Photoinhibition of photosynthesis in *Macrocystis pyrifera* (Phaeophyceae), *Chondrus crispus* (Rhodophyceae) and *Ulva lactuca* (Chlorophyceae) in outdoor culture systems

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Abstract

The effect of solar radiation on photosynthesis and chlorophyll fluorescence associated to photosystem II (PS II) was determined in the Phaeophyta *Macrocystis pyrifera*, the Rhodophyta *Chondrus crispus* and the Chlorophyta *Ulva lactuca* by oxygen evolution and pulse-amplitude-modulated (PAM) fluorescence. The algae were maintained in 1.2 m³ outdoor tanks with constant aeration and at 8, 26 and 100% incident irradiance (E₀). All three species showed a decrease in ΔF/F₀ values during solar noon compared to values in the morning and afternoon, suggesting a photoinhibition of photosynthesis. In general, photoinhibition was negatively correlated to increasing daily irradiance in all three species. Photoinhibition in *C. crispus* occurred in tissue incubated at 8, 26 and 100% E₀, while in *M. pyrifera* and *U. lactuca* a decrease in ΔF/F₀ values was only observed in tissue incubated at 100% E₀. This suggests that species that naturally grow at greater depths might be more susceptible to excessive light when cultured in shallow waters compared to species that naturally inhabit shallower depths. In *M. pyrifera*, ΔF/F₀ values were lower in the afternoon than those in the morning, suggesting slower repair mechanisms of the photosystem II compared to the other species. The results suggest that photoinhibition could be reduced by reducing incident irradiance to culture systems or increasing of biomass to promote self-shading. Gross oxygenic photosynthesis increased linearly at low electron transport rates after which it saturated in all three species. This suggests that chlorophyll fluorescence could be used as an indicator of the physiological status of macroalgae maintained in dense aquaculture systems. © 2000 Elsevier Science S.A. All rights reserved.

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1. Introduction

Spatial distribution and seasonal development of marine macroalgae is determined to a high degree by solar radiation. However, unlike some phytoplankton, marine macroalgae are attached to hard substrates and are unable to move to optimum photosynthetic light levels in the water column. Furthermore, it has been demonstrated that algal photosynthesis is light limited at low irradiances or stressed when algae are exposed to high light levels [1,2]. Marine macroalgae that are acclimated to low irradiance and are exposed to high incident irradiances levels may be subjected to serious light stress, which might decrease productivity [3]. Dynamic photoinhibition has been proposed as a mechanism that protects the photosynthetic apparatus by impairing photosystem II (PS II) so that an excess of absorbed energy is dumped through fluorescence and heat [4]. Such dynamic photoinhibition is often followed by a recovery of photosynthesis when algal tissue is incubated at low irradiances after an exposure to an excess of incident irradiation [5]. Chronic photoinhibition, however, generally damages reaction centers of the photosynthetic apparatus and reduces photosynthesis for longer periods of time [6].

Photoinhibition in marine algae is generally determined by measurements of oxygen evolution and/or pulse-amplitude-modulated (PAM) fluorescence [2,3,7]. In the last decade, chlorophyll fluorescence in algae from the Arctic, Antarctic, North sea, Chinese sea, Mediterranean, Atlantic and tropical waters has been reported [8–11]. Both maximal and effective quantum yield have been used as
indicators of photosynthesis and photoinhibition [12]. Some of these studies propose that chlorophyll fluorescence can be a reliable indicator of the primary light reactions, thylakoid electron transport reactions, dark-enzymatic reactions of the stroma and slow regulatory feedback processes of photosynthesis. Furthermore, studies have revealed a strong correlation between oxygen evolution and PAM fluorescence; however, under conditions of nutrient depletion and treatments under different light quality spectra little or no correlation has been found between these two methods [13]. The evaluation of chlorophyll fluorescence in algae through PAM fluorometry provides the opportunity to evaluate the photosynthetic performance rapidly without having to transport the seaweeds to the laboratory, reducing stress factors such as changes in temperature and irradiance [5]. Thus, a positive correlation between oxygen evolution and chlorophyll fluorescence could prove an efficient method for examining photosynthetic potential of algae under culture conditions. As a consequence, the investigation of the relationship between oxygen evolution and effective quantum yield of the PS II is critical to validate primary productivity measurements in cultured algae using fluorimetric techniques.

