End-of-Day Light Control of Growth and Pigmentation in the Red Alga *Porphyra umbilicalis* (L.) Kützing

F. L. Figueroa, J. Aguilera and F. X. Niell

Departamento de Ecología, Facultad de Ciencias, Universidad de Málaga, E-29071 Málaga, Spain

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The effects of end-of-day light pulses on thallus expansion, cell size, pigmentation and intracellular accumulation of C and N in the red alga *Porphyra umbilicalis* were analysed. Red (R) light pulses applied at the end of 12 h white light photoperiod for 14 d stimulated thallus expansion and produced an increase in cell size. Growth rate was higher after red (R) than after blue (B) light pulses. Red or blue light affect thallus expansion, but not the thallus weight, and was partially reversed by far-red (FR) light pulses.

Red light pulses promoted chlorophyll *a* accumulation whereas B light pulses stimulated the accumulation of phycoerythrin, phycocyanin and soluble protein. The effects of R or B light pulses on pigmentation were partially reversed by FR light, except for phycocyanin. Intracellular C content was similar under both R and B light treatments and higher than after FR light pulses and non-irradiated control. However, intracellular N content was higher after end-of-day R than after end-of-day B light pulses. Total absorptance (400–760 nm) of the thalli was higher after B than R light pulses. However, the ratio of total absorptance and concentration of total pigments (chlorophyll and biliproteins), used as index of light absorption efficiency, was higher after R than after end-of-day B light pulses. The efficiency of light absorption decreased after the application of FR light pulses. These observations explain the higher growth rate, in terms of thallus expansion, after R than after B end-of-day light pulses and FR treatments. The possible action of phytochrome and a B light photoreceptor in the control of growth and pigmentation in *Porphyra umbilicalis* is suggested.

Introduction

Light plays a very important role as a factor controlling both morphogenesis and metabolism in algae (Dring, 1988; Rüdiger and López-Figueroa, 1992). Light quantity and light quality have strong influence on vegetative development (Dring, 1988), reproductive induction (Dring and Lüning, 1983), growth (Duncan and Foreman, 1980; Lüning, 1992) and development of metabolic processes (Rüdiger and López-Figueroa, 1992). In algae, blue, green and red/far-red photoreceptors are involved in the control of morphogenesis and metabolism (Rüdiger and López-Figueroa, 1992). Most vegetative and reproductive development reported in algae are controlled by a blue light photoreceptor (Dring, 1988) but control by phytochrome of spore formation in *Porphyra* (Dring, 1967) and algal growth, for example, in various microalgae (Lipps, 1973) and in the macroalgae *Chara* (Rethy, 1968) and *Nereocystis* (Duncan and Foreman, 1980) have also been reported.

Among the metabolic responses affected by light quality, pigmentation has been extensively studied. Chl *a* accumulation is controlled by a B light photoreceptor *i.e.* in green microalgae (Senger and Bauer, 1987) and by phytochrome and a B light photoreceptor in the green macroalga *Ulva rigida* (López-Figueroa and Niell, 1989) and red macroalgae *Corallina, Porphyra* and *Chondrus* (López-Figueroa *et al.,* 1989 b; López-Figueroa, 1991). Biliproteins in red algae are controlled by red/green light, similar as in some cyanobacteria, and by phytochrome (López-Figueroa *et al.,* 1989 b; López-Figueroa and Niell, 1990; López-Figueroa, 1991).

Abbreviations: A, absorptance; A_{566}, A_{624} and A_{680} peak absorptances of phycoerythrin, phycocyanin and chlorophyll respectively; B, blue light; BP, biliprotein (PE + PC); Chl *a*, chlorophyll *a;* DA, disc area; DW, dry weight; FR, far-red light; PC, phycocyanin; PE, phycoerythrin; R, red light; RF, reflectance; SP, soluble protein; SRA, screen reference area; T, transmittance; TA, total absorptance (λ = 400–760 nm); TCA, total cell area; WL, white light.

