Oecologia (2004) 138: 193–201
DOI 10.1007/s00442-003-1424-z

ECOPHYSIOLOGY

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# Blue- and green-light signals for gamete release in the brown alga, *Silvetia compressa*

Received: 25 February 2003 / Accepted: 2 October 2003 / Published online: 5 November 2003
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**Abstract** The intertidal brown alga *Silvetia compressa* releases gametes from receptacles (the reproductive tissue) rapidly upon a dark transfer (following a photosynthesis-dependent period in the light, termed potentiation). In this study, the wavelength-dependence of this process was investigated. During the potentiation period in white light (WL), gametes are not released. However, gametes were released during potentiation in blue light (BL), or in low red light/blue light (RL/BL) ratios, but not in RL alone, high RL/BL ratios, or in broadband blue-green light (B-GL) (presence of BL, but absence of RL). RL was as effective as WL for potentiation, i.e., both lead to gamete release following transfer to darkness. Rates of linear photosynthetic electron transport were similar in RL and BL. Gamete release in BL was inhibited by equal amounts of additional narrow-waveband light between the green and red regions of the spectrum, with light-induced gamete release restricted between <491 nm and 509 nm. Very little light-induced gamete release occurred between 530 nm and 650 nm. It is proposed that a BL-responsive photoreceptor is responsible for light-induced gamete release. Transfer of WL-potentiated receptacles to GL near 530 nm resulted in significant de-potentiation and reduced gamete release during a subsequent dark transfer. This effect was not seen at 509 nm or 560 nm and revealed the presence of a second photoreceptor system repressing or counteracting potentiation in the light. We propose that the restriction of gamete release to periods when irradiance is blue-shifted may constitute a depth-sensing mechanism for this intertidal alga, allowing controlled release of gametes at high tide and/or less turbid periods, thus minimizing gamete dilution, and promoting fertilization success.

**Keywords** External fertilization · Intertidal seaweed · Photoreceptor · Reproductive biology · Spawning

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## Introduction

Synchronous release of gametes occurs in different groups of free-spawning marine organisms with external fertilization, often triggered by environmental factors (Harrison et al. 1984; Babcock et al. 1986; Yoshioka 1988; Morgan 1995; Pearson and Brawley 1996; Serrão et al. 1996; Clifton 1997; Pearson et al. 1998; Togashi and Cox 2001). This is interpreted as a mechanism ensuring that large numbers of gametes from different individuals are present in the water column at the same time, increasing the potential for fertilization. Fertilization success in natural populations of free-spawning organisms in the sea is often high, but the consequences of non-ideal conditions (e.g., non-synchronous release, high currents, low population density) can be severe (reviewed by Levitan and Petersen 1995; Yund 2000). Spawning synchrony under the lowest water motion conditions possible might be essential in turbulent environments, where external fertilization success is predicted to be very low due to gamete dilution (Denny and Shibata 1989). Intertidal rocky habitats are often under high water motion and, therefore, for intertidal organisms the timing of gamete release with respect to the phase of the tidal cycle is critical for fertilization success, as well as for the subsequent settlement and attachment of zygotes.

For intertidal marine plants and algae, such as fucoid seaweeds, light is a suitable signal for coordinating gamete release, since sophisticated light-detection systems have evolved in photosynthetic organisms, and because light quantity and quality varies in the marine environment on a variety of time scales, in predictable, as well as stochastic

and chaotic ways. The control of gamete release in fucoid algae is, however, complex, involving integration of inputs from several environmental variables on different timescales. During the reproductive season, gamete release is entrained by semi-lunar or tidal cycles (Brawley 1992; Pearson and Brawley 1996; but see Serrão et al. 1996). On the scale of single tides, it has been established that photosynthetic signals largely control gamete release. Blocking photosynthetic electron transport with DCMU prevents gamete release in all species tested (Serrão et al. 1996). An additional, and ecologically important, level of control over gamete release is water motion: release only occurs in calm conditions in these algae (Pearson and Brawley 1996; Serrão et al. 1996). This has led to the hypothesis that gamete release may occur at slack high tide, when water motion is at a minimum. This is supported by the available empirical data for both estuarine and marine dioecious fucoid algal populations, showing that slack high tide is the period of maximum gamete release (Brawley 1992; Berndt et al. 2002). Gamete release under calm conditions optimizes reproductive success in periodically turbulent environments, an idea supported by the high levels of fertilization success found in natural populations (Brawley 1992; Pearson and Brawley 1996; Serrão et al. 1996; Berndt et al. 2002). The "water motion detector" is linked to inorganic carbon supply for photosynthesis (Pearson et al. 1998); thus, gamete release occurs in the light during periods of carbon-limited photosynthesis.

*Silvetia compressa* [formerly *Pelvetia compressa* (Serrão et al. 1999)] was used as a model in this report. In such self-fertilizing, hermaphroditic species, low tide may be a favourable period for gamete release, if abiotic stresses can be avoided (Johnson and Brawley 1998; Brawley et al. 1999). Both out-crossing and dispersal/gene flow are restricted, but the problems associated with external fertilization outlined above are circumvented. Gamete release in this species can be controlled under laboratory conditions since, although a period of photosynthesis is required to precondition (=potentiate) release (as in other fucoids), synchronous gamete release occurs only after a transfer of receptacles (the reproductive tissue) to darkness (Jaffe 1954). This has allowed an assay system to be developed based on potentiation conditions and gamete release following a short transfer to darkness (Pearson and Brawley 1998).

Previous work suggested that light signalling via a non-photosynthetic pathway is involved in the gamete release response of *S. compressa* (Pearson and Brawley 1998). In this report, we characterize the effects of light quality on gamete release. Our results indicate the involvement of a photoreceptor(s) sensitive to broadband blue-green light (B-GL) and narrowband green light (GL) in both the positive and negative regulation of potentiation and gamete release.

