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Photosynthetic acclimation and biochemical responses of *Gelidium sesquipedale* cultured in chemostats under different qualities of light

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Abstract The red alga *Gelidium sesquipedale* (Clem.) Born. et Thur. has been cultured in chemostats to assess the effects of light quality and photon-fluence rate (PFR) on growth, photosynthesis and biochemical composition. Plants under blue and red light (BL and RL) showed higher growth rates than under white light (WL) of the same PFR (40 μmol m^{-2} s^{-1}). The light-saturated rate of photosynthesis was higher for algae grown under BL and RL than for algae grown under WL. When algae were transferred to WL of moderate PFR (100 μmol m^{-2} s^{-1}), the light-saturated rate of photosynthesis decreased, being higher in previously RL-grown algae than in previously BL- and WL-grown algae. The initial slope of photosynthesis–irradiance (PI) curves (α) was affected by PFR but not by light quality. Pigment content was little affected by light quality. Light-quality treatments also affected the biochemical composition of the alga; previous exposure to various light treatments activate or repress several metabolical pathways that are fully expressed in the subsequent phase of WL of moderate PFR. Thus, phycobiliproteins and soluble proteins increased for previously BL- and RL-grown algae, whereas insoluble carbohydrate concentration was reduced, indicating a change of the C-partitioning between carbon compounds and organic nitrogen compounds. Inorganic nitrogen metabolism was also affected by light: under WL of moderate PFR, NO_3^- was totally depleted from sea water, and maximal values of NO_3^- uptake were recorded. In addition, neither NO_2^- nor NH_4^+ was released. However, when algae were transferred to a low PFR, there was a drastic reduction of NO_3^- uptake under WL, which only partially recovered over time. It was accompanied by the release of NO_2^-, but not NH_4^+, to the culture medium. Under BL and RL, however, there was a transient enhancement of NO_3^- uptake that was followed by a net release of NO_2^- and NH_4^+. Growth rates were not correlated with PFR. This could be due to the the dynamics of internal carbon mobilization and accumulation in the algae. When algae were exposed to a moderate PFR of WL, carbon requirements for growth were satisfied by photosynthesis. Thus, there was a net accumulation of carbon in the tissue. In contrast, when algae were exposed to low PFRs of either WL, BL or RL, observed growth rates could not be maintained by photosynthesis and carbon was mobilized.

Introduction

The effects of light of defined spectral composition on photosynthetic acclimation are well known: in higher plants and in the green macroalga *Acetabularia mediterranea* Lamour. [*A. acetabulum* (L.) Silva], blue light (BL) has the same effects as high irradiance, whereas red light (RL) mimics low-irradiance conditions (Lichtenthaler et al. 1980; Schmid 1991). In unicellular green algae, the situation appears to be opposite (Humbeck et al. 1988; Senge and Senger 1991). The response of red algae to light quality in comparison to other plants is interesting, since they have a different pigment composition. In these organisms, Photosystem I (PSI) and Photosystem II (PSII) have very different, almost complementary action-spectra (Cunningham et al. 1990). Chlorophyll *a* is mainly associated with PSI, whereas phycobiliproteins are located in phycobilisomes,

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structurally linked to PSII (Gantt 1990). Acclimation to light preferentially absorbed by PSI or by PSII has been little studied in red algae (Ley and Butler 1980; Fujita et al. 1987; Cunningham et al. 1990, 1992; López-Figueroa 1991).

Light quality also affects carbon and nitrogen metabolism (Figueroa et al. 1995b), but very little is known about the interaction between photosynthetic acclimation and carbon and nitrogen metabolism under conditions of different light spectra in red algae. The aim of this study was to determine the effects of light quality and irradiance level on the photosynthesis and biochemical composition of the red alga *Gelidium sesquipedale* (Clem.) Born. et Thur. cultured in chemostats with a controlled flow of nutrients. In addition, we examined whether previous exposure to different light sources further affects the photosynthetic characteristics and biochemical composition of the alga.

Materials and methods

Plant material and culture conditions

Gelidium sesquipedale (Clem.) Born. et Thur. was collected from Punta Carnero, Algeciras (Southern Spain). Populations of this alga are located in crevices at the lowest spring-tide level (10 to 20 cm above datum). The thalli of *G. sesquipedale* consist of erect pennanted fronds with apical growth. This species is found along Atlantic coasts from Brittany to Morocco. A special morphotype grows on the southern coast of Spain that does not measure more than 10 cm. Algae were maintained in the laboratory for 4 d in natural sea water at a PFR of 100 $\mu mol\, m^{-2}\, s^{-1}$. Then, plants were acclimated to chemostat conditions for 3 d in artificial sea water (ASW) with a defined composition of nutrients (Woelkerling et al. 1983).

