Photomovement of the swarmers of the brown algae *Scytosiphon lomentaria* and *Petalonia fascia*: effect of photon irradiance, spectral composition and UV dose

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Abstract

Photomovement measurements were carried out with swarmers of the brown algae *Scytosiphon lomentaria* (Lyngb.) Link and *Petalonia fascia* (O. F. Mull.) as a function of irradiance direction, photon irradiance, spectral composition and ultraviolet radiation (UVR, $\lambda=280$–$400$ nm) dose. Swarmers from both species showed similar photomovement patterns: negative phototaxis occurred under photon irradiances of $10$–$90 \mu$mol photons m$^{-2}$ s$^{-1}$, and no movement was observed at $190 \mu$mol photons m$^{-2}$ s$^{-1}$. The translocational velocity measured between $10$ and $90 \mu$mol m$^{-2}$ s$^{-1}$ ranged from $100$ to $200 \mu$m s$^{-1}$. The accumulation of swarmers presented a peak at $450$ nm (waveband of $50$ nm), and smaller peaks at $400$ and $500$ nm; no effect was observed at wavelengths of $550$ nm and above. The decline in phototactic index (an estimator of photomovement response) of swarmers was linearly correlated with the logarithm of UVR doses. These data were correlated with levels of natural solar radiation in the field. It is hypothesized that motility of swarmers could be a critical factor in the survival of these species under a scenario of increased UVR.

Keywords: *Petalonia fascia*; Photomovement; *Scytosiphon lomentaria*; Swarmers; Ultraviolet radiation

1. Introduction

The biflagellate reproductive cells (swarmers) of many species of brown algae show phototaxis with a typical pattern of the so-called blue light responses [1,2]. The swarmers that have been found to show this behavior have a stigma and flagellar swelling [3–5] associated with photoreception by a green autofluorescent compound located only in the posterior flagellum [6].

Thinning of the stratospheric ozone layer has resulted in increased levels of ultraviolet B radiation (UVBR, $\lambda=280$–$315$ nm) at the Earth’s surface [7]. Although the thinning of the ozone layer was initially described for the Antarctic springtime, it also takes place in the northern hemisphere, where predictions for the next century suggest that UVBR will reach values similar to those in the southern hemisphere [8]. Therefore, there is at present great concern about the effect of current and increased UVBR on the biosphere.

It is known that the photo-orientation and motility of flagellated microalgae is impaired by solar [9–11] and artificial [12] ultraviolet radiation (UVR, $\lambda=280$–$400$ nm). It has been proposed that the inhibition of phototaxis is caused by a specific destruction of the photoreceptor protein–pigment complex [13]. Although no data are available on the effects of UVR on phototactic responses of swarmers of brown algae, this phenomenon must be addressed in order to understand the ecological role of present and increased UVBR levels on the structure and productivity of benthic algal populations [14,15]. Significantly, it has been demonstrated that the zonation pattern of large sublittoral kelps from the Arctic and the Strait of Gibraltar is strongly dependent on underwater UVBR levels that could affect the survival of zoospore and microthallus stages [16].

The aim of this work was to investigate the photomovement of the swarmers of the brown algae *Scytosiphon lomentaria* (Lyngb.) Link and *Petalonia fascia* (O. F. Mull.) Kuntze (Scytosiphonales). Specifically, the re-
2. Materials and methods

2.1. Collection site and algal material

Fertile, epiphyte-free thalli of *Scytosiphon lomentaria* (Lyngb.) Link and *Petalonia fascia* (O. F. Müll.) Kuntze (Scytosiphonales, Phaeophyceae) were collected in the eulittoral zone of the Araña beach, Málaga, southern Spain, in February and March 2000. Sea water temperature ranged from 13.9 to 15.5 °C during the collection period; thus, a temperature of 15±0.1 °C was used in the experiments carried out in the laboratory. At this period of the year, daily doses of solar radiation are close to 3500 kJ m⁻² photosynthetically active radiation (PAR, λ=400–700 nm), 450 kJ m⁻² ultraviolet A radiation (UVAR, λ=315–400 m) and 5 kJ m⁻² UVBR. Solar radiation data were collected by an ELDONET instrument (Real Time Computer, Erlangen, Germany) located on the roof of the building housing the Faculty of Sciences of the University of Málaga, at 10 km from the sampling site.

Five grams fresh weight of each species were precultivated during 4 days, in 70 l of natural filtered (Whatman GF/C) sea water from the same place where algae were collected; precultivation conditions were 250 μmol photons m⁻² s⁻¹ PAR, 12 h dark:12 h light, 15 °C.