Reprint requests to Prof. Dr. F. L. Figueroa.
Telefax: 3452 132000.

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The most frequently cited works used very artificial light treatments (short pulses followed by various hours in darkness, continuous light) and usually short periods (hours). The treatments used in this work are also artificial but are based in the fact that at dusk, the radiation is enriched in determined wavelengths, for example, at dusk blue:red ratio was greater and red:far-red ratio lower than at daylight (López-Figueroa, 1992). Dramatic changes of underwater R:FR and B:R ratios were detected at the depth where *Porphyra* grow (López-Figueroa, 1992); these changes were related with the daily changes of biliprotein concentration. Generally, on the other hand, growth, morphogenesis and pigmentation have separately been analyzed and there is little information on the relation between absorptance (*i.e.* pigmentation) and growth rate in macroalgae.

The aim of this work was to study growth and pigmentation simultaneously and to determine which photoreceptor systems act in these responses. In order to determine the possible action of phytochrome and/or a B light photoreceptor in the control of vegetative and metabolic development, end-of-day light pulses of different light qualities: red (R), far-red (FR), blue (B), R + FR and B + FR were applied to algae growing in 12 h white light (WL):12 h darkness cycles. The long-term experiments using end-of-day light pulses are an alternative approach to understand the photocontrol of algal growth because photosynthetic effects can be separated from photomorphogenesis.

Materials and Methods

Wintering specimen of the macroscopic phase of *Porphyra umbilicalis* (L.) Kützing were collected on rocky shores on coast of Lagos (Málaga, Spain) and incubated in the laboratory for 5–6 days at 15 °C in seawater with a low level of nitrogen (1–2 μM), under air agitation at a rate of 3 l min^{-1}, in 12 h light:12 dark regimes at photon fluence rate of 60 μmol m^{-2} s^{-1}. After this time, the algae presented the characteristics of N-limited algae: low content of pigments and a high C:N level (see "Initial" in Table I). Then, 80 circular pieces (discs) of *Porphyra* of 0.9 cm of diameter were transferred to flasks containing 3 l Provasoli medium (Starr and Zeikus, 1987). Algae were cultivated during the experiment under 12 h WL:12 h darkness for 14 days. Every day, at the end of the light period, 20 min pulses of different light qualities were applied: red (R), far-red (FR), red + far-red (R + FR), blue (B) and B + FR. Photon fluence rate between 400 and 800 nm was 60 μmol m^{-2} s^{-1} in WL, 23 μmol m^{-2} s^{-1} for B and R light and 80 μmol m^{-2} s^{-1} for FR light.

Spectra and measurements of light sources

Four Silvania F 20 W/CW fluorescent lamps were used as WL source. Blue light was obtained from four General Electric 40 W/B blue fluorescent lamps filtered throughout two plexiglass filters PG 627 and PG 602 (supplied from Rhöm). Red light was obtained from four General Electric 40 W/R fluorescent lamps filtered with two plexiglass PG 502 red filter (supplied from Röhm). Far-red light was provided simultaneously by: (1) Linestra lamps (Osram NU 4 20 W) filtered through one red plexiglass (PG 501) and two blue plexiglass (PG 627) and (2) halogen lamp (100 W) from a slide projector filtered with a Schott filter RG 715. The spectra are shown in Fig. 1. Photon fluence rates were obtained by means of a calibrated Licor 1800-UW spectroradiometer.