Cultures were maintained in three phases under different light-quality and irradiance conditions:

First phase. In this initial phase, algae were cultured for 10 d under 100 $\mu mol\, m^{-2}\, s^{-1}$ of WL with a NO_3^- concentration in the inflow of 61 μM ASW; nitrogen was supplied at a rate of 33.7 $\mu mol\ NO_3^-\ g^{-1}$ dry wt d^{-1}.

Second phase. The same plants cultured under the conditions specified in the first phase were transferred to a low PFR of 40 $\mu mol\, m^{-2}\, s^{-1}$ of three different spectral compositions (WL, BL and RL) for 14 d. NO_3^- concentration was 43 μM, and nitrogen was supplied at a rate of 64 $\mu mol\ NO_3^-\ g^{-1}$ dry wt d^{-1}, as there was a lower biomass than in the first phase (2.86 ± 0.06 and 1.05 ± 0.16 g fresh wt l^{-1}, respectively).

Third phase. The plants grown under low-irradiance BL, RL and WL were finally cultured at 100 $\mu mol\, m^{-2}\, s^{-1}$ of WL (Days 14 to 28) to determine if the effects of the second phase were maintained when irradiance was increased. In this third phase, the NO_3^- concentration was maintained at 43 μM.

The irradiance levels used in all treatments were below the light-saturation point of photosynthesis (Torres et al. 1991).

The cultures were maintained in chemostats, in accordance with the method of Vergara et al. (1993), with a photoperiod of 12 h light: 12 h dark and a temperature of 17°C. The renewal rate of the culture medium was 0.5 d^{-1} (total volume was 2.5 litres ASW), and mixing was achieved by bubbling air into the tank near the bottom at a rate of 4.4 litres min^{-1}. Algae were protected by enclosing them in a 0.5 cm-mesh cylinder to prevent them being tossed around, which would destroy the apical portions of their thalli. Biomass density was kept approximately constant to avoid growth limitation.

Since HCO_3^- was the only buffer in the culture medium, the pH was controlled to obviate the adverse effects that would result from too-high pH values. The pH of the inflowing ASW was 8.00; the pH of the outflowing ASW was maintained in steady state at 8.46 ± 0.02 in the first phase and 8.34 ± 0.02 in the second phase. Total dissolved inorganic carbon (DIC) was also maintained at sufficient levels for production: 2.33 mM for the inflowing ASW and 2.09 ± 0.02 mM for the outflowing ASW.

Light sources

Fig. 1 shows the spectra of the light sources used. WL was provided by Silvania F20 W/CW fluorescent lamps. To obtain a PFR of 40 $\mu mol\, m^{-2}\, s^{-1}$, the light source was attenuated through a Plexiglas neutral-density filter. Blue light (BL) was provided by General Electric 20 W/B blue fluorescent lamps filtered through two Plexiglas blue filters, Röhm PG627 and PG602, and red light (RL) by General Electric 20 W/R red fluorescent lamps filtered through a Plexiglas red filter, Röhm PG502. All light treatments had the same PFR of 40 $\mu mol\, m^{-2}\, s^{-1}$, determined with a Quantum Radiometer Li-Cor (Li-1000 data-logger) with spherical sensor (Li 193 SB). Photosynthetically usable radiation, PUR (Morel et al. 1987), was determined to make sure that the light absorbed by the thallus did not differ from that of photosynthetically active radiation (PAR). PUR was calculated using $A(\lambda)$ as a weighting function describing the probability that a photon of a given wavelength will be absorbed by the thallus of the algae (Morel et al. 1987). In the present study, this dimensionless variable was derived from the absorption spectrum of *Gelidium sesquipedale* (Fig. 1) by dividing the spectrum by the maximal absorption (a_{max}). For this species, a_{max} occurs in vivo at $\lambda = 550$ nm (due to R-phycoerythrin, RPE). Unlike the results obtained by Morel et al., the ratio PAR: PUR was close to 1 and there were no differences between the different light qualities, due to the pigment composition of red algae which covers all the spectral composition of light (Lüning and Dring 1985). The same effective PFR was incident for all cultures.

Growth and photosynthesis

Specific growth rates were determined as increases in the biomass of the cultures over time, as previously described by Vergara et al.