Subtidal macroalgae may show a lower tolerance to high irradiance and UV radiation than those living under high light level regimes. This may have detrimental effects if subtidal algae are transplanted to shallow water columns where the algae are exposed to high photosynthetic photon flux (PPF) as well as UV radiation [14–16]. Oxygenic photosynthesis and chlorophyll fluorescence patterns have been shown to differ between sun and shade adapted seaweeds as a result of elevated PPF levels. These studies demonstrate that the recovery of photosynthetic quantum yield is retarded in shade plants when transplanted to shallow waters [5].

Susceptibility of macroalgae to photoinhibition has generally been studied by either fluorescence methods or oxygen evolution methods, however, there are few studies that correlate oxygen evolution to fluorescence as a result of increasing irradiance [2,7]. Furthermore, there are few studies that evaluate the possible use of fluorimetric methods for estimating the photosynthetic performance of seaweeds in culture systems. As a consequence, the main objective of this study was to evaluate the effect of increasing irradiance on oxygen evolution and chlorophyll fluorescence in the Phaeophyta Macrocystis pyrifera, the Rhodophyta Chondrus crispus and the Chlorophyta Ulva lactuca in culture systems.

2. Material and methods

2.1. Plant material and culture conditions

Canopy blades Macrocystis pyrifera (<1 m depth) were collected at Campo Kennedy, Baja California, México and immediately transported (40 km) in an ice cooler (approximately 15°C) to the laboratory facility at Ensenada, Baja California. Chondrus crispus originally collected from the east coast of the US and Ulva lactuca from Northwestern Baja California were obtained from dense (>12 kg m⁻³) experimental cultures maintained for more than 1 year at 17°C and natural irradiance levels at the Ensenada facility. A mixture of 6 kg FW of C. crispus, 100 g of U. lactuca and 10 blades of M. pyrifera (without pneumatocysts) were added to each of three 1200 l outdoor tanks. The enriched seawater (250 μM NO₃) of the culture system was kept at 17°C and the PPF of individual tanks was adjusted to 8, 26 and 100% incident irradiance (Eᵣ) by using layers of black screen mesh. The reduction of PPF of was determined by measuring the irradiance levels above and below the screen mesh in each tank with a 4π quantum sensor ( Biospherical Instruments). Seaweeds were kept in suspension by constant aeration and circulated to the top of the tanks approximately every 15 s. While the biomass of C. crispus in each tank was greater than the other two species, individuals from all three species had similar exposure time to the surface of the culture system. The algae were acclimated for 3 days at the different light conditions before the measurements were conducted.

Incident photosynthetically active radiation (PAR) above three culture tanks was monitored every minute throughout the experiment with a LI-190SA 2π quantum sensor attached to a LI-COR data logger. The attenuation coefficient (K) in the 100% Eᵣ tank was determined at minute intervals by measuring PAR subsuperficially and at 0.5 m depth throughout the experiment with LI-193SA spherical quantum sensors and a LI-COR data logger. The similar biomass density provided equal K values in all three tanks.