## Materials and methods

### Collection, maintenance and general experimental conditions for *S. compressa*

Branches of *S. compressa* bearing mature receptacles (reproductive tissue) were collected fortnightly from the intertidal zone at Pigeon Point, Calif., USA. The material was wrapped in moist paper and shipped cool in styrofoam containers to Marburg, Germany by express mail (2 days). After arrival, the receptacle-bearing tissue was kept in darkness wrapped in moist paper at 15°C. Individual receptacles were selected for each experiment from this "stock" and used within 10 days. Prior to most of the experiments reported here, receptacles were pre-treated by shaking in seawater (SW) in white light (WL; ca. 30 μmol $m^{-2}$ $s^{-1}$) on a rotary shaker at 120 rpm for 3–4 h. This ensured that receptacles were fully de-potentiated at the beginning of a given experiment [i.e., following this treatment a transfer to darkness did not stimulate gamete release, because water motion de-potentiates receptacles: see Fig. 2a in Pearson and Brawley (1998)].

### Absorbance spectra and photosynthetic action spectra

Absorbance spectra were measured by covering the sensor of a Li-Cor radiometer (Li-Cor, Lincoln, Neb., USA) with a series of interference filters (401–702 nm). Light was provided by a halogen lamp fed through a fibre optic light guide. Incident irradiance was recorded with and without algal tissue (a receptacle, or apical vegetative thallus) between the light and filter/sensor assembly. Absorbance at each wavelength was calculated based on the Lambert-Beer law as $A_\lambda=\log_{10}[I_0/I(x)]$, where $A_\lambda$ is the absorbance of algal tissue at wavelength λ, $I_0$ is the incident irradiance and $I(x)$ is the irradiance after passing through the algal sample. Absorbance spectra were repeated four times and the results shown are data normalized to $A_{681\ nm}=1$.

A modulated chlorophyll fluorometer (PAM 101, Waltz, Germany) was used to estimate relative rates of electron transport (ETR) in receptacles illuminated under a range of wavelengths. Narrow waveband light was delivered to the sample via a fibre-optic light guide by placing interference filters between the light source (slide projector) and the fibre optic. A different branch of the same fibre-optic system carried the modulated light from the fluorometer and the fluorescence signal from the sample. The receptacle was held perpendicular to the fibre optic in a glass vessel containing 25 ml SW with stirring provided by a magnetic stirrer. The entire apparatus was shielded from external light by two layers of black cloth. After dark-adaptation for 15 min, minimum and maximum fluorescence ($F_o$ and $F_m$) were determined to allow calculation of the maximum quantum yield $[(F_m-F_o)/F_m=F_v/F_m]$. Photosynthesis versus irradiance response curves at wavelengths between 431 nm and 650 nm were then constructed by measuring $F_t$ (steady-state fluorescence at time $t$) and $F_m'$ (maximum light-adapted fluorescence), allowing calculation of the effective quantum yield $[(F_m'-F_t)/F_m'=\Delta F/F_m'$; Genty et al. (1989)] at photon flux density (PFD) between 5 and 20 μmol $m^{-2}$ $s^{-1}$. Relative ETR at each PFD were calculated as $\Delta F/F_m'\times PFD\times 0.5\times 0.84$, and the slope of the photosynthesis versus irradiance response was calculated. A linear rate increase at each wavelength was obtained, indicating that photosynthesis was light-limited.

### Red-light: blue-light gradient experiments

RL:BL gradients used horizontal light from two projectors (one above and one below the sample) deflected with mirrors at 45° vertically up and down onto the Petri dish containing the receptacles. Coloured light was obtained by using the appropriate glass filters (BG12 for BL, RG630 for RL), and the intensity of the projector lamp output was adjusted to obtain RL:BL ratios of 0:50,

3:47, 6:44, 12:38, 25:25, 38:12, 44:6, 47:3, and 50:0 μmol $m^{-2}$ $s^{-1}$. WL controls were at 50 μmol $m^{-2}$ $s^{-1}$. In the first experiment, pre-treated receptacles (see above) were first allowed to potentiate for 7 h in RL/BL, and then transferred to a new Petri dish in darkness for 30 min. In the second experiment, potentiation was first carried out in WL (50 μmol $m^{-2}$ $s^{-1}$) for 7 h, followed by RL/BL for 30 min, before transferring the receptacles to darkness for 30 min. Egg release was quantified for the light (potentiation) and dark periods by counting eggs (or a sub-sample) under the dissecting microscope.

### Effects of other wavelengths on BL-mediated gamete release

Receptacles were incubated in 25 μmol $m^{-2}$ $s^{-1}$ of either BL, or broad wavelength B-GL, using glass filters (BG12 and BG38, respectively). For treatments, additional narrow waveband light of 2.5 μmol $m^{-2}$ $s^{-1}$ between 495 and 651 nm was added to the background BL (experiment 1), or 25 μmol $m^{-2}$ $s^{-1}$ between 495 and 590 nm (experiment 2). Narrow waveband light was produced by passing WL through interference filters.

### Effects of specific wavelengths on potentiated receptacles

Pre-treated receptacles (see above) were allowed to potentiate in WL (30 μmol $m^{-2}$ $s^{-1}$) for 7 h. They were then transferred to narrow waveband colored light between 491 and 650 nm, produced by passing WL through interference filters, for 30 min (30 μmol $m^{-2}$ $s^{-1}$). Controls were transferred after potentiation either to WL or to broad waveband BL (BG12 glass filter). All treatments were then transferred to darkness, and gamete release was assessed at each stage (WL potentiation, colored light transfer, and dark transfer) by counting eggs released under a dissecting microscope. A further control was maintained in darkness throughout, with appropriate transfers of SW medium at the same time as the other treatments. In other experiments, the specific effects of green wavelengths were investigated by comparing the effectiveness for receptacle potentiation of narrow waveband light at 530 and 560 nm compared with the effectiveness of WL.