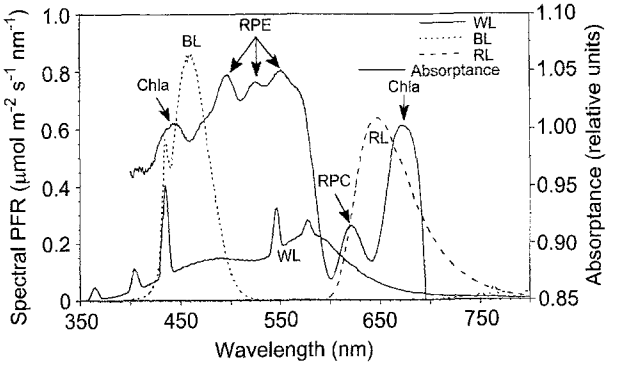

Fig. 1 *Gelidium sesquipedale.* Spectral photon-fluence rate (*PFR*) of light sources of 40 $\mu mol\, m^{-2}\, s^{-1}$ (*WL, BL, RL*) used in cultures and in vivo absorption spectrum (*WL* white light; *BL* blue light; *RL* red light; *RPC* R-phycocianin; *RPE* R-phycoerytrin; *Chla* chlorophyll *a*)

(1993). The net rate of photosynthesis was estimated as oxygen evolution (Hansatech, Kings Lynn, Norfolk, England). Samples of 0.030 g fresh wt were transferred from the cultures to a measurement chamber containing 2 ml ASW (pH 8.0, DIC = 2.33 m*M*). Light was provided by a fibre-optic cable, and was attenuated with neutral-density filters to obtain a range of PFRs from 12 to 970 $\mu mol\, m^{-2}\, s^{-1}$. Each photosynthesis–irradiance curve (PI) for every sample took about 1 h to generate. The initial oxygen concentration in the ASW was maintained at ~ 50% of saturation by bubbling with N_2. Temperature in the measurement chamber was the same as in the culture media (17°C).

PI curves were recorded in WL in plants cultured for 14 d at 40 $\mu mol\, m^{-2}\, s^{-1}$ under different light qualities, and in plants cultured for 14 d at 100 $\mu mol\, m^{-2}\, s^{-1}$ of WL (Day 28), as well as in control plants ($n = 3$) not submitted to culture conditions. Curves were plotted according to Edwards and Walker (1983) and fitted by iteration (Kaleida Graph for Macintosh, Abelbeck software). The photosynthetic efficiency (α) was calculated in the linear phase of the curve (from 0 to 48 $\mu mol\, m^{-2}\, s^{-1}$). Besides PI curves, the photosynthetic rates of algae grown at each light quality were estimated with WL, BL and RL at a PFR of 42 $\mu mol\, m^{-2}\, s^{-1}$ on Day 11 of culture, using red and blue filters in the case of BL and RL, respectively.

Nutrients

NO_3^- and NO_2^- and NH_4^+ concentrations were determined in samples taken from the inflow and from the outflow of the chemostats with an automatic analyzer (Bran & Luebbe Technicon Traacs 800): NO_3^- according to Wood et al. (1967), NO_2^- according to Shinn (1941), and NH_4^+ according to Slawyck and MacIsaac (1972).

The net rate of uptake of NO_3^-, or of release of NO_2^- and NH_4^+ (when this was below zero) for a given interval of time (i, $i + 1$) was determined according to:

$$[\mu mol\ N\ g^{-1}\ dry\ wt\ d^{-1}]_i^{i+1} = \frac{Cout_i\ V + Q\ Ci\ \Delta t - Q(Cout_i + Cout_{i+1})/2\,\Delta t - Cout_{i+1}\ V}{B\ \Delta t}, \quad (1)$$

where Ci and $Cout$ = concentrations of nitrogen in the inflow and the outflow of the chemostat (μM), respectively; V = volume (litres), Q = flow of ASW (litres d^{-1}); B = total biomass (g dry wt $litre^{-1}$) during time interval (Δt, days) considered.

Biochemical composition

Samples were frozen in liquid N_2 and stored until analysis. Triplicate samples were ground in liquid N_2 and homogenized in phosphate buffer (0.1 *M*, pH 6.5) at 4 °C. Phycobiliproteins, soluble proteins and amino acids were determined as described by Vergara and Niell (1993). Soluble carbohydrates (included floridoside and other low molecular weight compounds) and insoluble carbohydrates (comprising floridean starch and cell-wall polysaccharides) were determined according to Vergara et al. (1995). Insoluble proteins were extracted by treating the pellet resulting from the insoluble carbohydrate digestion with 1 *N* NaOH overnight (Bird et al. 1982), followed by centrifugation at 19000 ×*g* for 15 min. Chlorophyll *a* concentration was spectrophotometrically determined in triplicate at the end of the culture periods (14 and 28 d) with a Beckman DU-7 spectrophotometer, according to Talling and Driver (1963). Biochemical composition was expressed on a dry weight basis. The ratio fresh wt : dry wt remained constant throughout the cultures with a value of 0.32 (slope of the regression line = 0.32; $r = 0.96$, $n = 72$).