2.2. Fluorescence measurements

Diurnal fluctuations of in vivo fluorescence in M. pyrifera, C. crispus and U. lactuca exposed to 8, 26 and 100% Eᵣ were evaluated with a pulse amplitude modulated (PAM) fluorometer (PAM 2000; Waltz, Effeltrich, Germany) as described by Schreiber and Neubauer [17]. After 3 days in the culture tanks, individuals from C. crispus and U. lactuca and disks (15 mm) of M. pyrifera were collected from the culture tanks from dusk to dawn at 1 h intervals. The algae were transported in a black container with seawater (17°C) to the laboratory nearby and chlorophyll fluorescence was determined within 2 min of collection. It has been demonstrated that effective quantum yield values do not vary within 10 min of the collection of the algae from the field [18]. Tissue from approximately the middle of the thallus of C. crispus and U. lactuca and the middle of the blade of M. pyrifera was placed horizontally in a seawater cuvette with a cooling jacket (Walz, Germany) and fastened at the end of the fiber optics of the fluorometer. Effective quantum yield was determined in the algae submerged in seawater under white fluorescent light (Osram DL, 20 W) at an irradiance of 50 μmol
quanta m\(^{-2}\) s\(^{-1}\). The algae were placed at a 45\(^\circ\) angle and the fiber optic of the fluorometer was placed at a distance of 2 mm. Effective quantum yield was calculated as \(\Delta F/F_m^\prime\) where \(\Delta F=F_m^\prime-F_m\), \(F_m^\prime\) is the maximal fluorescence and \(F_m\) is the basal steady-state fluorescence. Values of \(F_m^\prime\) in \(M.\ pyrifera\) and \(U.\ lactuca\) were determined at a very low intensity pulse of red light (650 nm, 0.3 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\)), while \(F_m^\prime\) was induced with a saturating white light pulse (0.4 s, approximately 9000 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\)). Measurements were conducted on a minimum of six individuals. Fluorescence emission in red algae is complicated by the presence of phycobilines where the onset of maximal fluorescence after a saturating light pulse occurs very quickly, and is associated with the excitation of PS I and delay of the \(F_m^\prime\) decline. As a consequence, \(Chondrus\ crispus\) samples were submitted to a 5 s low irradiance (30 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\) far-red pulse, followed by a 5 min darkness period. The stabilization of the fluorescence signal was assured by applying a short red actinic pulse (5 s; 655 nm; 8 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\)), improving the measurement of \(\Delta F/F_m^\prime\) [19]. Finally, \(F_m\) and \(F_m^\prime\) were evaluated as described above.

The apparent photosynthetic electron transport rate (ETR) was determined in \(M.\ pyrifera\), \(C.\ crispus\) and \(U.\ lactuca\) under 8, 26 and 100\% \(E_o\) after 3 days in the culture tanks. Values of ETR were estimated in the three species by relating the chlorophyll fluorescence emission and the intensity of the actinic irradiance (red-light-emitting diode, LED). Tissue samples were placed horizontally in a seawater cuvette with a cooling jacket (Walz, Germany) and fastened at the end of the fiber optics of the fluorometer at a distance of 2 mm. Samples \((n=6)\) were exposed to 11 steps of increasing irradiances (0–400 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\) at 30 s intervals). Electron transport rates were calculated at each irradiance using the following equation:

\[
ETR = \Delta F/F_m^\prime \cdot E_{LED} \cdot A \cdot 0.5
\]

where \(E_{LED}\) is the irradiance of the actinic light; \(A\) is the thallus absorbance (fraction of the light effectively absorbed); and 0.5 is the correction factor due to the fact that the transport of one electron requires the absorption of two quanta by PSI and PSII, respectively [17]. Average thallus absorbance within the PAR range was determined by covering a 2\(\pi\) quantum sensor (LI-190SA, LI-COR) with one layer of seaweed tissue used in the fluorimetric assays and subtracting the transmittance of natural irradiance through the tissue from 1. Maximum fluorescence and initial slope of the irradiance vs. ETR curves were determined by a non-linear direct fitting algorithm (Sigma Plot, Jandel Scientific) of the data to the exponential equation described by Webb et al. [20].

### 2.4. Pigment analysis

Chlorophyll \(a\) levels were determined in the thallus of \(M.\ pyrifera\), \(C.\ crispus\) and \(U.\ lactuca\) exposed for 3 days to 8, 26 and 100\% \(E_o\) in the morning, noon and afternoon to evaluate diurnal fluctuations in pigment levels. Pigments were extracted by disrupting the tissue (approximately 0.2 g FW) with a glass tissue homogenizer and 90% \((v/v)\) acetone. The extracts were centrifuged at 2000 \(\times\) g for 15 min and the concentration of chlorophyll \(a\) in the three species was determined by spectrophotometry using the equations of Jeffrey and Humphrey [21].