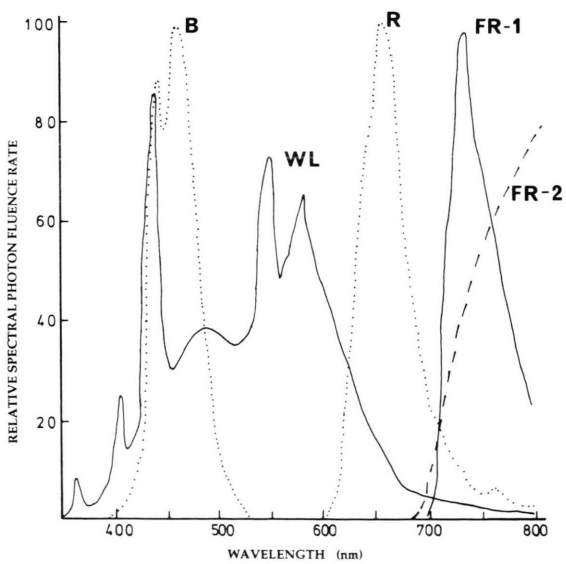

Fig. 1. Relative spectral photon fluence rate of the light sources used, B = blue light, R = red light, WL = white light, FR-1 = halogen lamp with Schott RG 715 filter, FR-2 = Linestra lamps with 2 PG 627 and 1 PG 501 plexiglass filters (see Materials and Methods).

Pigment and soluble protein determinations

Photosynthetic pigments were determined at the beginning of the treatments (initial) and after 14 d incubation. Four replicates from two different experiments were obtained. Chlorophyll *a* (Chl *a*) was extracted in acetone (90%) neutralized with Na_2CO_3 and determined spectrophotometrically using a Beckman DU-7 spectrophotometer according to the equations of Jeffrey and Humphrey (1975). Biliprotein, phycoerythrin (PE) and phycocyanin (PC) and soluble protein (SP) were extracted in phosphate buffer 0.1 M, pH 6.5 at 4 °C containing 10 mM EDTA-Na_2 and 4 mM phenylmethylsulfonil fluoride (PMSF). Biliprotein concentration was determined spectrophotometrically according to Beer and Eshel (1985). Soluble protein was determined by Coomassie Blue G-250 according to Bradford (1976).

Intracellular C and N

Total intracellular C and N contents were determined by means of a Perkin-Elmer analyzer model 2400 CHN.

Thallus absorptance

Porphyra thallus absorptance spectra (3–4 replicates) were determined using a spectroradiometer of Li-Cor model Li-1800 UW connected to an integrating sphere (Li-1800-12). Wet discs of *Porphyra* located between two slide covers were used. The transmittance (T) and the reflectance (RF) of the thallus were determined at 1 nm intervals between 400 to 760 nm. The absorptance (A, the fraction of light absorbed by the thallus) was determined by the expression A = 1-T-RF. Peak absorptances of PE (λ = 566 nm), PC (λ = 624 nm) and Chl *a* (λ = 680 nm) were obtained by subtraction of the values of absorptance at 760 nm. The total absorptance (TA) was determined by numerical integration of absorptance between 400 and 760 nm for each 1 nm.

Morphometric measurements of whole thallus and cells

Ten to fifteen discs of two experimental sets were collected for analysis. They were weighed in a microbalance Slater AND (ER-GOA) with a sensibility of 0.1 mg in order to obtain fresh weight (FW). The data are expressed in dry weight (DW) according to the expression FW = 3.453 DW. The diameter of the discs was determined using a rule and the disc area (DA) was estimated considering *Porphyra* discs as circles (DA = π r^2). Cell arrangement and shape in surface view of the thalli (fixed in 80% glycerol) were observed by means of a Fluovert FV Leitz microscope connected to a Leica Quantimet 500 image analysis equipment. Cell area (CA) was determined for each light treatment, at least from 150 cells on 8–10 sets from three different discs in a screen reference area (SRA) of 8346 μm^2. The number of cells per SRA and the percentage of the screen reference area occupied by cells (TCA) were determined.

Statistics

The results are expressed as the mean value ± standard deviation (SD). Data were analysed by model 1-one way ANOVA followed by a multirange test by Fisher's protected least significance (LSD).

