Photobiological characteristics and photosynthetic UV responses in two *Ulva* species (Chlorophyta) from southern Spain

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Abstract

The effect of different wavebands of artificial UV (UVB and UVA) and photosynthetically active radiation (PAR) was assessed in two species of the genus *Ulva*, *U. olivascens* and *U. rotundata*, from southern Spain in order to test for possible differences in acclimation of photosynthesis. Both species share similar morphology but are subject to different light environments: *U. rotundata* is an estuarine alga, inhabiting subtidal locations, while *U. olivascens* is an intertidal, sun-adapted organism. Algae were exposed to three different UV conditions, PAR + UVA + UVB, PAR + UVA and PAR for 7 d. Short-term exposure (6 h) was also carried out, using two PAR levels, 150 and 700 μmol m⁻² s⁻¹. Pigment contents and photosynthesis vs. irradiance curves from oxygen evolution were used to contrast sun- and shade adaptation between these species. O₂-based net photosynthesis (*P* max) and PAM-chlorophyll fluorescence (optimal quantum yield, *F* v/*F* m) were used as parameters to evaluate photoinhibition of photosynthesis in the experiments. The results underline different photobiological characteristics among species: the subtidal *U. rotundata* had higher contents of pigments (Chl *a*, Chl *b* and carotenoids) than the sun-adapted *U. olivascens*, which resulted in higher thallus absorptance and *P*–*I* parameters characterized by higher photosynthetic efficiency at limiting irradiances (α) and lower saturating points for photosynthesis (*E* k). After 7 d exposure, photoinhibition of *F* v/*F* m was close to 40–45% in both species. Differences between UV treatments were seen in *U. rotundata* after 5 d and after 7 d in *U. olivascens*, in which PAR + UVA impaired strongly photosynthesis (80%). Such patterns were correlated with a progressive decrease in pigment contents, specially chlorophylls. In short-term (6 h) exposures, combinations of UVA + UVB and high PAR level resulted in high rates of photoinhibition of chlorophyll fluorescence (68–92%) in *U. rotundata*, whereas in *U. olivascens* photoinhibition ranged between 42% and 53%. Photoinhibition under low PAR combined to UV radiation was lower than observed under high PAR. Net O₂–*P* max revealed similar response among the species, with maximal photoinhibition rates close to 60% in algae incubated under high PAR + UVA + UVB. In the case of UV exposure in combination with low PAR, the highest photoinhibition rates were measured in *U. rotundata*.

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Keywords: *Ulva*; Chlorophyll fluorescence; Photoinhibition; Photosynthesis; UV radiation

1. Introduction

Much of the ecology of seaweeds is determined by their ability to absorb and efficiently manage the incident radiant energy [1]. Because seaweeds are sessile organisms, in contrast to the situation of phytoplankton, they have to acclimate (and adapt) to the prevailing light conditions in their natural habitat. In terms of its spectral composition, sun light is not uniform and photosynthetically active radiation (PAR) irradiances that primarily excite light harvesting pigments (chlorophylls and accessory pigments), which are mainly involved in photoacclimation, are simultaneously accompanied by UVB and UVA irradiance. Thus, algae have to use efficiently light by optimizing on the one hand the absorption of photons required in photochemistry (PAR) and on the other hand avoiding the detrimental effects of absorbed short wavelengths, mainly UVB, on target molecules [2]. In eulittoral and
shallow environments, extreme and varying conditions of combined PAR and UV radiation set different physiological strategies in macroalgae. One of them is to occupy habitats where algae can reach optimal performance, e.g. photosynthesis. In this sense, acclimation and related responses to light in macroalgae as for example, the capability of photoinhibition of photosynthesis, appear to be habitat-conditioned phenomena that are related to distribution of macroalgae [3,4]. This is particularly notorious in species from a same genus, which, despite having similar morpho-functional characteristics, attain contrasting photobiological responses. For example, a study examining fluorescence kinetics of two morphologically similar members of the red algal genus Gelidium, G. sesquipedale and G. latifolium, which co-exist at intertidal zones in the Atlantic-Mediterranean transition coast in southern Spain, revealed completely different rates of photoinhibition and recovery capacity after exposure to variable doses of solar radiation [5]. These findings confirmed the micro-distribution patterns of both species. G. sesquipedale is a shade-adapted species, preferentially inhabiting rocky crevices or canopies. In contrast, G. latifolium occupies light exposed zones. Similar response patterns were reported for sun and shade-morphs of the genus Porphyra (Rhodophyta) growing at different positions on the shore [6]. These findings challenge the hypothesis that algae attaining similar morphology share also similar ecological strategies [7].