### 2.5. Statistical analysis

Significance of daily variations in fluorescence and oxygenic photosynthesis parameters was determined by one-way analysis of variance (ANOVA) after testing for normality and homoscedasticity of the data [22]. Tukey’s
tests were conducted to evaluate statistical differences between samples at specific sampling periods.

3. Results

Daily incident PAR in the culture tanks reached values of almost 2000 µmol quanta m\(^{-2}\) s\(^{-1}\) throughout the experimental period (Fig. 1a). There was a sharp irradiance increase in the morning and decrease in the afternoon as a result of shadowing of the wall tanks of the culture system. The values of \(K\) in the 100% \(E_o\) culture tank fluctuated from low values in the morning and noon to high values in the afternoon with mean values of approximately 3.5 m\(^{-1}\) (Fig. 1b). The increase of \(K\) values in the afternoon was probably the result of changes in the angle of incidence of the sunlight and shadowing of the culture tank walls. The increase in \(K\) values suggests much lower subsurface irradiances in the culture tanks during the afternoon.

There was a daily cycle of \(\Delta F/F_m\) in the three species studied, with greatest values in the morning and afternoon and minimum values at solar noon (Fig. 2). In \(M. pyrifera\), \(\Delta F/F_m\) decreased significantly \((P<0.05)\) at midday in tissue exposed to 100% \(E_o\), however, values did not decrease in tissue exposed to 8 or 26% \(E_o\) throughout the day. Maximum rates of oxygen evolution in \(M. pyrifera\) incubated at 100% \(E_o\) followed a similar pattern to that of \(\Delta F/F_m\), maximum values were observed in the morning decreasing at noon and increasing in the afternoon, however, afternoon values did not reach \(P_{\text{max}}\) rates observed in the morning. Values of \(P_{\text{max}}\) in \(M. pyrifera\), \(C. crispus\) and \(U. lactuca\) at 8 and 26% \(E_o\) did not fluctuate throughout the day (data not shown). In \(C. crispus\), there was a general and significant \((P<0.05)\) decrease of \(\Delta F/F_m\) values in individuals exposed to 8, 26 and 100% \(E_o\). Values of \(\Delta F/F_m\) in individuals exposed to 8 and 26% \(E_o\), however, were approximately 35% greater than tissue exposed to 100% \(E_o\). Similarly to \(M. pyrifera\), \(P_{\text{max}}\) values in \(C. crispus\) incubated at 100% \(E_o\) were low at noon, and maximum in the morning and afternoon, following a similar pattern to that of \(\Delta F/F_m\). Oxygen evolution and \(\Delta F/F_m\) values in \(U. lactuca\) only decreased significantly at midday in tissue exposed to 100% \(E_o\). In contrast to \(M. pyrifera\), \(\Delta F/F_m\) values in \(C. crispus\) and \(U. lactuca\) incubated at 100% \(E_o\) did not reach values observed in
tissue incubated at 8 and 26% $E_o$ during the morning and afternoon.

Values of $\Delta F/F'_m$ for $M. pyrifera$, $C. crispus$ and $U. lactuca$ determined through the day were plotted against the surface irradiance of the 8, 26 and 100% $E_o$ tanks at which the individuals were exposed just prior to the sampling period. The values of $\Delta F/F'_m$ showed a negative correlation ($P<0.05$) with respect to incubation irradiance in the three species studied (Fig. 3). In $M. pyrifera$, $\Delta F/F'_m$ values decreased as incubation irradiance increased above 500 μmol quanta m$^{-2}$ s$^{-1}$ in the culture tanks. In $M. pyrifera$, $\Delta F/F'_m$ values in the afternoon were significantly ($P<0.05$) lower than those in the morning, suggesting slower PS II repair mechanism compared to the other species. Values of $\Delta F/F'_m$ in $C. crispus$ and $U. lactuca$ also decreased as irradiance increased above 400 μmol quanta m$^{-2}$ s$^{-1}$, however, there were no differences between morning and afternoon values.

Simultaneous calibration of ETR vs. oxygenic photosynthesis showed a positive correlation at low ETR values in $M. pyrifera$, $C. crispus$ and $U. lactuca$ (Fig. 4). Gross oxygenic photosynthesis showed an initial linear increase with an increase of $\Delta F/F'_m$ in the three species studied.