## Results

### Absorption spectra and photosynthetic action spectra

Absorbance spectra for reproductive receptacle and apical vegetative tissue (adjusted for $A_{681\ nm}$=1) are similar between 460 and 700 nm (Fig. 1). However, receptacles (inset picture, Fig. 1) absorb BL between 400 and 460 nm more effectively than the morphologically similar vegetative apices.

Plotting the slope of ETR versus irradiance curves against wavelength, for wavelengths between 431 and 650 nm, showed that photosynthetic electron transport was most efficient at 431 nm and between 591 and 650 nm, and least efficient at 531 and 560 nm (Fig. 2). The values of ΔETR were very similar at the latter two wavelengths.

### Effects of RL:BL on potentiation and gamete release

Light-induced gamete release (i.e., during potentiation) occurred prior to a dark transfer in BL alone, but was negligible in RL alone or in WL (Fig. 3). This was true

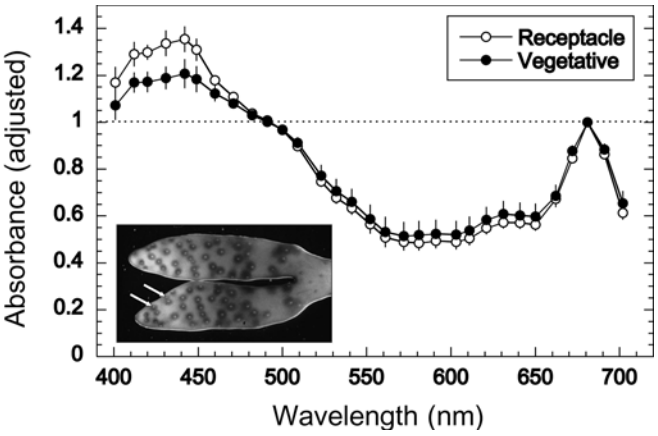


**Fig. 1** Absorbance curves for *Silvetia compressa* receptacles (*open circles*) and vegetative apices (*filled circles*) between 400 and 700 nm. The data were normalized by setting absorbance at 681 nm=1. Values are means±SE ($n$=4). The *inset* shows a

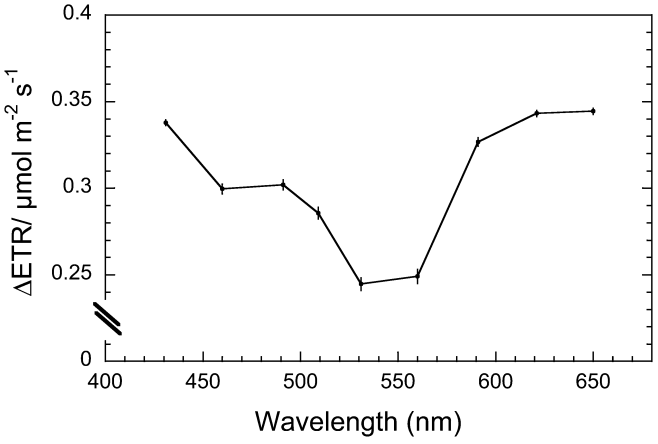


**Fig. 2** Initial slopes of electron transport rate (ETR) versus irradiance curves for *S. compressa* receptacles in narrow waveband light between 430 and 650 nm. ETR was calculated from the effective quantum yield of PSII photochemistry ($\Delta F/F_m'$) for several irradiances in each waveband. Values are means±SE ($n$=3 independent response curves)

both in experiment 1, where receptacles were potentiated in RL:BL (Fig. 3a), and in experiment 2, where potentiation was in WL followed by a short period (30 min) in RL:BL (Fig. 3b). However, the total numbers of eggs released (light period plus dark transfer) did not differ between treatments in either experiment (ANOVA experiment 1 $F_{9,20}$=0.952, $P$=0.505; experiment 2 $F_{9,20}$=0.674, $P$=0.723). In experiment 1 there was a significant difference in light-induced gamete release between BL and all the other treatments ($F_{9,20}$=2.65, $P$=0.033; Fisher's LSD tests of differences between means; Fig. 3a). The percentage of egg-release stimulated in low RL:BL in experiment 1 was between ca. 30% for BL alone and ca. 10% in the presence of RL between 3–38 μmol $m^{-2}$ $s^{-1}$ (Fig. 3c). In contrast, following 7 h in WL, a 30 min transfer to BL stimulated almost 90% of the total egg release in experiment 2 (Fig. 3c). A linear decline in light-induced release was observed with increasing RL:BL

between 3:47 and 25:25 μmol $m^{-2}$ $s^{-1}$ RL:BL (Fig. 3c). Statistical analysis indicated a highly significant effect of decreasing RL:BL on light-induced (but not total) egg release ($F_{9,20}$=11.79, $P$≤0.0001). The results indicate either that BL has a specific stimulatory effect on gamete release that is inhibited by RL (implying that the RL:BL ratio of WL is sufficient to inhibit BL-mediated release), or that RL has a specific inhibitory effect on gamete release in the light (but not on potentiation for subsequent gamete release in the dark).