Case studies on species of the genus Ulva (Chlorophyceae) from southern Spain reveal complex UV-dependent physiological responses, which have been documented in populations exposed to high natural solar radiation. In situ growth (during 7 d) of U. rigida from Gibraltar Strait was strongly affected by high PAR levels rather than enhanced UVB radiation. However, after 20 days, algae acclimated to current solar radiation [8]. In the case of U. rotundata from a salt marsh area in Cádiz Bay, detrimental effects of UVB radiation on photosynthesis and RUBISCO activities were seen to occur in top layers of mat-like canopies, whereas subcanopy algae were well shielded from harmful UV radiation. In both cases, the results highlight the synergistic effects of UV and PAR promoting canopy arrangements of Ulva in these environments [9]. In general, such studies point out that physiological performance of algae reflect inherent genetic vs. ambient interactions.

In the present study, two Ulva species, U. rotundata and U. olivascens, collected from different light environments (intertidal and subtidal) in southern Spain were examined for their photosynthetic characteristics and tolerance capacity against different levels of UV radiation in the laboratory. We tested the hypothesis that related algae inhabiting contrasting environments have different potential acclimation to UV radiation, which results in differential photoinhibition. The inherent capacity for photoinhibition of photosynthesis (measured as O2 evolution rates and PAM-fluorescence) was examined after long (7 days) and short-term (6 h) exposures to different combinations of PAR and UV radiation.

2. Material and methods

2.1. Collecting sites

Ulua olivascens P.A. Dangeard was collected at La Araña (30 km east of Málaga, 36°45′N 4°18′W), southern Spain, from the intertidal rocky shores, while U. rotundata Blid. was collected in an estuary area, Palmones River estuary in Cádiz, southern Spain (36°13′N 5°27′W) at the depth of 1.5 m. The two localities exhibit different environmental characteristics. The location of La Araña has a salinity of 35–37 PSU and almost no tidal variations. On the other hand, the estuary of the Palmones River (salinity between 33 and 10 PSU) is characterized by high contents of particles and detritus, which due to the tidal action are remobilized resulting in marked temporal and local variations in water transparency [10,11].

2.2. Light sources and experimental set up

After collection, algae were transported in an icebox to the laboratory, where they were maintained for at least one day in 5 l glass cylinders with aeration at 15 °C under 12:12 h L:D conditions. The irradiance was 50 μmol m−2 s−1 (Osram LW40). These algae were used as the initial control.

For experimentation, thallus discs (diameter 10 mm) were punched out and put into transparent plastic containers containing 0.75 l of filtered seawater with aeration at a temperature of 15 °C. Algae (80 sample discs) were maintained circulating and illuminated continuously with a combination of three different lamps: PAR (daylight fluorescent lamps, Osram DL), UVB (Philips TL-12, 40 W) and UVA (Q panel-340). The dose rates of PAR and UV were measured using a Licor-1800 spectroradiometer (Licor, USA). Algae were finally exposed to three different conditions in an Ibercex F-4 chamber (A.S.L., S.A., Spain):
(a) PAR alone by using Ultraphan foils (Digefra, Munich, Germany), which cutoff wavelengths shorter than 395 nm.
(b) PAR + UVA with a 320 nm Folex foil (Folex, Dreieich, Germany).
(c) PAR + UVA + UVB with a 295 nm Ultraphan foil.
The spectral characteristics of lamps and filters are described by Figueroa et al. [12]. Experimental and weighted dose according the biologically effective weighting function described by Jones and Kok [13] rates used in the experiments are summarized in Table 1. In general, the dose rates of UV radiation used in the experiments are ecologically relevant and match UV levels at this latitude [5].

Algae were maintained for 7 days under these conditions and samples were taken after 1, 5 and 7 days for measurement of pigments and quantum yield of fluorescence \( F_v/F_m \) as described below. To avoid nutrient depletion seawater was changed every 2 days.