Fig. 3. Values of $\Delta F/F'_m$ for $Macrocystis pyrifera$, $Chondrus crispus$ and $Ulva lactuca$ as a function of incubation irradiance. Open symbols (X±S.D., n=6) represent samples taken in the morning and closed symbols (X±S.D., n=6) represent samples taken in the afternoon.

Fig. 4. Relationship of electron transport rate vs. oxygen evolution assayed simultaneously for $Macrocystis pyrifera$, $Chondrus crispus$ and $Ulva lactuca$ exposed to 8, 26 and 100% incident irradiance.

Oxygenic photosynthesis, however, was saturated at approximately 20 ETR units, which is consistent with the decrease of $\Delta F/F'_m$ observed in the cultured specimens at high irradiances.

Oxygen evolution and chlorophyll fluorescence responses to increasing irradiances were determined independently in $M. pyrifera$, $C. crispus$ and $U. lactuca$. The photosynthetic response to irradiance levels showed a different response between the oxygen evolution and fluorescence methods in the three species (Fig. 5). Oxygenic photosynthesis was saturated at approximately 200 μmol m$^{-2}$ s$^{-1}$ in the three species while ETR was not saturated at 450 μmol quanta m$^{-2}$ s$^{-1}$ in the species studied. Maximum oxygen evolution rates were significantly greater ($P<0.05$) in $U. lactuca$ than in $M. pyrifera$ and $C. crispus$, however, such difference was not observed in the maximum ETR values. Furthermore, maximum oxygen evolution rates were significantly greater ($P<0.05$) in tissue incubated at 100% $E_o$, however, no clear pattern was observed in maximum values of ETR.

Maximum values of $P_{max}$ followed a different pattern to that of ETR values in $M. pyrifera$, $C. crispus$ and $U. lactuca$ (Fig. 6). Values of $P_{max}$ in $U. lactuca$ were 2- to 5-fold greater ($P<0.05$) than those observed in $M. pyrifera$ and $C. crispus$. In general, $P_{max}$ values were slightly
greater in tissue incubated at 100% $E_o$ than at 8 and 26% $E_o$. In *M. pyrifera* and *C. crispus*, $ETR_{max}$ values were slightly greater in individuals incubated at 100% $E_o$ than at 26% $E_o$. However, values of $ETR_{max}$ in individuals incubated at 8% $E_o$ could not be calculated due to the lack of saturation of the ETR vs. irradiance curves. There was also a variation in $\alpha$ values among species and among irradiance treatments. In general, values of $\alpha$ were greatest in *U. lactuca* and lowest in *M. pyrifera* (Fig. 7). In *M. pyrifera* and *C. crispus*, there was no significant difference in $\alpha$ values from individuals incubated at 8, 26 and 100% $E_o$, however, $\alpha$ values in *U. lactuca* were greater in tissue incubated at 8 and 26% $E_o$ relative to individuals incubated at 100% $E_o$. In contrast to the oxygenic photosynthetic efficiency, $\alpha$ETR values were slightly lower in *U. lactuca* and there were no significant differences in tissue incubated at different irradiances.