Results

Cell area and weight of *Porphyra* discs were significantly ($p < 0.05$) higher in algae exposed to end-of-day R light pulses than after to B light pulses or control (no end-of-day light pulses) (Table I). R or B light effects on thallus expansion but no on disc weight were partially reversed when a FR light pulse applied immediately after the R or B light pulse (Table I). End-of-day FR pulses applied after R and B light pulses slightly increased the relation between weight and area of the thalli.

End-of-day R light pulses increased the cell area (about 60%) and this effect was partially reversed by FR light (Table I). Thus, red light stimulated thallus expansion and increased cell size, consequently high cell density per thallus area was produced. Approximately 76% of the screen reference area was occupied by cells in R light treatments, however, in FR light treatments only presented 59–64% (Table I). After 14 days end-of-day B light pulses, the area occupied by cells was also high (68%) but smaller than after R light pulses. B light effects on TCA/SRA were not reversed by FR light (Table I). Intercellular spaces are greater in algae submitted to partial starvation

Table I. Disc area (DA), dry weight (DW) and relation between weight and disc area (DW/DA) of *Porphyra* thallus, cell area, number of cells counted on a screen reference area (SRA) of 8346 μm^2, total cell area (TCA) per SRA after 14 d cultivation in 12 h white light:12 h darkness. Every day, at the end of the light period, 20 min far-red (FR), 20 min red (R), 20 min R + 20 min FR, 20 min blue (B) or 20 min B + 20 min FR were applied. A control without light pulses (white light control) was also conducted. The values of the LSD test at 95% significance level are indicated.

Light treatments	Disc area [cm^2]	Disc weight [mg DW]	DW/DA [mg/cm^2]	Cell area [μm^2]	Cell numbers per SRA	TCA/SRA
Initial	0.63 ± 0.01	1.3 ± 0.23	2.1 ± 0.025	96 ± 6.0	49 ± 6.6	0.56 ± 0.065
WL control	2.56 ± 0.1	7.8 ± 0.40	3.0 ± 0.20	115 ± 10	41 ± 4.0	0.56 ± 0.050
WL (+FR)	2.60 ± 0.2	6.0 ± 0.30	2.3 ± 0.15	105 ± 12	46 ± 3.0	0.59 ± 0.045
WL (+R)	3.50 ± 0.3	8.8 ± 0.53	2.5 ± 0.17	153 ± 16	42 ± 3.4	0.76 ± 0.070
WL (+R + FR)	2.90 ± 0.15	8.0 ± 0.40	2.7 ± 0.12	110 ± 10	47 ± 3.8	0.64 ± 0.063
WL (+B)	2.86 ± 0.2	7.7 ± 0.32	2.7 ± 0.25	115 ± 12	50 ± 2.5	0.68 ± 0.069
WL (+B + FR)	2.50 ± 0.2	7.8 ± 0.21	3.1 ± 0.20	100 ± 9.0	57 ± 3.5	0.68 ± 0.059
LSD at 95%	0.25	0.4	0.18	12.1	3.0	0.06

(initial) than in algae collected directly from rocky shores (with no apparent nutrient deficient conditions). Similar effects are obtained when end-of-day FR light treatments are compared to end-of-day R and B treatments.

Both R and B light pulses stimulated the accumulation of pigments in algae previously starved (see the low level of pigments and the low ratio biliprotein/soluble protein in the initial algae). Chl *a* accumulation was significantly ($p < 0.05$) higher after R than after B light pulses and both R and B light effects were partially reversed by FR light. Biliprotein and soluble protein accumulation was higher ($p < 0.05$) after B than after R light pulses. Both, R and B light effects were significantly ($p < 0.05$) reversed by FR light except for PC. The relation of PC/PE increased after end-of-day FR light pulses and no significant differences were found between end-of-day R and end-of-day B light pulses (Table II). The relation BP/SP was similar in all light treatments. Intracellular C accumulation was similar in both end-of-day R and B light pulses. R and B light effects were partially reversed by FR light. However, intracellular N content was significantly ($p < 0.05$) higher after R than after B light pulses and the effects were reversed by FR light (Table II). Similar pattern of pigmentation and accumulation of C and N was found when the contents of pigments, C and N were expressed in terms of disc area (data not shown) instead of disc dry weight (Table II).