## Effects of other wavelengths on BL-mediated gamete release

We tested the effects of various wavelengths of light on the putative BL-mediated gamete release mechanism. Egg release from receptacles incubated for 6 h in 50 μmol $m^{-2}$ $s^{-1}$ BL or B-GL (BG12 and BG38 glass filters, respectively), or 25 μmol $m^{-2}$ $s^{-1}$ BL plus 25 μmol $m^{-2}$ $s^{-1}$ light at 495, 515, 530, 550, or 590 nm is shown in Fig. 4a. No significant differences in total egg release (following dark transfer) were found, except for controls kept in darkness, where release was negligible (results not shown). As a percentage of the total, light-induced release was not significantly different between the BL control and BL+495 nm, and BL+515 nm treatments, but lower in BG38 light, BL+530 nm, BL+550 nm, and BL+590 nm, relative to the BL control (Fig. 4a; one-way ANOVA of arcsine-transformed data $F_{6,14}$=12.1, $P$<0.001; and Dunnett's tests of the treatment means with BL control). In a second experiment, the added irradiance was reduced from 25 to 2.5 μmol $m^{-2}$ $s^{-1}$. No inhibition of gamete release in the BL background (25 μmol $m^{-2}$ $s^{-1}$) was observed between 495 and 590 nm, whereas gamete release appeared to be inhibited at wavelengths ≥611 nm (Fig. 4b; one-way ANOVA of arcsine-transformed data $F_{9,20}$=3.569, $P$=0.009). However, comparisons of means (Dunnett's test) only detected a significant difference between the BL control and 651 nm at the $P$=0.05 level. The results indicated that (1) green and orange wavelengths are sufficient to inhibit the BL-stimulated release, and therefore that (2) inhibition of light-induced egg release is not a specific RL effect. (3) Longer wavelengths (orange/red) were more effective in counteracting the BL-mediated gamete release at lower irradiance relative to shorter green and orange wavelengths.

## Effects of specific wavelengths on potentiated receptacles

To investigate further the wavelength response of light-induced gamete release, we potentiated receptacles in WL (30 μmol $m^{-2}$ $s^{-1}$ for 7 h) and then transferred them to narrow waveband light between 491 and 650 nm for 30 min. The same receptacles were then transferred to darkness for 30 min. The relative numbers of eggs released during potentiation in WL, transfer to coloured light and

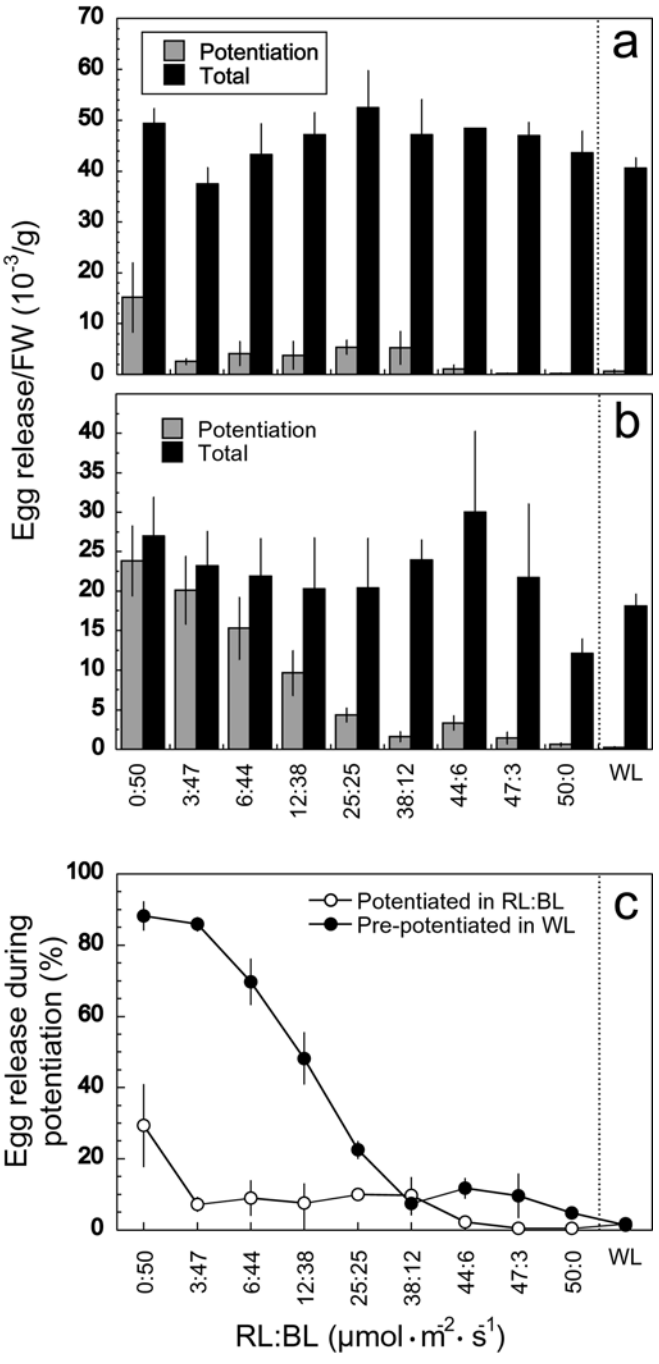


**Fig. 3a–c** Gamete release from receptacles in response to red (RL) and blue (BL) light, either alone or in combination. **a** Potentiation was carried out for 7 h in RL/BL at various ratios (plus WL control) and gamete release was assessed at the end of this period (*grey bars*) and following a transfer of the potentiated receptacles to darkness for 30 min (*black bars*). **b** Potentiation was carried out for 7 h in WL, followed by a transfer to RL/BL for 30 min, and a second transfer to darkness for 30 min. Gamete release was assessed at the end of the WL+RL/BL potentiation (*grey bars*), and after the dark transfer (*black bars*). A control was potentiated in WL only for 7.5 h, and transferred to darkness. **c** Data from experiments shown in **a** and **b** expressed as the percentage of total gamete release occurring during potentiation in the light. Values are means±SE ($n$=3)