Chlorophyll $a$ levels in *C. crispus* were slightly lower at noon with respect to morning and afternoon levels, while there was no clear pattern of pigment fluctuation in *M. pyrifera* and *U. lactuca* (Fig. 8). Levels of chlorophyll $a$ in *U. lactuca* were 2- to 4-fold greater ($P<0.05$) than those determined in *C. crispus* and *M. pyrifera*. In general, chlorophyll levels were significantly greater ($P<0.05$) in
Marine algae are often cultured under shallow water columns thus increasing the irradiance levels at which they grow under natural conditions [14–16]. Photoinhibitory processes dissipate such excess of absorbed energy through fluorescence and heat, and functions as a protective mechanism for the photosynthetic apparatus. In marine algae and vascular plants, it is generally accepted that the $\Delta F/F^m$ values of the photosynthetic apparatus decrease as a result of high irradiances resulting in photoinhibition [5,7]. Photoinhibition here appears to increase at approximately 400–500 $\mu$mol quanta m$^{-2}$ s$^{-1}$ for the three species assayed under the culture conditions. Such decrease in $\Delta F/F^m$ values could be accompanied by a decrease in photosynthetic rates as observed in other species [7]. Assuming a daily average $K$ value of 3.5 m$^{-1}$ in the culture tanks and a maximum threshold for irradiance saturated photosynthesis of 100 $\mu$mol quanta m$^{-2}$ s$^{-1}$ in all three species, the depth in the tanks at which photosynthesis is saturated can be calculated as described by Kirk [23]. In the tanks exposed to 100% $E_o$, photosynthesis was saturated up to 80% of the depth of the tank for approximately 10 h of the day. While greater irradiance can lead to an increase in photosynthesis ($P_{max}$) and probably growth, photo-inhibition also increased in the tank with 100% $E_o$. This suggests that high irradiance levels promoting saturated photosynthesis and rapid growth rates within the culture tanks should be supplied to algae in an aquaculture facility to obtain maximum yields. However, critically high PPF levels should be avoided to reduce photoinhibition of photosynthesis that might reduce productivity. Photoinhibition was diminished in seaweeds by applying filters that reduced natural PPF levels. Similar techniques could be used at aquaculture facilities to reduce photoinhibition of photosynthesis during daily periods of critically high PPF levels. It is likely that increasing the attenuation coefficient of the water column by augmenting the biomass within the culture tanks and promoting self-shading would also decrease photoinhibition levels.

The results from this study indicate that there is a differential photoinhibition of photosynthesis in seaweed species grown in culture systems. Dynamic or rapidly reversible photoinhibition in $C. crispus$ was observed even in plants exposed to 8% incident irradiance, while in $M. pyrifera$ and $U. lactuca$ 8 and 26% $E_o$ did not cause a significant decrease in $\Delta F/F^m$. $C. crispus$ generally lives from the intertidal zone to 10–15 m in depth [24] and might be more susceptible to higher irradiances than $M. pyrifera$ and $U. lactuca$ that are generally found subsuperficially or in the intertidal zone, respectively [25,26]. Prior to the experiments, $C. crispus$ was maintained for 1 year in very dense cultures (>12 kg m$^{-3}$) which might also have acclimated these individuals to low irradiances, making them more susceptible to photoinhibition when exposed to increasing irradiance. These results suggest a pho-adaptive mechanism of species exposed to high PPF rates in the field, either through photorepair mechanisms or the synthesis of photoprotective compounds, as observed in other studies [27–29]. The results here also indicate that dynamic photoinhibition might increase in some species as a result of increasing solar irradiances during low tides.

Algae generally experience daily cycles of dynamic
photo inhibition as a result of daily variations of PPF levels. During these dynamic photo inhibitory processes, thermal energy dissipation increases and the yield of PS II is diminished for short periods of time. However, algae growing at low irradiances and transplanted to high light environments often suffer chronic photo inhibition. Such photodamage is characterized by decrease of photosynthetic potential, proteolysis and uncoupling of D1 proteins within the reaction centers [30]. Thus, photodamage occurs when the rate of photodamage of D1 proteins exceeds the rate of repair processes [31]. In contrast to C. crispus and U. lactuca, there was a dramatic decrease of $P_{\text{max}}$ values in M. pyrifera at noon that did not fully recuperate in the afternoon which is indicative of a more chronic photo inhibition compared to the other species. Furthermore, the reduction of PPF levels decreased $P_{\text{max}}$ fluctuations in all species, indicating that the photosynthetic apparatus could likely be protected and the overall productivity yields increased by using filters in the culture tanks and reducing critically high irradiance levels at noon. This also indicates that canopy blades of M. pyrifera in the field might reduce their photosynthetic performance as a result of photoinhibition during periods of high PPF levels. The full recovery of the $\Delta F/F'_m$ values to pretreatment levels after a strong photoinhibition in M. pyrifera at all PPF treatments and C. crispus and U. lactuca at 8 and 26% $E_a$ is more indicative of a dynamic photosynthesis, and is consistent with the results observed in other species [5]. However, $\Delta F/F'_m$ values for C. crispus and U. lactuca incubated at 100% $E_a$ in the morning were lower than for individuals incubated at 8 and 26% $E_a$, suggesting that photodamage repair in species exposed to high irradiance levels was not completed during the night. This is indicative of a more chronic photo inhibition in these species incubated at high irradiances, and is consistent with results observed in other studies where it took 2 days for the tissue to recover full photosynthetic activity after a strong photoinhibition [2].

