradiance since only a slight photoinhibition (decrease of F_v/F_m) occurred at noon, while in other macroalgal cultures daily photoinhibition fluctuations have been observed (Aguirre-von Wobeser et al. 2000, Cabello-Pasini et al. 2000, Altamirano et al. 2004). The low photoinhibition and rapid recovery of photosynthesis in tank cultivated *Falkenbergia rufolanosa* could be explained by the protection promoted by N-replete conditions in fishpond effluents. Nutrient availability has a pronounced effect on pigment composition and photosynthetic performance, and consequently may affect light sensitivity of algae (Geider et al. 1993). Nutrient limitation has been associated with increased vulnerability to photoinhibition by PAR and UV in microalgae (Litchman et al. 2002, Shelly et al. 2002) and macroalgae (Döhler et al. 1995, Korbee Peinado et al. 2004, 2005).

The decrease of photosynthetic activity in *Falkenbergia rufolanosa* after three overnight periods without aeration may be due to nutrient deficiency and/or hypoxia. Aeration inside the tanks is essential to avoid clumping of thalli on the bottom and to promote mixing of the water column. Available data on the oxygen concentration indicates that there was hypoxia during the three night periods that preceded the chlorophyll fluorescence measurements (data not shown).

The density range was not large enough to detect differences in the photosynthetic rate. The irradiance decrease with density is not high (Table 1). In fact, this was only observed at very low densities of 1.5 g l^{-1} (Mata et al. 2006).

Acknowledgements

This work was financed by the UE research project SEAPURA Q5RS-2000-31334, the Spanish Ministry of Science and Technology (Project AGL2005 02655) and Junta de Andalucía (Research group RNM-295). We thank Dr. Jorge Santinha, director of the fish farm Aquamarim, for supporting this research.

References

Aguirre-von Wobeser, E., F.L. Figueroa and A. Cabello-Pasini. 2000. Effect of UV radiation on photoinhibition of marine macrophytes in culture systems. *J. Appl. Phycol. 12*: 159–168.

Altamirano, M., A. Murakami and H. Kawai. 2004. High light stress in the kelp *Ecklonia cava*. *Aquat. Bot. 79*: 125–135.

Beer, S. and A. Eshel. 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous extracts of red algae. *Aust. J. Mar. Freshw. Res. 36*: 785–792.

Beer, S., C. Larsson, O. Poryan and L. Axelsson. 2000. Photosynthetic rates of *Ulva* (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. *Eur. J. Phycol. 35*: 69–74.

Björn, L.O., A. Cunningham, Z. Dubinsky, M. Estrada, F.L. Figueroa, F. García Pichel, D.-P. Häder, D. Hanelt, G. Levavasseur and K. Lüning. 1996. Technical discussion I. Underwater light measurement and light absorption by algae. *Scientia Marina 60*: 293–297.

Büchel, C. and C. Wilhelm. 1993. *In vivo* analysis of slow chlorophyll fluorescence induction kinetics in algae: progress, problems and perspectives. *Photochem. Photobiol. 58*: 137–148.

Cabello-Pasini, A., A. Aguirre-von Wobeser and F.L. Figueroa. 2000. Photoinhibition of photosynthesis in *Macrocystis pyrifera* (Phaeophyta), *Chondrus crispus* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) in outdoor culture systems. *J. Photochem. Photobiol. 57*: 169–178.

Döhler, G., E. Hagmeir and C. David. 1995. Effects of solar and artificial UV radiation on pigment and assimilation of ^{15}N ammonium and ^{15}N nitrate by macroalgae. *J. Photochem. Photobiol. B 30*: 179–187.

Figueroa, F.L. and I. Gómez. 2001. Photosynthetic acclimation to solar UV radiation of marine red algae from the warm-temperate coast of southern Spain: a review. *J. Appl. Phycol. 13*: 235–248.

Figueroa, F.L., R. Conde-Álvarez and I. Gómez. 2003. Relations between electron transport rates determined by pulse amplitude modulated chlorophyll fluorescence and oxygen evolution in macroalgae under different light conditions. *Photosynth. Res. 75*: 259–275.

Flameling, I.A. and J. Kromkamp. 1998. Light dependence of quantum yields for PSII charge separation and oxygen evolution in eucaryotic algae. *Limnol. Oceanogr. 43*: 284–297.

Franklin, L.A. and R.M. Forster. 1997. The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *Eur. J. Phycol. 32*: 207–232.

Geider, R.J., J. LaRoche, R.M. Greene and M. Olaizola.1993. Response of photosynthetic apparatus of *Phaeodactylum tricornutum* (Bacillariophyceae) to nitrate, phosphate, or iron starvation. *J. Phycol. 29*: 755–766.

Genty, B., J. Briantais and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta 990*: 87–92.