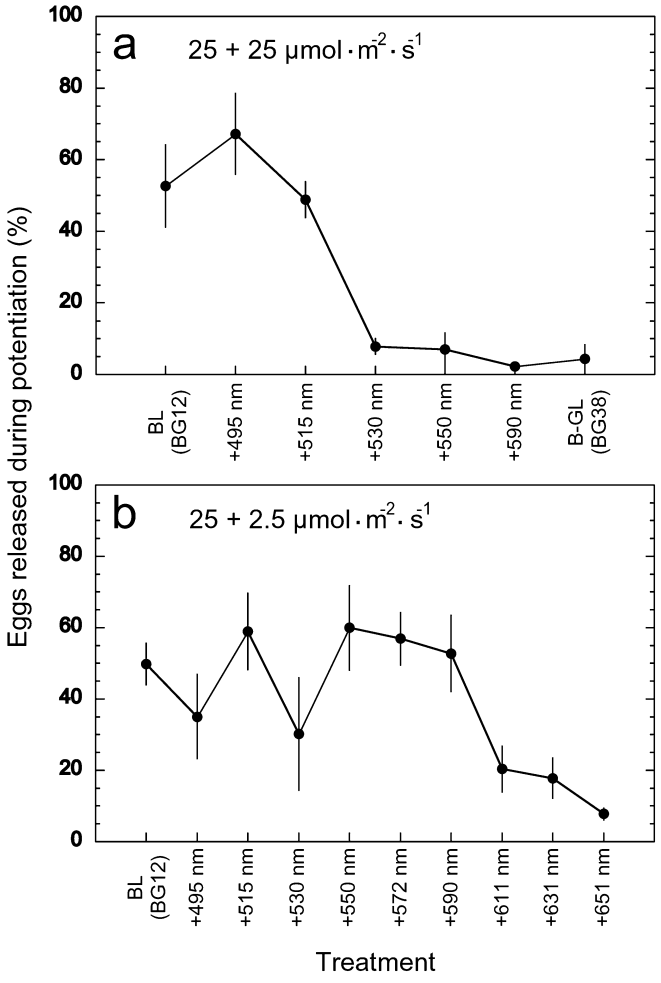


**Fig. 4a, b** Effects of additional narrow waveband irradiance on gamete release in a BL background. **a** Effects of BL or BL+specified narrow waveband at the same irradiance. Broadband B-GL (without transmission in the red region of the spectrum) was used as a control. **b** Effects of BL or BL+specified narrow waveband at 0.1×irradiance. Broadband B-GL (without transmission in the red region of the spectrum) was used as a control. Values are means±SE ($n$=5)

transfer to darkness was used to assess the effectiveness of a particular waveband in stimulating gamete release. WL and wavebands between 531 and 650 nm induced very little light-induced release, whereas broad waveband BL, and wavebands centred around 491 and 509 nm, induced over 90% of the total gamete release after transfer from WL to coloured light (Fig. 5; ANOVA of release during transfer $F_{8,18}$=20.341, $P$<0.0001, Fisher's LSD tests). Thus, the cut-off in effectiveness of the light-induced gamete release lay between 509 nm and 531 nm.

Total gamete release was significantly lower at 531 nm than at either lower (BL, 491 nm, 509 nm) or higher (560 nm, 591 nm) wavelengths (ANOVA of total release $F_{8,18}$=3.265, $P$<0.018, Fisher's LSD test). This result suggested the existence of a GL effect near 530 nm that acts to reverse the potentiation effect of WL. WL and GL at 560 nm were equally effective in potentiation, since similar numbers of eggs were released following a dark transfer (Fig. 6). However, following potentiation in GL at

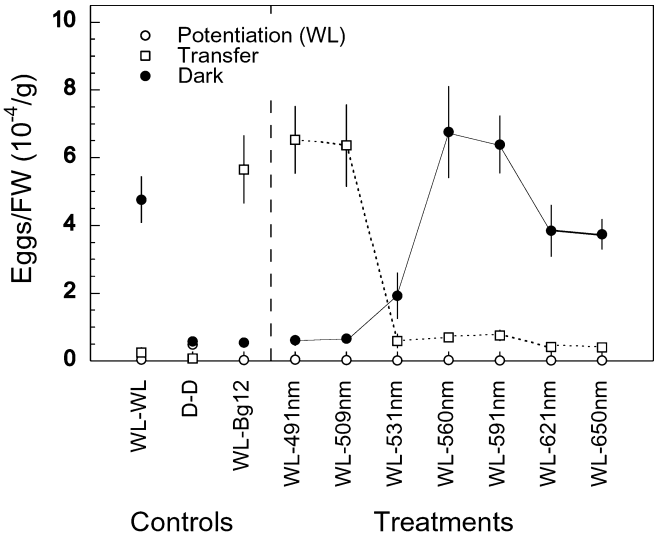


**Fig. 5** Specific triggering of gamete release by BL and B-GL. Effects of narrow waveband irradiation on gamete release during potentiation (*grey bars*) and following transfer to darkness (*black bars*). Receptacles were initially potentiated in WL for 7 h (gamete release shown by *open bars*), then transferred to coloured light for 30 min, followed by 30 min in the dark. Controls were kept either in WL or in darkness for the duration of the experiment. Values are means±SE ($n$=5)

530 nm, gamete release in darkness was reduced by ca. 50% (ANOVA of total gamete release $F_{3,8}$=61.446, $P$<0.0001, Fisher's LSD test). The effect appears not to be directly linked to reduced photosynthetic efficiency in GL, since ΔETR values are similar at 530 and 560 nm (Fig. 2). The data therefore suggest that light of wavelengths near 530 nm specifically inhibits and reverses potentiation (Figs. 6, 7). Furthermore, GL inhibition of potentiation occurs at wavelengths just longer than the cut-off for BL-mediated light-induced gamete release (Fig. 5).