The effect of two PAR levels in combination with different UV wavelengths on photosynthesis \( O_2 \)-based \( P_{\text{max}} \), and \( F_v/F_m \) was also assessed in a separate short-term (6 h) experiment. Here, 10 sample discs of similar diameter as previously described were put into Petri dishes (8.5 cm diameter, 100 ml). The two PAR levels, 150 and 700 \( \mu mol \cdot m^{-2} \cdot s^{-1} \), were provided by an Optimarc lamp (Duro-test, USA). The lower irradiance was set by covering the Petri dishes with a neutral shading screen to reduce irradiance. It must be emphasized that 700 \( \mu mol \cdot m^{-2} \cdot s^{-1} \) represent an intensity lower than those recorded commonly during noon at Málaga (close to 2000 \( \mu mol \cdot m^{-2} \cdot s^{-1} \)), however, is an intensity largely higher than average PAR levels at which intertidal algae are exposed between 11:00 and 17:00 h [5].

2.3. \( O_2 \)-based photosynthesis

Measurements of oxygen evolution were performed using polarographic YSI 5531-\( O_2 \) electrodes (Yellow Springs Instrument, USA) connected to a computer aided-OXY M5 \( O_2 \)-meter (Real Time Computer, Germany). The system consisted of a measuring Plexiglas chamber (9 ml) fitted with a Clark-type electrode and a magnetic stirrer. The temperature within the chamber was maintained constant (17 °C) by means of a water cooling system. To generate the photosynthesis vs. irradiance \( (P-I) \) curves, increasing irradiances were set up using different neutral gray filters (Lee filters, UK). The signal of the first 120 s from OXY M5 (except the five first values) were used to calculate the initial slope of the curve, expressed as \( \mu mol \cdot O_2 \cdot m^{-2} \cdot s^{-1} \). The light source was a white fluorescent lamp (Compact Trueelite, Duro Test, USA). The irradiance passing through the neutral gray filter and Plexiglas cross-section was measured with a spherical PAR quantum sensor (Zemoko, The Netherlands) connected to a data Logger model Licor-1000 (Li-cor Ltd, USA). In order to express the \( O_2 \) evolution as unit fresh weight (FW), after every measuring series, the thallus piece was weighted in a micro-balance.

2.4. PAM chlorophyll fluorescence

In vivo chlorophyll fluorescence was determined with a portable pulse-amplitude modulated fluorometer, PAM-2000 (Walz, Effeltrich, Germany) connected to a laptop [14]. Measurement was first conducted in eight sample discs previously incubated for 30 min in darkness to ensure that all reaction centers are open \( (F_0, \text{all } Q_A \text{ molecules are oxidized}) \). Fluorescence was induced by a weak red light source. Thereafter, a short (800 ms) pulse of white light was applied to quickly reduce the electron transport chain between PSII and PSI. As a result, all reaction centers become closed \( (Q_A \text{ molecules are reduced}) \) resulting in maximal fluorescence \( (F_m) \). The maximal or optimal quantum yield is expressed as \( F_v/F_m \), \( F_v \) being the variable fluorescence \( (F_v = F_m - F_0) \). This ratio is a good indicator of maximal algal photosynthetic efficiency and of photoinhibition when algae are exposed to high irradiances [15].

2.5. Pigment contents and optical characteristics

The quantification of Chl \( a \), Chl \( b \) and carotenoids was based on an extraction with \( N,N \)-dimethylformamide following the protocol described by Inskeep and Bloom [16]. After an incubation period of 24 h at 4 °C in the dark, the extinction was measured in a Beckman DU-7 spectrophotometer.

The absorbance or optical density due to Chl \( a \) was measured in one algal three sample discs according to the opal glass (OG) technique [16]. Firstly the difference between the in vivo maximal absorbance at 678 nm and the absorbance at 750 nm was determined in a spectrophotometer (Beckman DU-7).

Absorp_{\text{Chl}\_a} = \text{Absorp}_{678\text{nm}} - \text{Absorp}_{750\text{nm}}.
The absorptance \( [A_{\text{Chl,a}}(\text{OG})] \) was thereafter obtained from the formula:
\[
A_{\text{Chl,a}}(\text{OG}) = 1 - 10^{-\text{Absorbance}_{\text{Chl,a}}}.
\]

The absorptance determined by the opal glass procedure is converted in a integrating sphere (IS), which takes into account the reflectance. Finally, the total absorptance (TA) in the range 400–700 nm was determined by using the formulas reported by Mercado et al. [17]
\[
A_{\text{Chl,a}}(\text{IS}) = -0.002 + 0.965A_{\text{Chl,a}}(\text{OG}), \\
\text{TA} = 0.012 + 0.486A_{\text{Chl,a}}(\text{IS}).
\]

2.6. Data treatments and statistical analysis

Data set was analyzed using two-way ANOVA, species and UV treatments being the factors. In the case of short-term exposure using two PAR levels, a three-way ANOVA was performed. When differences were detected, means were compared using Tukey–Kramer multicomparative analysis [16]. In some cases, percentage and ratio data were arcsin transformed in order to meet normality and homocedasticity. Differences in pigments, TA and \( \text{O}_2 \)-based \( P-I \) parameters between species were examined by \( T \)-test.