![Fig. 1. (A) Experimental device for the investigation of photomovement of swarmers from *Scytosiphon lomentaria* and *Petalonia fascia*. 1, Source of white light; 2, lightguide; 3, lens; 4, interference filter; 5, lightguide; 6, microscope glass with concave dip depression; 7, microscope. (B) Width (d) of the mass of accumulated cells at the edge of the concave depression of the microscope, in front of the lateral light.](image-url)
with a calibrated ocular (Periplan GF10×/18M, Leitz, Germany) by measuring the time taken for swarmers to traverse a distance of 500 μm.

The phototactic index (PI) proposed in Ref. [17] was used for the phototaxis measurements:

$$PI = \frac{(n_{\uparrow} - n_{\downarrow})}{(n_{\uparrow} + n_{\downarrow})},$$

where \(n_{\uparrow}\) and \(n_{\downarrow}\) are the amounts of swimming cells that are moving within a 30° sector towards and away from the source of light, respectively. The sectors were obtained by cutting out two opposite direction 30° angles on a foil of tracing paper which was placed on the scale disc of the calibrated ocular. All the measurements were carried out in 30 min after the release of swarmers.

The spectral sensitivity of the phototactic response of the swarmers was estimated as the dependence of the width \(d\) (relative units corresponding to divisions of a Periplan GF10×/18M calibrated ocular) of the mass of accumulated cells at the edge of the concave depression of the microscope slide (Fig. 1B), on the wavelength. The value of \(d\) is a good estimator of the spectral sensitivity of swarmers because the concentration of cells was the same in all the measurements and they accumulated in a single layer. A set of coloured glass interference filters with central transmission at 400, 450, 500, 550, 600, 650 and 700 nm, and waveband of 50 nm each (Oriel, USA), were used. A photon irradiance of 2–3 μmol photons m⁻² s⁻¹ was used in all the measurements. The measurement of \(d\) was carried out in 1 h after the release of swarmers. It was checked that all the swarmers stand motionless after this time period.

### 2.4. UVR treatments

Two different light treatments were used in the experiments: PAR+UVR, and PAR-only (Table 1). Macrothalli of algae were illuminated with four Duro-Test (True-Lite, Richmond Hts., OH, USA) and three UVA-340 (Q-Panel, Cleveland, OH, USA) fluorescent lamps; the obtained irradiances were 150 μmol photons m⁻² s⁻¹ PAR, 17 W m⁻² UVR, and 0.8 W m⁻² UVBR, which were measured with a spectroradiometer (LI-1800UW, Li-Cor Inc., Lincoln, NE, USA) by integrating the spectral irradiances between the wavelength limits of each waveband. The UVBR levels were underestimated because the device is not able to measure below 300 nm; therefore, the UVBR levels shown in the experimental set-up correspond to the irradiances measured between 300 and 315 nm. For the PAR+UVR treatment, the cultures were covered with a filter with a transmission cut-off at \(\lambda<295\) nm, whereas a filter with a transmission cut-off at \(\lambda<395\) nm (both cut-off filters from Ultraphan, Digebla GmbH, Germany) was used for the PAR-only treatment. The spectral transmission characteristics of the cut-off filters were shown in Ref. [18]. Different doses were achieved by subjecting the cultures to different times of exposure: 1, 2 and 6 h. Then the swarmers were isolated as described above.

In order to estimate the wavelength-dependent effectiveness of the UVR treatments (i.e. biologically effective dose, BED), spectral irradiances in the range 300–400 nm were weighted using the action spectrum for the generalized plant damage [19] (Table 1). The weighted irradiance dose rate (\(R\)) was calculated as follows [20]:

$$R = \int_{300 \text{ nm}}^{400 \text{ nm}} I(\lambda)e(\lambda) \, d\lambda,$$

where \(I(\lambda)\) and \(e(\lambda)\) are the spectral irradiance and the biological response derived from the action spectrum at \(\lambda\) nm, respectively. Time integration (from initial, \(t_0\), to final, \(t_f\), time period of exposure) of this quantity gives the BED:

$$BED = \int_{t_0}^{t_f} \int_{300 \text{ nm}}^{400 \text{ nm}} I(\lambda)e(\lambda) \, d\lambda \, dt.$$

### 2.5. Statistical analysis of data

Not less than 10 separate samples (one single measurement per sample) were used in the measurements of the translocational velocity, PI as a function of light intensity, and spectral sensitivity of phototactic response.

Ten measurements, in separate samples, of PI per combination of UVR treatment and time of exposure were made. Coefficients of variation of PI measurements were lower than 16% of the mean. The PI data were compared by a two-way (UVR condition and time of exposure) model I ANOVA. The arcsin transformation was applied to the PI percentages. The homogeneity of variances was checked by Bartlett’s test.