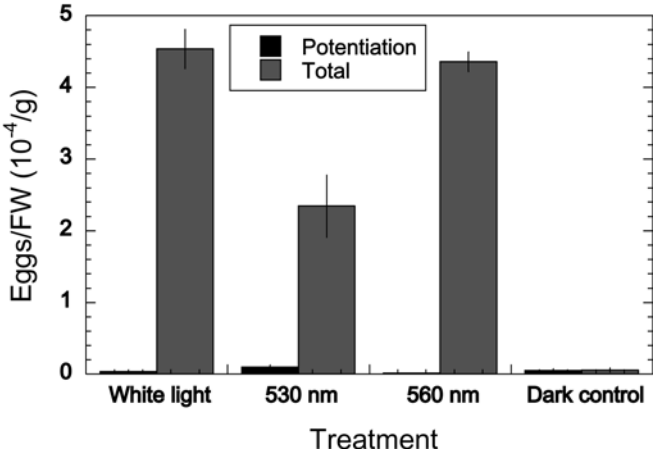


**Fig. 6** Wavelength-specific depotentiation of gamete release in response to GL near 530 nm. Receptacles were potentiated in equal irradiances of WL, GL at 530 nm, or GL at 560 nm, before being transferred to darkness for 30 min to stimulate gamete release. Gamete release was quantified during light (*black bars*) and dark periods (*grey bars*). Controls were kept in darkness throughout the experiment. Values are means±SE ($n$=5)

## Discussion

In this study we report on the role of photosynthesis-independent light signaling for gamete release in the brown alga *S. compressa*, preliminary evidence for which has been published previously (Pearson and Brawley 1998). This work extends our understanding of the environmental signals controlling gamete release in this alga. The major findings were that:

1. Light-induced gamete release is a specific BL response (contradicting previous findings—see below) with upper wavelength limit between 515 and 530 nm, although photosynthesis-dependent potentiation of dark-induced gamete release occurs in PAR between 430–680 nm (wavelength range tested);
2. BL-induced gamete release (but not potentiation) is inhibited non-specifically by additional irradiance at all wavelengths between the green and red regions of the spectrum;
3. Potentiation of dark-induced gamete release is partially inhibited by narrow-waveband GL near 530 nm, and WL-potentiated receptacles are partially de-potentiated by short exposures to 530 nm irradiance;
4. The BL and GL effects on gamete release do not appear to be directly related to photosynthesis, since the initial slopes of photosynthetic ETR versus irradiance curves are similar in BL and RL; and
5. Since the initial slopes of light-limited photosynthetic ETR versus irradiance curves are similar at 530 nm, which inhibits potentiation, and at 560 nm, which does not.

Experiments in RL and BL gradients showed that total gamete release (light-induced + dark-induced) was not significantly different in RL, BL, and WL. However, light-induced gamete release was limited to BL or to low RL:

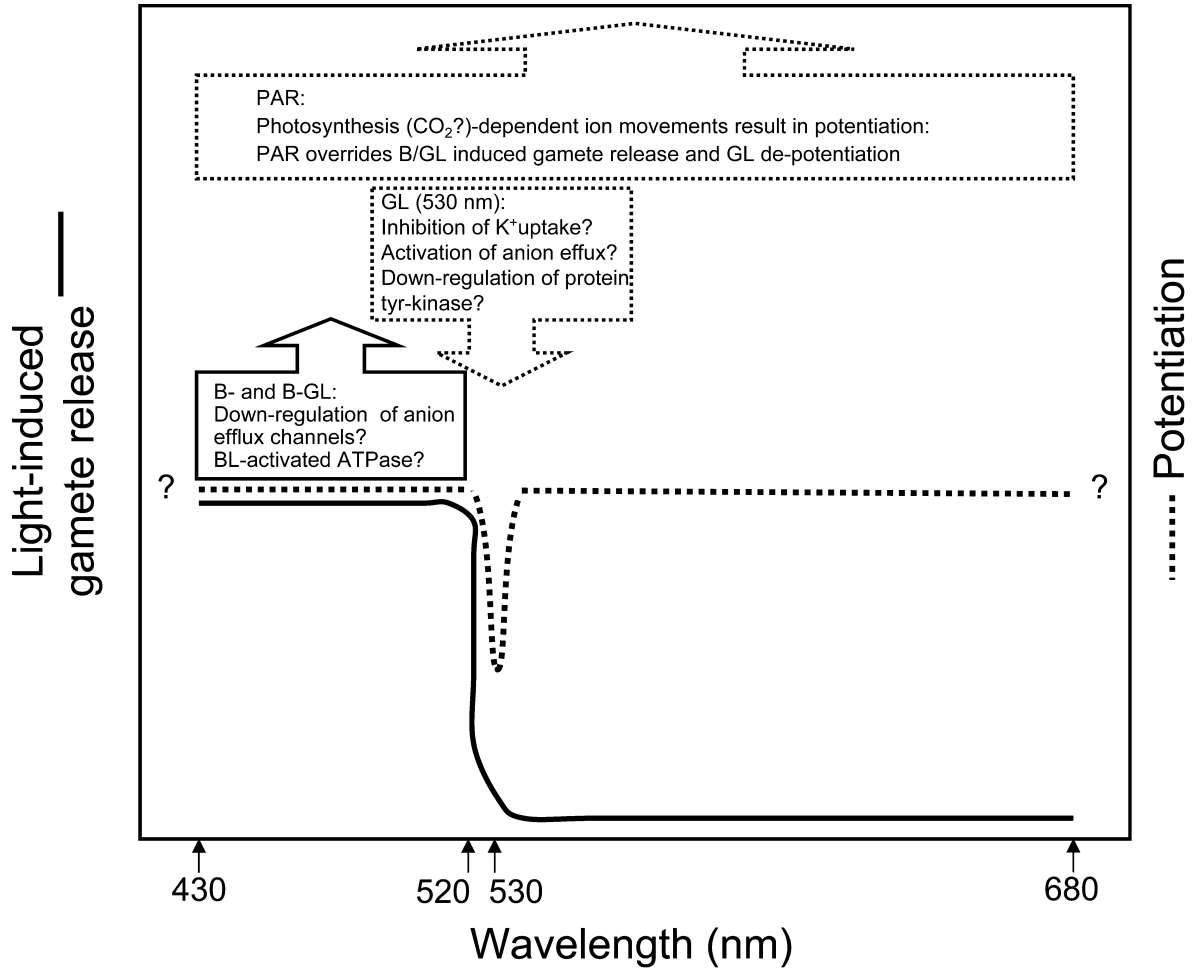


**Fig. 7** Model of the regulation by light quality of light-induced gamete release (*solid line*) and potentiation (*broken line*) in *S. compressa*. Direction of *arrows* on text boxes indicates positive (*up*) or negative (*down*) regulation. The model integrates previously published observations/data to indicate possible downstream targets of the light quality effects identified. BL and B-GL below ca. 520 nm induces gamete release in the light, implicating a possible link to negative regulation of S-type anion channels (Pearson and Brawley 1998), perhaps involving a BL-activated ATPase. Narrow-band GL around 530 nm inhibits potentiation, as do the $K^+$-channel inhibitor $TEA^+$, L-(-)malate, and a specific inhibitor of protein tyrosine kinases (Pearson and Brawley 1998). Photosynthetically active radiation (PAR) leads to potentiation under favourable (low $CO_2$) conditions without light-induced release, and thus overrides the antagonistic effects of BL and GL signals