3. Results

3.1. Initial pigment contents and \( \text{O}_2 \) based \( P-I \) curves

The species of \( \text{Ulva} \) could be characterized as sun- and shade-type organisms according to different optical and photosynthetic parameters (Fig. 1 and Table 2). Pigment contents (Chl \( a \), Chl \( b \) and total carotenoids) and TAs were significantly higher in \( \text{U. rotundata} \) than in \( \text{U. olivascens} \) (\( p < 0.05 \), \( t \)-test). Specific Chl \( a \) absorptance (Abs\textsubscript{676}) was 57% for \( \text{U. rotundata} \) and 31% for \( \text{U. olivascens} \). Photosynthesis vs. light curves (Fig. 1) revealed different light adaptations: \( \text{U. olivascens} \) showed higher \( P_{\text{max}} \) (20.8 \( \mu \text{mol} \text{O}_2 \text{ g}^{-1} \text{ FW h}^{-1} \)), lower \( z \) (0.17 \( \mu \text{mol} \text{ O}_2 \text{ g}^{-1} \text{ FW h}^{-1} \) (\( \mu \text{mol photon m}^{-2} \text{ s}^{-1} \text{ yr}^{-1} \)) and higher light saturation point (\( E_k \), 116 \( \mu \text{mol photon m}^{-2} \text{ s}^{-1} \)) than \( \text{U. rotundata} \) (Table 2). In this species, which attains shade-adapted characteristics, \( P_{\text{max}} \) had values close to (5.9 \( \mu \text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1} \)), whereas \( z \) and \( E_k \) reached 0.25 \( \mu \text{mol} \text{ O}_2 \text{ g}^{-1} \text{ FW h}^{-1} \) (\( \mu \text{mol photon m}^{-2} \text{ s}^{-1} \text{ yr}^{-1} \)) and 23 \( \mu \text{mol photon m}^{-2} \text{ s}^{-1} \), respectively.

3.2. Photoinhibition after short-term (6 h) exposure to different UV and PAR conditions

After 6 h exposure there were clear differences in photosynthetic responses between the different UV treatments (Table 3). In \( \text{U. olivascens} \), light saturated net photosynthesis (\( P_{\text{max}} \)) measured at 250 \( \mu \text{mol photon m}^{-2} \text{ s}^{-1} \) was close to 16 \( \mu \text{mol} \text{ O}_2 \text{ g}^{-1} \text{ FW h}^{-1} \) and decreased by 62% and 50% (\( p < 0.05 \)) in PAR + UVA + UVB treatments with high and low PAR intensities, respectively. Exposures to PAR + UVA resulted in 50% photoshination of photosynthesis under both PAR intensities (\( p < 0.05 \), Tukey). Decreases of \( P_{\text{max}} \) in samples incubated under treatment deprived of UV were close to 22% and 12% under high and low PAR level, respectively.

In \( \text{U. rotundata} \), responses of \( P_{\text{max}} \) to PAR + UVA + UVB treatments varied depending of the PAR level: high PAR resulted in 56% reduction compared to 36% recorded in samples incubated under low PAR (Table 3). On the other hand, photoinhibition of photosynthesis under PAR + UVA and PAR was close to 30% and 50% and did not vary significantly in relation to PAR intensity (\( p > 0.05 \), Tukey). Dark respiration...
Differences due to species and PAR levels were all statistically significant at similar letters indicate non-significant differences of means between light treatments for each species after Tukey–Kramer multicomparison test.