The values of width \(d\) of the mass accumulated swar-

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**Table 1**

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>Experimental irradiance (W m⁻²)</th>
<th>UVAR</th>
<th>UVBR</th>
<th>Weighted irradiance (W m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without filter</td>
<td>35.7</td>
<td>17</td>
<td>0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Ultraphan 295 (PAR + UVR)</td>
<td>35.7</td>
<td>15.7</td>
<td>0.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Ultraphan 395 (PAR-only)</td>
<td>34.9</td>
<td>0.3</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>
mers of both species as a function of wavelength were compared by a two-way (wavelength and species) model I ANOVA.

It was found that the linear best-fit relating the variables was achieved with PI as a function of the logarithm of BED. The regressions of both species were compared with a test of equality of slopes and analysis of covariance.

All the statistical analyses were carried out according to Ref. [21].

3. Results

The movements of the swarmers from *S. lomentaria* and *P. fascia* showed similar features. In the absence of lateral light, or under a photon irradiance $<10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, the movement of swarmers was characterized by a chaotic, random trajectory of circles, semicircles, and spiral forms. However, the increasing photon irradiance from 10 to 90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ stimulated a strong negative phototaxis (Fig. 2), characterized by the movement of the swarmers oriented along a linear trajectory away from the light source, with a speed ranging from 100 to 200 $\mu\text{m s}^{-1}$ (Fig. 3). No movement of the swarmers was detected at 190 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 3).

The spectral sensitivities of the phototactic responses of the swarmers of *S. lomentaria* and *P. fascia* showed similar patterns, with a significant ($P<0.001$) peak at 450 nm, and smaller significant peaks ($P<0.001$) at 400 and 500 nm, respectively. In both species no accumulation of swarmers was observed at wavelengths of 550 nm and above (Fig. 4).

Under the PAR-only treatment, the PI values of swarmers from both species did not change with time of exposure (overall mean of $-0.74\pm 0.03$ for *P. fascia* and $-0.75\pm 0.03$ for *S. lomentaria*). By contrast, the PAR+UVR treatment significantly affected the values of PI in both algae ($P<0.001$ in the two ANOVAs, one per species), as well as the movement patterns of swarmers which were characterized by random trajectory of circles and spiral forms. Absolute value from PI decreased with increasing UVR doses, and a dose of 23.1 kJ m$^{-2}$ eliminated phototactic orientation almost completely (Fig. 5). The inhibition of phototaxis in both species showed similar features, i.e. significant similar intercepts and slopes in the linear fit of PI as a function of logarithm of BED. Therefore, their data were pooled and analysed together. According to the BED computed by using the action spectrum for generalized plant damage [19], a UVR dose of 4.1 kJ m$^{-2}$ was found to cause 50% inhibition in PI (Fig. 5).
Fig. 5. Response of the phototactic index (PI) of swarmers from 
Scytosiphon lomentaria (open circles) and Petalonia fascia (filled circles) 
as a function of logarithm of biologically effective dose (BED), computed 
according to the action spectrum for generalized plant damage [19]. The 
horizontal dotted line shows the overall mean PI value from both species 
under the PAR-only treatment.

4. Discussion

The photomovement performances of swarmers of S. lomentaria and P. fascia analysed in this work agree closely. The similarity of the phototactic responses could be due to the fact that both species share the habitat and, generally, appear together. Therefore, it could be supposed that the swarmers of these species have similar features.

Swarmers from S. lomentaria and P. fascia showed negative phototaxis at photon irradiance levels of 10–90 
μmol m⁻² s⁻¹. This differs from the pattern found in the swarmers of other brown algae: the male gametes of Ectocarpus siliculosus (Dillw.) Lyngbye [1] and the zoospores of the primitive kelp Pseudochorda gracilis Kawai 
et Nabata [22] have mostly positive phototactic responses. Although the orientation of phototactic responses in microalgae can change depending on irradiance levels [12,17,23,24], the direction of the phototactic response in swarmers of brown algae do not show a clear pattern. Negative phototaxis of swarmers seems to be more common than positive phototaxis, even in the deep water species Desmarestia tabacoides Okamura [25] and the sublittoral kelps Akkesiphycus lubricus Yamada et Tanaka [26]. Thus, a possible relationship between vertical distribution of species and direction of phototactic response cannot be hypothesized.