The stimulation of pigment accumulation by end-of-day R and B light pulses was also demon-

Table II. Concentration of soluble protein (SP) and photosynthetic pigments: chlorophyll *a* (Chl *a*), phycoerythrin (PE) and phycocyanin (PC) and total C and N in *Porphyra umbilicalis*, all expressed in mg per g algal DW, after 14 d cultivation in 12 h white light:12 h darkness. Every day, at the end of the light period, pulses of 20 min far-red (FR), 20 min red (R), 20 min R + 20 min FR, 20 min blue (B) or 20 min B + 20 min FR were applied. A control without light pulses (white light control) was also conducted. The values of the LSD test at 95% significance level are indicated.

Light treatments	Chl *a*	SP	PE	PC	PC/PE	BP/SP	C	N	C:N
Initial	1.32 ± 0.08	41.3 ± 1.1	4.3 ± 0.5	2.7 ± 0.2	0.62 ± 0.07	0.17 ± 0.02	218 ± 20	17.8 ± 2.0	12.2 ± 0.9
WL control	2.4 ± 0.1	100 ± 9.0	15.6 ± 1.8	6.4 ± 0.7	0.41 ± 0.04	0.22 ± 0.02	218 ± 20	27.0 ± 3.0	8.1 ± 0.8
WL (+FR)	2.9 ± 0.2	89.2 ± 6.8	13.0 ± 1.6	5.1 ± 0.6	0.35 ± 0.04	0.22 ± 0.02	211 ± 20	23.0 ± 1.6	9.2 ± 0.8
WL (+R)	3.9 ± 0.3	99.2 ± 6.0	17.2 ± 2.0	5.6 ± 0.3	0.30 ± 0.03	0.21 ± 0.02	308 ± 23	39.0 ± 23	7.9 ± 0.7
WL (+R + FR)	3.1 ± 0.3	88.2 ± 4.0	12.8 ± 0.8	5.2 ± 0.5	0.38 ± 0.04	0.22 ± 0.03	210 ± 22	22.8 ± 2.1	9.2 ± 0.8
WL (+B)	3.5 ± 0.3	126 ± 12.0	20.6 ± 2.6	6.6 ± 0.4	0.32 ± 0.03	0.22 ± 0.03	306 ± 31	33.0 ± 3.6	9.3 ± 0.9
WL (+B + FR)	2.4 ± 0.2	87.7 ± 2.3	14.6 ± 1.7	6.6 ± 0.4	0.46 ± 0.04	0.24 ± 0.03	227 ± 3.0	26.0 ± 3.1	8.7 ± 0.9
LSD at 95%	0.4	8.1	3.2	0.6	0.05	0.02	25	4.1	0.7

Table III. Total absorptance (TA = 400–760 nm) of *Porphyra* thalli, peak absorptances of PE (A_{566}), of PC (A_{624}) and of Chl *a* (A_{680}), total absorptance per total pigment solution (TA/TP), A_{680} per Chl *a* solution, A_{624} nm per PC solution and A_{566} per PE solution after 14 d cultivation in 12 h WL:12 h darkness. Every day, at the end of the light period, pulses of 20 min FR, 20 min R, 20 min R + 20 min FR, 20 min B or 20 min B + 20 min FR. A control without light pulses (white light control) was also conducted. SD represented between 8 and 10%. The values of the LSD test at 95% significance level are indicated.