Table 3
Effects of short term (6 h) exposure to three light treatments (PAR + UVA + UVB, PAR + UVA and PAR alone) on maximal O₂-based photosynthesis (Pmax) in U. olivascens and U. rotundata from two sites in southern Spain.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>U. olivascens</th>
<th>U. rotundata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pmax (μmol O₂ g⁻¹ FW h⁻¹)</td>
<td>Decrease (%)</td>
</tr>
<tr>
<td>Initial</td>
<td>16.4 ± 2.0ᵃ</td>
<td>4.5 ± 1.1ᵃ</td>
</tr>
<tr>
<td>HP</td>
<td>6.2 ± 2.0ᵇ</td>
<td>62.1 ± 19.9</td>
</tr>
<tr>
<td>PAR + UVA + UVB</td>
<td>7.7 ± 2.6ᵇ</td>
<td>52.7 ± 17.9</td>
</tr>
<tr>
<td>PAR</td>
<td>12.8 ± 2.8ᶜ</td>
<td>22.3 ± 5.0</td>
</tr>
<tr>
<td>LP</td>
<td>8.2 ± 3.1ᶜ</td>
<td>50.0 ± 18.8</td>
</tr>
<tr>
<td>PAR + UVA + UVB</td>
<td>7.7 ± 1.5ᵈ</td>
<td>52.7 ± 10.3</td>
</tr>
<tr>
<td>PAR</td>
<td>14.5 ± 0.2ᵃ</td>
<td>12.0 ± 0.1</td>
</tr>
</tbody>
</table>

Incubations were carried out using PAR intensities of 700 (High PAR, HP) and 150 (Low PAR, LP) μmol photon m⁻² s⁻¹. Percentage decreases relative to initial controls are also given. Negative percentages correspond to values higher than controls. Data are means ± S.D. (n = 3 discs). Similar letters indicate non-significant differences of means between light treatments for each species after Tukey–Kramer multicomparison test. Differences due to species and PAR levels were all statistically significant at p = 0.05.

Table 4
Effects of short term (6 h) exposure to three light treatments (PAR + UVA + UVB, PAR + UVA and PAR alone) on PAM-based optimal quantum yield (Fv/Fm) of U. olivascens and U. rotundata from two sites in southern Spain.

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<tbody>
<tr>
<td></td>
<td>Fv/Fm</td>
<td>Photoinhibition (%)</td>
</tr>
<tr>
<td>Initial</td>
<td>0.76 ± 0.04ᵃ</td>
<td>0.75 ± 0.01ᵃ</td>
</tr>
<tr>
<td>HP</td>
<td>0.36 ± 0.004ᵇ</td>
<td>52.6 ± 0.54</td>
</tr>
<tr>
<td>PAR + UVA + UVB</td>
<td>0.38 ± 0.01ᶜ</td>
<td>50.5 ± 1.46</td>
</tr>
<tr>
<td>PAR</td>
<td>0.45 ± 0.01ᵈ</td>
<td>41.5 ± 1.38</td>
</tr>
<tr>
<td>LP</td>
<td>0.57 ± 0.005ᵉ</td>
<td>24.7 ± 0.34</td>
</tr>
<tr>
<td>PAR + UVA</td>
<td>0.62 ± 0.004ᶠ</td>
<td>18.6 ± 0.10</td>
</tr>
<tr>
<td>PAR</td>
<td>0.66 ± 0.005ᵍ</td>
<td>13.1 ± 1.2</td>
</tr>
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Photoinhibition relative to initial controls are also given. Incubations were carried out using PAR intensities of 700 (high PAR, HP) and 150 (low PAR, LP) μmol photon m⁻² s⁻¹. Data are means ± S.D. (n = 10 discs). Similar letters indicate non-significant differences of means between light treatments for each species after Tukey–Kramer test following a two-way ANOVA. Differences due to species and PAR intensities were all statistically significant at p = 0.05.

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Percentage photoinhibition relative to initial controls are also given. Incubations were carried out using PAR intensities of 700 (high PAR, HP) and 150 (low PAR, LP) μmol photon m⁻² s⁻¹. Data are means ± S.D. (n = 10 discs). Similar letters indicate non-significant differences of means between light treatments for each species after Tukey–Kramer test following a two-way ANOVA. Differences due to species and PAR intensities were all statistically significant at p = 0.05.

measured in U. rotundata was enhanced in all treatments (p < 0.005, Tukey), with the exception of a slight decrease in samples exposed to PAR + UVA + UVB and high PAR background.

An exposure for 6 h to the different UV treatments and PAR levels caused significant photoinhibition of Fv/Fm in both species (Table 4). In general, algae exposed to PAR + UVA + UVB exhibited the highest photoinhibition rates (p < 0.05). In U. olivascens, photoinhibition ranged between 42% and 53% in treatments supplied with high PAR. Under low PAR, photoinhibition rates ranged between 13% and 25%. In U. rotundata, Fv/Fm strongly decreased, mainly when algae were incubated in the presence of high PAR (68–92%). Although the values of Fv/Fm were lower after exposures to UV and low PAR intensity than measured under high PAR (42–61%), a photoinhibitory effect was evident.