BL ratios. After prior potentiation in WL, BL or low RL: BL had a triggering effect, i.e., a relatively rapid expulsion of gametes occurred within 30 min of transfer to coloured light. BL alone and a RL:BL ratio of 3:47 μmol $m^{-2}$ $s^{-1}$ were the most effective treatments for the response, with the proportion of total gamete release in the light decreasing from nearly 90% in BL to ca. 20% in 50:50 RL:BL. Light-induced gamete release was much less efficient when receptacles were potentiated in mixtures of RL and BL; even in BL alone, only ca. 30% of gametes were released in the light (Fig. 3b, c) although this figure reached ca. 50% in other experiments (see Fig. 4).

Light-induced gamete release involves a specific response to BL, since additional wavelengths added to a BL background inhibited light-induced release over a broad range between the green and red regions of the spectrum (Figs. 3, 4). Similarly, when WL-potentiated receptacles were transferred to narrow waveband light, gamete release was induced at 495 and 515 nm, but not at wavelengths between 530 and 650 nm. Potentiation is a photosynthesis-dependent process that does not occur in darkness (Serrão et al. 1996; Pearson and Brawley 1998; Pearson et al. 1998), while the data from this study show that light-induced gamete release is a specific BL response. Gamete release is triggered from potentiated receptacles upon transfer to both BL and darkness (Pearson and Brawley 1998; this study). However, unlike darkness, BL is also sufficient to potentiate receptacles, presumably via a photosynthetic mechanism. Thus the experimental dissection of the BL response is complicated by the co-occurrence of gamete release and potentiation in BL, with only the release of gametes being a specific (and presumably non-photosynthetic) BL response. It remains unclear, however, how BL-mediated gamete release is inhibited in the presence of other wavelengths.

Short pulses of BL increase rates of RL-saturated photosynthesis in brown algae (Dring 1989; Schmid and Dring 1992), although members of the fucales display the lowest levels of stimulation (10–15%; Forster and Dring 1992; Schmid et al. 1994). BL activates the release of inorganic carbon from internal stores (Schmid and Dring 1996), as well as a rapid surface acidification (Schmid and Dring 1993) that may serve to increase $CO_2$ supply. Given the dependence of potentiation on inorganic carbon supply (Pearson et al. 1998), it is reasonable to ask whether these aspects of BL stimulation of photosynthesis and BL-induced gamete release are related.

Figure 7 presents a model of the effects of light quality on potentiation and light-induced gamete release, together with possible downstream components of the signaling pathway found previously (Pearson and Brawley 1998; Pearson et al. 1998). PAR is a positive regulator of potentiation, acting via the photosynthesis-dependent pathway that involves inorganic carbon supply (Pearson et al. 1998). BL is also a positive regulator because it stimulates light-induced gamete release. The effect of BL is similar to the effects of slow-type (S-type) anion channel inhibitors (Pearson and Brawley 1998). Thus, BL-signaling and inhibition of (putative) S-type anion channels within the *Silvetia* receptacle have similar downstream effects on gamete release. In higher plant guard cells, turgor gain occurs via the BL-stimulated activity of a plasma-membrane $H^+$-ATPase (Assmann 1993). However, S-type anion channels also play an active role in controlling guard cell opening, as well as closure, and inhibition of S-type anion channels in *Vicia faba* guard cells resulted in stomatal opening even under low KCl conditions that would usually prevent it (Schwartz et al. 1995). Thus, for guard cells, the same outcome in terms of turgor regulation (i.e., stomatal opening) can be obtained experimentally by stimulating the $H^+$-ATPase with BL, or by preventing anion efflux via S-type anion channels. However, the presence of BL-activated anion channels in higher plants (Cho and Spalding 1996) means that such a correlation must remain speculative at the present time, until the characteristics of (putative) anion channels in *S. compressa* are resolved.

The results obtained in this study partially contradict previous results (Pearson and Brawley 1998), which suggested that BL was ineffective for potentiation, although it was found in the same study that BL triggered rapid gamete release from WL-potentiated receptacles. Dichroic colour-separation BL filters were used in Pearson and Brawley's (1998) study, which can transmit longer wavelengths if light incident on the filter deviates from normal. The BL glass and interference filters used in this present work are, therefore, more reliable than dichroic filters, and this could account for the discrepancy in the results. The effects of BL reported here were consistently verified in several independent experiments with different batches of receptacles.

GL near 530 nm caused a de-potentiation and subsequent reduction in the total number of gametes released from WL-potentiated receptacles (Fig. 5). This was not a photosynthetic effect since GL at 560 nm was as effective as WL for potentiation, while GL at 530 nm was only 50% as effective (Fig. 6), despite 530 and 560 nm being similarly effective for photosynthetic electron transport (Fig. 2). The $K^+$ channel inhibitor, TEA (tetraethylammonium chloride), as well as malate [an activator of anion channels in guard cells (Hedrich and Marten 1993; Hedrich et al. 1994, 2001)], partially inhibit potentiation in WL (Pearson and Brawley 1998). The partial inhibition, and reversal, of potentiation by GL at 530 nm that we observed further suggests a possible link between light signals and the turgor-related ion movements required for gamete expulsion (Fig. 7). Regulatory effects of GL might involve inhibition of protein tyrosine kinase(s), since a specific inhibitor of tyrosine kinases blocked potentiation in Pearson and Brawley's (1998) study, and/or activation of anion channels.