Light treatments	TA (400–760 nm)	A_{680}	A_{624}	A_{566}	TA/TP	A_{680}/Chl *a*	A_{624}/PC	A_{566}/PE
Initial	0.25	0.22	0.19	0.21	0.030	0.17	0.068	0.047
WL control	0.46	0.47	0.41	0.47	0.020	0.33	0.064	0.030
WL (+FR)	0.46	0.48	0.43	0.47	0.021	0.16	0.084	0.036
WL (+R)	0.69	0.85	0.72	0.76	0.030	0.22	0.130	0.044
WL (+R + FR)	0.44	0.47	0.42	0.46	0.020	0.14	0.076	0.036
WL (+B)	0.78	0.76	0.75	0.83	0.024	0.21	0.113	0.039
WL (+B + FR)	0.46	0.50	0.49	0.46	0.019	0.21	0.063	0.033
LSD at 95%	0.06	0.08	0.09	0.05	0.004	0.07	0.014	0.004

strated *in vivo* (Table III). The total absorptance (TA) of the thalli after 14 d end-of-day light pulses was significantly ($p < 0.05$) higher in B or R treatments than in the WL control or after FR light pulses (Table III). This corresponded to a higher peak absorptance of PE (566 nm) after B than after R light pulses. The peak absorptance of Chl *a* (680 nm) was higher after R than after B light and no significant differences were found in the peak absorptance of PC (624 nm). R and B light effects on TA and on the peak absorptances were reversed by FR light pulses (Table III).

In spite of total absorptance and the peak absorptance of PE were higher after B than after R light pulses, the ratios of the peak absorptance of PC per concentration of PC and peak absorptance of PE per concentration of PE, used as index of the efficiency of light absorption, were significantly ($p < 0.05$) higher after R than after B light pulses (Table III). This could explain the greater thallus expansion after end-of-day R than after end-of-day B light pulses. The efficiency of light absorption was decreased after the application of FR light pulses, this explained the lower algal growth after FR than after R or B light pulses (Table III).

Discussion

End-of-day light pulses of different light qualities affected growth rate and pigmentation in *Porphyra*. Red light pulses stimulated growth, namely thallus expansion and cell size and Chl *a* accumulation whereas B light mainly stimulated the accumulation of N compounds: soluble protein and biliprotein. The effects of R light on growth and pigmentation were partially reversed when a short FR pulse was applied immediately after the R pulse. B light effects on pigment accumulation was also reversed by a subsequent FR pulse except for phycocyanin. These results suggest an end-of-day light control through phytochrome. Control by phytochrome of Chl *a* accumulation in dark-adapted *Porphyra umbilicalis* after short R light pulses followed by up to 4 h incubation in darkness has been demonstrated (López-Figueroa and Niell, 1989). In this work, the use of both long-term cultivation period under WL and of end-of-day short pulses of different light qualities provide more conclusive information on the photoreceptor system than the treatments used before in *Porphyra* (López-Figueroa and Niell, 1989, 1990). Because of FR reversibility, R stimulation of Chl *a* synthesis seems to be controlled by phytochrome. However, the higher stimulation of PE and soluble protein by B light than by R light pulses seem to be due to the action of specific B light photoreceptor. Because of partial reversibility of B effect, co-action with phytochrome is suggested. Previously, under light pulses followed by short dark incubation, the involvement of phytochrome in the control of chlorophyll synthesis was suggested in algae partially starved (López-Figueroa and Niell, 1989). Under continuous light (4–5 h), R and G

control of PC and PE respectively was observed (López-Figueroa and Niell, 1990). Phytochrome has been isolated and detected by monoclonal antibodies from different micro- (Cordonier *et al.*, 1986; Kidd and Lagarias, 1990; Ruyters *et al.*, 1990) and macroalgae (López-Figueroa *et al.*, 1989a). On the other hand, flavins and pterins has been proposed as B light photoreceptors in algae (Galland and Senger, 1988a, b) and rhodopsin as green light photoreceptors (Uhl and Hegemann, 1990).