3.3. Effects of long-term (7 days) UV exposure on pigment contents and Fv/Fm

In terms of their pigment contents, exposure of algae for 7 days to UV treatments revealed different responses (Fig. 2). Chl a contents measured in U. olivascens decreased by 50% after 7 days exposure to the three UV treatments, whereas in U. rotundata, values at the end of the experiment these decreases were close to 30%. For the Chl b contents, differences were even more accentuated: in U. olivascens, Chl b concentrations remained constant over the time, contrasting with the marked decrease observed in U. rotundata, where Chl b values were 20% relative to the control (p < 0.05). In the case of the carotenoids, two different patterns were found: in U. olivascens, the carotenoids decreased only during the first 5 days (30%), remaining constant two days later (p > 0.05). In U. rotundata these accessory pigments had
marked fluctuations averaging 0.07 mg g\(^{-1}\) FW during the incubation time. Overall, although some differences could be seen at a particular time, we did not detect in general significant effects of the UV treatments on the pigment contents.

The results from \(F_v/F_m\) measurements (Fig. 3) indicated a progressive photoinhibition, which reached values close to 20% during the first day in both species. At this time no differences between treatments were found. After 5 days, however, a clear decrease in \(F_v/F_m\) (60%) was measured in *U. rotundata*, mainly in samples incubated under PAR + UVA + UVB treatment (*p* < 0.05). At the end of the experiment, photoinhibition in this species was close to 40%, similar as in PAR + UVA treatment. In *U. olivascens*, the decrease in \(F_v/F_m\) due to PAR + UVA + UVB was linear over the time and after 7 days, photoinhibition was close to 45% (*p* < 0.05). However, the highest rates of photoinhibition were measured in samples incubated under PAR + UVA condition.

The correlation between the percentage photoinhibition vs. weighted doses from the Jones and Kok [13] which include the UVA region, revealed that *U. rotundata* was photoinhibited at lower doses (2400 kJ m\(^{-2}\)) than *U. olivascens*, which, extrapolated from the linear curve, may require doses higher than 4000 kJ m\(^{-2}\) to decrease \(F_v/F_m\) by 50% (Fig. 4).

Fig. 2. Pigment contents (Chl \(a\), Chl \(b\) and total carotenoids) in *U. olivascens* and *U. rotundata* after incubations for 7 d under three different treatments: PAR + UVA + UVB (PAB), PAR + UVA (PA) and PAR (P). Data are means ± S.D., *n* = 3.

Fig. 3. Photoinhibition of photosynthesis measured as optimal quantum yield of chlorophyll fluorescence (\(F_v/F_m\)) in *U. olivascens* and *U. rotundata* incubated for 7 d under three different treatments: PAR + UVA + UVB (PAB), PAR + UVA (PA) and PAR (P). Data are means ± S.D., *n* = 10.
4. Discussion

Our results confirmed the hypothesis that *U. olivascens* and *U. rotundata* differ in their physiological responses under similar UV environments in the laboratory. The optical and photosynthetic characteristics of both species appear to be key factors to interpret such responses. *U. olivascens*, a sun-adapted species, showed low pigment contents and TA, while *P–I* curves revealed low photosynthetic efficiency (*v*) and high light saturation point (*E_s*). In contrast, *U. rotundata* exhibited parameters resembling shade-adapted macroalgae.

4.1. Susceptibility to UV radiation

Both species exposed for 7 days to different UV conditions in the laboratory exhibited marked photoinhibition at the end of the experiments. Photoinhibition rates close to 60% were recorded in *U. rotundata* after 5 days of incubation under PAR + UVA + UVB, while in *U. olivascens*, maximal photoinhibition occurred two days later in the PAR + UVA treatment. Two major findings may be emphasized in the light of our results. Firstly, biologically effective doses of UVA + UVB (weighted after Jones and Kok [13]) required to cause 50% photoinhibition of photosynthesis after 7 d are higher in the sun-adapted *U. olivascens* (4000 kJ m$^{-2}$) than those necessary to reach similar effect in the shade alga *U. rotundata* (2400 kJ m$^{-2}$). Secondly, a marked effect of UVA irradiances (PAR + UVA) was observed, mainly in *U. olivascens*. These results are in agreement with the data reported by Dring et al. [4] who emphasized that UVA was responsible for an important fraction of the reduction in photosynthesis of various red algae from the North Sea, which undoubtedly has ecological relevance as solar radiation reaching the earth surface contains much more UVA than UVB. In our study, similar trend was found after short-term (6 h) exposures. PAR + UVA treatment resulted in similar or slightly lower rates of photoinhibition as in samples incubated under PAR + UVA + UVB condition (see below).