Recent observations of $K^+$ and $Cl^-$ localization within *Silvetia* receptacles using X-ray microanalysis has indicated that the efflux of both ions is not limited to periods of gamete release, but also occurs during potentiation. However, periods of particularly extensive ion efflux into the extracellular matrix after dark-transfer of potentiated receptacles were observed, and it was suggested that

crossing this threshold might trigger an irreversible commitment to gamete release (Speransky et al. 2001). The same authors hypothesize that cycles of ion efflux, which may be part of a turgor control mechanism in fucoid algae (Nuccitelli and Jaffe 1975, 1976), may have been adapted for controlling gamete release in receptacle (reproductive) tissue.

Based on our results, chlorophylls *a* and *c*, which absorbs RL and BL, and fucoxanthin, which absorbs broadly in the blue and green regions, can be discounted as photoreceptors for BL-induced gamete release and GL depotentiation. Several BL or UV-BL photomorphogenetic or phototropic effects have been documented in brown algae, e.g., thallus growth form (Dring and Lüning 1975), BL stimulation of photosynthesis (Dring 1989; Schmid and Dring 1992), zygote polarity (Kropf 1992), and chloroplast movements (Haupt 1983; Nultsch 1984). The BL-dependent photopolarization of *Silvetia* zygotes is coupled to cyclic GMP (Robinson and Miller 1997), and eggs of *S. compressa* were recently reported to contain relatively high concentrations of retinal, a compound only found in biological systems such as the chromophore of opsin-type photoreceptors (Robinson et al. 1998). It has, therefore, been suggested that opsin-like photoreceptors may be present in fucoid algae (Robinson et al. 1998, 1999). Certain bacterial halorhodopsins may operate in response to both BL and GL, with BL-induced proton pumping and GL-induced chloride transport in the same direction across the membrane (Bamberg et al. 1993). Although it is possible that opsins may play a role in controlling gamete release in *S. compressa*, experiments with cGMP analogues and guanylyl cyclase inhibitors have so far failed to produce any effects on potentiation and gamete release (G. Pearson and R. Schmid, unpublished data).

Flavin-binding phototropins appear to have been confirmed as the principal photoreceptors in the guard cell response to BL (Kinoshita et al. 2001). Currently unresolved is the additional role of the chloroplastic carotenoid zeaxanthin, which was previously implicated (Zeiger and Zhu 1998; Frechilla et al. 1999), but see Eckert and Kaldenhoff (2000). In particular, the GL reversal of BL-stimulated stomatal opening, recently reported by Frechilla et al. (2000), hints at the existence of striking parallels between stomatal regulation in plants and the control of gamete expulsion in brown algal.

## Consequences of light-quality control of gamete release in natural populations

In order for free-spawning (externally fertilizing) marine organisms to achieve successful fertilization in the sea, avoidance of gamete dilution in the water column, and synchronous gamete release from many individuals, are desirable traits to avoid sperm limitation. This, and previous work, shows that fucoid algae possess at least two mechanisms to control and synchronize gamete release:

1. A photosynthesis-dependent pathway based on sensing inorganic carbon, that is inhibited by high water motion (Pearson and Brawley 1996, 1998; Serrão et al. 1996; Pearson et al. 1998);
2. A photosynthesis-independent pathway sensitive to the spectral characteristics of the water column (this study).

Taken together, this suggests that the B-GL environment in many coastal waters may induce gamete release from receptacles at slack high tide, if water motion is below inhibitory levels. *S. compressa* also releases gametes within the canopy when exposed to air at low tide (Johnson and Brawley 1998; Brawley et al. 1999). This is presumably due to the triggering effect of darkness on potentiated receptacles. On shores where calm periods are infrequent, even at high tide, gamete release at low tide may be an advantage for a self-fertilizing hermaphrodite, by assuring the possibility of reproduction during what might be the only calm window of opportunity for successful external fertilization. The balance between release at high tide and low tide may have important consequences for the population genetic structure in this alga, by influencing gamete dispersal and, therefore, the level of outcrossing and gene flow. A recent field investigation of gamete release from a dioecious (obligate outcrossing) fucoid species, *Fucus vesiculosus*, has also suggested that gamete release is largely restricted to a short period close to peak high tide (Berndt et al. 2002). There is no dark-induction of gamete release in *Fucus* species (G. Pearson, personal observation). The proximity of the effective wavelengths for the opposing processes of light-induced gamete release (effective in BL and B-GL to at least 515 nm) and de-potentiation (maximum measured effectiveness at 530 nm) appears at first curious. We propose, however, that the ability of receptacles to de-potentiate in GL reflects the relationship between turbidity (and therefore often turbulence) and a shift in light transmission in the water column towards longer wavelengths. If these hypotheses are supported by empirical field studies of natural populations, the photosynthesis-dependent and -independent pathways may interact to provide a high degree of control over gamete release, reflecting the selective pressure for synchronous gamete release under conditions favorable for successful external fertilization.

**Acknowledgements** G.P., E.S., and M.D. express our gratitude to Professor Paul Galland for hosting our visits to his laboratory in Marburg, his generosity in allowing us access to equipment, as well as helpful discussions. Many and ever-reliable algal collections were made by Diana Steller, University of California at Santa Cruz, Calif., USA. Silvia Busch (Philipps-Universitaet, Marburg, Germany) provided fine selections of bread for breakfast. Funding by the COST-49 program (EU), the Portuguese Science Foundation (FCT) project GAMETE (POCTI/BSE/39431/2001), and an FCT and European Social Fund (FSE) fellowship to G.P.

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