In addition to pigmentation, end-of-day light seem to control the growth rate of *Porphyra.* End-of-day R light pulses stimulate thallus expansion and increase cell size. Although, light quality effects on algal growth, reproduction and pigmentation have been reported (Dring, 1988), the photoreceptors involved are still object of controversy (Dring, 1988; Rüdiger and López-Figueroa, 1992) in contrast to the clear phytochrome effect on stem elongation in higher terrestrial plants (Smith, 1982). Under continuous light, with photosynthetic interference the involvement of B light photoreceptor in the control of growth and pigmentation of micro- and macroalgae has been proposed (Dring, 1988; Senger and Bauer, 1987). In this work, applying end-of-day light pulses, photosynthetic effects were clearly separated from morphogenetic effects and the photoreceptors involved can be determined: growth rate in *Porphyra umbilicalis* is controlled at least by phytochrome. Red and B light have been proposed as a cause of enhancement growth in phytoplankton (Humphrey, 1983). Red light stimulated and FR depress growth in several microalgae probably through phytochrome (Dring, 1988), as was observed in *Porphyra.* Control by phytochrome of growth has been reported in other algae, for example, *Nereocystis* (Duncan and Foreman, 1980), *Spyrogira* (Virgin, 1978) and *Chara* (Lipps, 1973). Recently, Lüning (1992) obtained higher growth rate in R than in B light (12 h light : 12 h darkness cycles) in pieces of *Porphyra* but the photoreceptor system was not indicated.

The higher growth rate in R compared to B treatment can be explained by higher chlorophyll concentration and higher efficiency of light absorption after R than in B end-of-day light pulses. In spite of the total absorptance (400–760 nm) and the peak absorptance of PE were larger in B than in R light treatments, the ratios of peak absorptance of PE per concentration of PE and peak absorptance of PC per concentration of PC were higher after R than after B light pulses. A direct relation between growth and peak absorptance ratios, *i.e.* PE/Chl *a* has been shown in *Porphyra abbottae* (Hannach, 1991).

C accumulation patterns were similar after 14 d cultivation in both treatments but intracellular nitrogen content was higher after R than after B light pulses. These results contrast with those published for several microalgae, which present high accumulation of N compounds under continuous B light and high accumulation of carbohydrates in continuous R light (Kowallik, 1982). The higher content of N after R compared after B light pulses can be attributed to a high structural protein concentration according to the higher cell size and thallus area after R than after B light.

Light quality effects on growth were the opposite for those reported in higher plants in which end-of-day R and B light pulses suppress growth and FR stimulated stem elongation (Casal and Smith, 1989; Cosgrove and Green, 1981). This different pattern has a clear ecological implication. In shade terrestrial habitats, R : FR is low and the spectra is poor in B wavelengths due to chlorophyll absorption, however, underwater shade habitats (deep waters), R : FR is high due to very rapid FR light extinction and B : R ratios increased (in clear waters). Thus, the promotion of growth in aquatic shade habitats by R and B light and the growth suppression by FR light through phytochrome could be an adaptation to the underwater light field since FR : R is greater in sun than in shade habitats (deep waters). The greatest growth reported in aquatic shade habitats in both macroalgae (Santelices, 1978; Mathienson and Burns, 1975) and marine angiosperms (Tomasko, 1992) could be controlled by light proportion of R : FR (in algae growing in emersion as *Porphyra*) and B : R as it has been suggested for pigmentation in *Porphyra* and *Chondrus* (López-Figueroa, 1992). Recently, Tomasko (1992) proposed that R : FR can act as an underwater light signal of shade controlling elongation and rhizome growth rates in aquatic angiosperms. A similar proposal has been reported for macroalgae (Duncan and Foreman, 1980).

More investigations are needed in order to find out more detail about underwater light field where macroalgae grow, relating the changes of light quality proportions to growth and metabolic responses.

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