It is controversial to assign a marked photoinhibitory role of UVA wavelengths. Other studies carried out in macroalgae from high solar radiation environments indicate that UVA doses have not only have less detrimental effects on photosynthesis, but also it can have a beneficial role [9,19,20]. The ameliorative effects of UVA radiation reported on e.g. photosynthesis of the warm-temperate green alga *Dasycladus vermicularis* [30] or on nitrate reductase and carbonic anhydrase activities of various macroalgae from southern Spain [20] have been discussed in terms of the experimental lamp design. In general, laboratory approaches using combinations of artificial PAR + UV sources reproduce well the UVA/UVB ratios, but not accurately natural UV/PAR conditions [4]. Although the use of a high UV/PAR ratio (PAR < 100 μmol m$^{-2}$ s$^{-1}$) certainly avoids a possible masking of UV effects by PAR, C assimilation can be impaired in species characterized by high light requirements for photosynthesis due to a low energy supply. The idea that UVA can become a compensatory energy source for photosynthesis has recently been evaluated. Pérez-Rodríguez et al. [19] reported a decrease in chlorophyll fluorescence and O$_2$ evolution-based photosynthesis in the green alga *Dasycladus vermicularis* exposed to low PAR alone (32 μmol m$^{-2}$ s$^{-1}$), whereas photosynthesis of plants under PAR + UVA radiation significantly increased. These authors suggested that a fraction of the UVA region could be used in photosynthesis. Apparently, UVA activates secondary photosynthetic reactions via stimulation of some Calvin cycle enzymes, as has been demonstrated in brown algae [21]. However, such a hypothesis is ruled out in our study since PAR intensities used in our incubations were at saturating levels (150 μmol photon m$^{-2}$ s$^{-1}$).

The decrease in *F$_r$/F$_m*$ after 7 days under different conditions of UV radiation was concomitant with a gradual decrease in pigments contents. This was particularly accentuated for Chl *a* and *b*, mainly in *U. rotundata*. Although in general, a slower response of pigments (chlorophylls) to light variations compared to other physiological parameters has been recognized in previous reports on *Ulva* [22–24], exposure periods longer than 24 h (as in our study) can give important information on changes in light harvesting capacity of algae. In our study, pigment concentrations measured at the beginning of the study did not change with short term exposure to UV radiation (data not shown), indicating that 6 h is not enough to cause changes in...
pigmentation. Moreover, we were not able to conclude any effect due to UVB as concentrations were similar between the different treatments, confirming data reported for other intertidal species of Ulva e.g. U. expansa [24]. Apparently, content of pigments gives fundamental information on the light harvesting, and indirectly, some insights into possible UV responses, however, because pigment concentration is uncoupled of e.g. photosynthetic UV acclimation, it is regarded as a weak biological indicator of effects of UV radiation in this type of experimental designs.

4.2. The role of PAR intensity in short-term response

Measurements of O2-based photosynthesis and chlorophyll fluorescence outlined different responses after short-term (6 h) exposure to different UV climates. Whereas reductions of O2-based $P_{\text{max}}$ were similar or even higher in the intertidal, sun-adapted U. olivascens, photoinhibition of PAM-based $F_v/F_m$ was much more accentuated in the estuarine U. rotundata than U. olivascens. Although both parameters reflected well the existence of photoinhibition at levels above 30% in treatments including UV wavebands, the differences between the species confirm the discrepancies found when O2 evolution and chlorophyll fluorescence data are confronted [25]. In U. rotundata, rates of photoinhibition of $P_{\text{max}}$ was always lower than photoinhibition of $F_v/F_m$, suggesting that net saturated O2 production only begin to decrease when the down-regulation of the photoschemistry process, in this case expressed as a decreased in $F_v/F_m$, reaches rates higher than 30%. On the other hand, net $P_{\text{max}}$ is a parameter linked more to the C assimilation and Calvin cycle rather than to the processes related to photosynthetic efficiency or quantum yield at limited irradiances [26].