The relationship between effective quantum yield of carbon assimilation or oxygen production with the quantum yield of fluorescence has been demonstrated in higher plants [17,32] and in both micro and macroalgae [8,33]. This relation validates chlorophyll fluorescence as an indirect determination of photosynthetic performance. In *Palmaria palmata* and other red seaweeds, however, no linear relationship between oxygen and fluorescence has been observed [34]. In addition to carbon assimilation processes, nitrate assimilation as nitrite reduction or nutrient limitation can also lower the relation between oxygen and fluorescence [35]. Energy misbalance between PS I and PS II as consequence of incubation under light qualities preferentially absorbed by one photosystem have also been found as responsible of low correlation between quantum yield of oxygen and quantum yield of fluorescence [36]. Light response curves determined by oxygen evolution and fluorescence here showed similar patterns in the three species studied, however, saturation was much lower in samples assayed polarographically. This is consistent with results obtained by Silva et al. [11] and suggests an uncoupling of oxygen evolution and electron transport rates in these species. It is possible that the decrease of oxygen evolution and the lack of correlation of both methods at high ETR values might be the result of photorespiratory processes at high irradiances. As a consequence, our results here indicate that electron transport rate in cultured seaweeds is only a good estimator of oxygenic photosynthesis at low ETR values.

Values of $P_{\text{max}}$ obtained though oxygen evolution and ETR$_{\text{max}}$ fluorometry covaried in M. pyrifera and C. crispus incubated at 26 and 100% $E_a$ which is further evidence of the relationship of both sampling methods. This indicates that the non-intrusive and easier fluorimetric method could potentially be used for the evaluation of photosynthetic performance of some algae in culture systems. Values of $\alpha$ did not vary as a function of incubation irradiance when measured through oxygenic or fluorimetric methods which is consistent with results reported for other species [11]. It is likely that a clearer pattern could develop between both methods if seaweeds are incubated under the same treatment for a longer period of time.

It is possible that the decrease in $\Delta F/F'_m$ as a function of increased irradiance in C. crispus could be the result of daily variations of phycoerythrin and phycocyanin as observed in other Rhodophytes [3]. The noon decrease in chlorophyll $a$, however, did not result in a decrease of oxygenic photosynthesis or electron transport rates in C. crispus. Furthermore, chlorophyll $a$ levels did not vary or increased slightly in M. pyrifera and U. lactuca suggesting that the decrease in $\Delta F/F'_m$ values at noon in all species might be the result of a direct damage to the PS II rather than fluctuations in pigment levels in the tissue. It is also possible that the photosynthetic apparatus is being photo-protected through the synthesis of UV absorbing compounds as observed in a number marine algae in the field or under culture conditions [29,37].

In this paper, the potential usefulness of chlorophyll fluorescence as an indicator of photosynthesis in macro-algae in aquaculture systems is shown. The results also show that PAM fluorometry could be used as a non-intrusive technique in aquaculture systems to evaluate the physiological status of seaweeds especially in dense cultures where productivity is reduced due to self-shading or through the use of irradiance filters. The evaluation of the photosynthetic status of the seaweed through PAM fluorometry is relatively fast and could be used to plan strategies to avoid photo inhibition of photosynthesis in algae exposed to natural levels and cycles of solar radiation. A clear daily decrease in $\Delta F/F'_m$ values was shown to occur in algae under normal culture conditions indicating that a decrease of daily productivity might occur as a result of high solar radiation in the culture tanks. It is expected that future interaction between basic photosynthetic research
and technology will improve the management of algal aquaculture.

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