The translocational velocity measured for the swarmers of S. lomentaria and P. fascia (from 100 to 200 μm s⁻¹) was higher than that observed in the zoospores of P. gracilis (from 100 to 130 μm s⁻¹) [22]. For a given morphology, larger cells can swim faster than smaller cells, a performance linked to the Reynolds number which is a function of organism size [27]; however, the size of swarmers from Scytosiphonales (i.e. isogametes from S. lomentaria reach 6.1–8.4×2.7–3.8 μm) [28] is smaller than the zoospores of P. gracilis (11–12×8 μm) [29] and, therefore, any explanation linked to hydrodynamic properties of swarmers cannot be applied.

Although no studies on action spectra of the phototactic response of swarmers of S. lomentaria and P. fascia were carried out, the photoaccumulation of both species showed a peak at 450 nm, and no effect was found at a wavelength of 550 nm, as is typical in a blue-light response [30,31]. The wavelengths between 430 and 450 nm were found to be the most effective for photoaccumulation in the male gametes of E. siliculosus [32], and this was later corroborated by the action spectrum of their phototactic response [1]. Moreover, the action spectrum for phototaxis in zoospores of P. gracilis had two peaks at 420 and 460 nm, whereas wavelengths above 500 nm were not effective in changing the swimming direction of the zoospores [22]. Possible photosensing pigments that can be responsible for light-induced motile reactions of algae are flavins, rhodopsin, pterins and carotenoids [33], and it has been shown that the photoreceptor in the swarmers of S. lomentaria is riboflavin-4’,5’-cyclic phosphate [34], which is located in the posterior flagellum of the cell [6].

Our laboratory results can be compared with exposures under field conditions because the doses of UVR were normalized as BEDs. In fact, this is the only way in which results delivered by artificial light sources can be compared with solar UVR. Therefore, by knowing the spectral irradiance in situ it was possible to calculate the time period that must be run to achieve a dose similar to the BED producing 50% inhibition of PI. For example, when the BEDs computed with the action spectrum for generalized plant damage were applied, 50% inhibition PI in both species was reached in the field after 3–4 h (daily UV AR and UVBR doses of 450 and 5 kJ m⁻², respectively). These data suggest that the motility of swarmers could be a critical factor in the survival of these algae under the scenario of increased UVBR. In particular, the zooids from S. lomentaria and P. fascia can be isogametes, anisogametes or non-sexual (parthenogenetic or neutral) gametes [5]. If the swarmers work as gametes, fertilisation may not take place under high UVR, blocking the life history; on the other hand, if they work as non-sexual gametes, the recruitment of new thalli by asexual reproduction could be diminished, as has been demonstrated in several species of kelps [16]. However, it must be emphasized that the relationships between BED and PI could be unrealistic because they were derived from the action spectrum for generalized plant damage [19]. This approach was used because an action spectrum focused on photomovement of algae is not available; anyway, it could be expected that the BED figures based on a hypothetical action spectrum for photomovement were lower than those obtained with the action spectrum for generalized plant damage because single-celled stages of macroalgae are structurally much more simple than higher plants.
From the results of this work it could be hypothesized that the winter-ephemeral character of both species [35] could be related to the survival and motility of swarmers, because the highest percentage of cloudy days occurs in winter so that the lowest solar radiation levels [36] coincide with the reproductive period of these algae.

It is difficult to estimate future possible effects of increased UVR on ecosystems on the basis of a single biological process because the biological targets of UVR are many (reviewed by Ref. [14]). For example, the germination of zoospores and growth of the microscopic gametophytic phase of kelps is correlated with UVR doses occurring in the habitat where these species grow, thus explaining the upper limit of their vertical distribution [16]. However, swarmers of most kelps lack a stigma or eyespot [37,38] and are not phototactic. Therefore, the harmful influence of UVR enhancement will affect different cellular targets in zooids of kelps, in contrast to what will possibly occur with the swimming behavior of swarmers from Sctyosiphonales.

5. Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>BED</td>
<td>biologically effective dose</td>
</tr>
<tr>
<td>PAR</td>
<td>photosynthetically active radiation ((\lambda = 400-700) nm)</td>
</tr>
<tr>
<td>PI</td>
<td>phototactic index</td>
</tr>
<tr>
<td>R</td>
<td>weighted irradiance dose rate</td>
</tr>
<tr>
<td>UVR</td>
<td>ultraviolet radiation ((\lambda = 280-400) nm)</td>
</tr>
<tr>
<td>UVAR</td>
<td>ultraviolet A radiation ((\lambda = 315-400) nm)</td>
</tr>
<tr>
<td>UVBR</td>
<td>ultraviolet B radiation ((\lambda = 280-315) nm)</td>
</tr>
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</table>

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References