In the case of U. olivascens, UV treatments combined to a high PAR level (700 μmol photon m$^{-2}$ s$^{-1}$) resulted in similar rates of photoinhibition of net $P_{\text{max}}$ and $F_v/F_m$, whereas under low PAR level, UV wavelengths affected more strongly $P_{\text{max}}$. Similar results were reported in the sun adapted green alga Dasycladus vermicularis [18]. Such responses are difficult to interpret, and apparently, the mechanisms involved in shade and sun adaptation are part of a suite of metabolic adjustments operating in different ways depending on the photosynthesis level: light harvesting (antenna, photoreceptors), photochemical (electron transport) or C-assimilation. Flammeling and Kromkamp [27] have suggested two possible reasons to explain some discrepancies between O2 evolution and chlorophyll fluorescence: (A) net oxygen production would be influenced by processes that consume oxygen or affect linear electron transport, e.g. cyclic electron transport around PSII, pseudocyclic electron transport in the Mehler reaction, Rubisco oxygenase activity and light dependent mitochondrial respiration. (B) At saturating irradiances, changes in photosynthesis turnover time may occur, and thus effective quantum yield does not match steady-state O2 yield. In addition to variations in carbon assimilation processes, nitrate assimilation processes or nutrient limitation can also affect the relation between oxygen and fluorescence. On the other hand, recently it was demonstrated that the activity of Calvin cycle enzyme Rubisco in U. rotundata was impaired only when UV radiation was accompanied of PAR [9]. Furthermore, Rubisco is UV targeted independently of the other primary photosynthetic reactions, e.g. quantum yield of fluorescence [28].

As was mentioned above, the photoinhibition was greater under high PAR level of 700 μmol m$^{-2}$ s$^{-1}$ than under 150 μmol m$^{-2}$ s$^{-1}$, which was particularly accentuated in U. rotundata. In the case of U. olivascens, photoinhibition, mainly of $F_v/F_m$, in samples incubated under high PAR was in general 50% higher than the counterparts maintained under low PAR condition. Such findings reinforce the idea of a sun-adaptation of U. olivascens, which is exposed at its natural habitat to solar PAR irradiances at noon as high as 2000 μmol m$^{-2}$ s$^{-1}$, but also indicate that in shade algae, as U. rotundata, PAR is important as a trigger of the down-regulation of the photosynthetic apparatus. For example, in U. rotundata from a salt marsh in Cádiz, UVB radiation only exerted an adverse effect on photosynthesis in combination with high PAR level [9]. The same authors using filters that cutoff PAR radiation, reported the synergistic action of UV and PAR, suggesting that UV alone does not cause obvious detrimental effects on photosynthesis in e.g. U. rotundata. The importance of PAR has often been recognized in this type of studies, not only in macroalgae from geographical regions with high solar radiation [12,20,29], but also from polar algae where PAR levels are generally low [30,31]. The marked effect of PAR on photoinhibition of photosynthesis has been interpreted as a photoprotective strategy that primarily induces the down-regulation of the photosynthetic apparatus in order to avoid that simultaneous incident shorter wavelengths (UV) harm D1 protein turnover [32].

4.3. Concluding remarks: ecological implications

The different sensitivity of Ulva species to UV radiation reflects well the acclimation of each species to its natural environment. U. rotundata occurs at subtidal sites in the Palmones estuary and is submitted to drastic changes in light environment, including very shady conditions as consequence of the high turbidity of the water column [10]. Thus the highest inhibition of photosynthesis in U. rotundata, particularly under exposure including high PAR level, may be explained by a less developed photoprotective mechanisms com-
pared to *U. olivascens*. However, not only incident irradiances can govern photosynthetic UV responses of algae. Nutrients, e.g. low nitrogen levels have been seen to exacerbate photoinhibition in *U. rotundata* [22,23]. Similarly, high CO2 levels (1%) affect light relations in algae, e.g. the red alga *Porphyra leucosticta* [33]. In this study, *U. rotundata* was the species with the highest discrepancies between fluorescence and oxygen evolution, which could be associated to differences in nutrient metabolism. Although no limitation due to nitrogen is expected in this study during the short incubation time, the history of nutrient status in *U. rotundata* in the field, which is characterized by strong fluctuations [10], could affect in part the pattern of photoinhibition. In the case of the intertidal *U. olivascens*, it not only must be able to cope with daily changes in solar radiation but also have to withstand simultaneously changes in temperature, salinity or wave action during emersion periods. Thus, the capacity for light stress tolerance forms part of the whole suite of ecological strategies of these species.

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