Gómez, I. and F.L. Figueroa. 1998. Effects of solar UV stress on chlorophyll fluorescence kinetics of intertidal macroalgae from southern Spain: a case study in *Gelidium* species. *J. Appl. Phycol. 10*: 285–294.

Gómez, I., F.L. Figueroa, I. Sousa-Pinto, B. Viñegla, E. Pérez-Rodríguez, C. Maestre, S. Coelho, A. Felga and R. Pereira. 2001. Effects of UV radiation and temperature on photosynthesis measured by PAM fluorescent in the red alga *Gelidium pulchellum* (Turner) Kützing. *Bot. Mar. 44*: 9–16.

Gómez, I., F.L. Figueroa, P. Huovinen, N. Ulloa, V. Morales and S. Hess. 2005. Photosynthesis of the red alga *Gracilaria chilensis* under natural solar radiation in an estuary in southern Chile. *Aquaculture 244*: 369–382.

Häder, D.-P. and F.L. Figueroa. 1997. Photoecophysiology of marine macroalgae. *Photochem. Photobiol. 66*: 1–14.

Hanelt, D. and W. Nultsch. 1995. Field studies on photoinhibition show no correlation between oxygen and fluorescence measurements in the Arctic red alga *Palmaria palmata*. *J. Plant Physiol. 145*: 31–38.

Hanelt, D., C. Wiencke and W. Nultsch. 1997. Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. *Mar. Ecol. Progr. Ser. 149*: 255–266.

Inskeep, W.P and P.R. Bloom. 1985. Extinction coefficients of chlorophyll *a* and *b* in N,N-dimethylformamide and 80% acetone. *Plant Physiol. 77*: 483–485.

Jassby, A.D. and T. Platt. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr. 21*: 540–547.

Kirk, J.T.O. 1994. Light and photosynthesis in aquatic ecosystems. CUP, Cambridge. pp. 509.

Korbee Peinado, N., R. Abdala-Díaz, F.L. Figueroa and E.W. Helbling. 2004. Ammonium and UVR stimulate the accumulation of mycosporine-like amino acids (MAAs) in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. *J. Phycol. 40*: 248–259.

Korbee Peinado, N., P. Huovinen, F.L. Figueroa and J. Aguilera 2005. Availability of ammonium influences photosynthesis and the accumulation of mycosporine-like amino acids in two *Porphyra* species. *Mar. Biol. 146*: 645–654.

Litchman, E., P.J. Neale and A.T. Banaszak. 2002. Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates. Photoprotection and repair. *Limnol. Oceanogr. 47*: 86–94.

Mata, L., J. Silva, A. Schuenhoff and R. Santos. 2006. The effects of light and temperature on the photosynthesis of the *Asparagopsis armata* tetrasporophyte (*Falkenbergia rufolanosa*), cultivated in tanks. *Aquaculture 252*: 12–19.

Schreiber, U., U. Schliwa and W. Bilger. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res. 10*: 51–62.

Schreiber, U., T. Endo, H. Mi and K. Asada. 1995. Quenching analysis of chlorophyll fluorescence by saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. *Plant Cell Physiol. 36*: 873–882.

Schuenhoff, A., L. Mata and R. Santos. 2006. The tetrasporophyte of *Asparagopsis armata* as a novel seaweed biofilter. *Aquaculture 252*: 3–11.

Shelly, K., P. Heraud and J. Beardall. 2002. Nitrogen limitation in *Dunaliella tertiolecta* (Chlorophyceae) leads to increased susceptibility to damage by UV-B radiation but also increased repair capacity. *J. Phycol. 38*: 713–720.

Silva, J., R. Santos, J. Serôdio and R.A. Melo. 1998. Light response curves *for Gelidium sesquipedale* from different depths, determined by two methods: O_2 evolution and chlorophyll fluorescence. *J. App. Phycol. 10*: 295–301.

Sokal, R.R. and F.J. Rohlf. 1995. Introducción a la bioestadística. Editorial Reverté. Barcelona. pp. 362.

Van de Poll, W.H., M.A. von Leeuwe, J. Roggeveld and A.G.J. Buma. 2005. Nutrient limitation and high irradiance acclimation reduce PAR and UV-induced viability loss in the Antarctic diatom *Chaetoceros brevis* (Bacillariophyceae). *J. Phycol. 41*: 840–850.

Villafañe, V.E., K. Sundbäck, F.L. Figueroa and E.W. Helbling 2003. Photosynthesis in the aquatic environment as affected by UVR. *In*: (E.W. Helbling and H. Zagarese, eds) *Aquatic organisms and ecosystems.* Comprehensive series in photochemical and photobiological sciences. RSC Press, Cambridge. pp. 357–397.

Young, E.B. and J. Beardall. 2003. Rapid ammonium- and nitrate-induced perturbations to Chl *a* fluorescence in nitrogen-stressed *Dunaliella tertiolecta* (Chlorophyta). *J. Phycol. 39*: 332–342.

Received 30 November, 2005; accepted 20 May, 2006