Results

Growth

Biomass production of *Gelidium sesquipedale* was affected by light quality, but appeared to be fairly independent of PFR in the range of photon-fluence rates tested. At low PFR (40 $\mu mol\, m^{-2}\, s^{-1}$), growth rates of algae grown under BL and RL were higher than of those algae grown under WL (Table 1), and were in fact even higher than at 100 $\mu mol\, m^{-2}\, s^{-1}$ (first phase). Subsequently, when the algae were shifted up to 100 $\mu mol\, m^{-2}\, s^{-1}$ of WL, their growth rate decreased in previously WL- and BL-grown algae, but not in previously RL-grown algae.

Photosynthesis

Photosynthetic performance under WL was a function of the various light fields used (Table 2). The light-saturated rate of photosynthesis (V_{max}) was higher for BL- and RL-grown algae than for WL-grown algae. Subsequently, when algae were exposed to a WL of 100 $\mu mol\, m^{-2}\, s^{-1}$, V_{max} decreased, but the differences observed in the previous light treatments were maintained. The initial slope of the PI curves (α) was affected by PFR but not by light quality. It was higher for algae grown under a low PFR than for those grown under a moderate PFR, independent of light quality. The decrease in α under moderate PFR was greater for previously WL-grown algae than for BL- and RL-grown algae.

The net photosynthetic rate was also determined at various light qualities of PFRs similar to those used for the cultures (42 $\mu mol\, m^{-2}\, s^{-1}$). The results obtained by Beer and Levy (1983) for the red algae *Gracilaria* sp. under similar light conditions are shown for comparison in Table 3. The light quality during measurement and the light quality of the culture both affected the

Table 1 *Gelidium sesquipedale*. Growth rates (% d^{-1}) of algae cultured in different light qualities and irradiance levels. Standard error and goodness of fit (*r*, *in parentheses*) are also indicated (*WL* white light; *BL* blue light; *RL* red light)

Light treatment	Growth rate (% d^{-1})
100 $\mu mol\, m^{-2}\, s^{-1}$ of:	
WL	1.27 ± 0.13 (0.96)
40 $\mu mol\, m^{-2}\, s^{-1}$ of:	
WL	1.33 ± 0.12 (0.97)
BL	1.78 ± 0.12 (0.97)
RL	1.86 ± 0.12 (0.97)
100 $\mu mol\, m^{-2}\, s^{-1}$ of WL:	
WL-previously grown	0.79 ± 0.12 (0.98)
BL-previously grown	1.38 ± 0.05 (0.99)
RL-previously grown	1.79 ± 0.09 (0.99)

Table 2 *Gelidium sesquipedale.* Photosynthetic parameters of photosynthesis–irradiance (PI) curves in white light of algae after 14 d of culture in different light qualities of 40 $\mu mol\,m^{-2}\,s^{-1}$ and after 14 d in white light of 100 $\mu mol\,m^{-2}\,s^{-1}$. Maximum net photosynthetic rate (V_{max}), expressed on fresh weight basis, light-compensation point (L_{CP}), half-saturation constant (K_s) and photosynthetic efficiency (α) and r of the fitted curve are shown; standard errors are given in parentheses

Light conditions	V_{max} ($\mu mol\,O_2\,g^{-1}$ fresh wt min^{-1})	L_{CP} ($\mu mol\,m^{-2}\,s^{-1}$)	K_s ($\mu mol\,m^{-2}\,s^{-1}$)	r	α (mol O_2 mol^{-1} photons)
40 $\mu mol\,m^{-2}\,s^{-1}$ of:					
WL	0.70 (0.02)	24.6 (1.4)	119.1 (10.1)	0.99	0.040 (0.004)
BL	1.04 (0.12)	18.7 (7.4)	228.8 (77.6)	0.99	0.035 (0.003)
RL	1.05 (0.08)	19.5 (4.5)	153.8 (39.5)	0.99	0.036 (0.002)
100 $\mu mol\,m^{-2}\,s^{-1}$ of WL:					
WL-previously grown	0.47 (0.06)	34.6 (7.0)	154.5 (55.2)	0.98	0.020 (0.002)
BL-previously grown	0.52 (0.06)	19.0 (5.4)	74.2 (31.5)	0.97	0.023 (0.003)
RL-previously grown	0.71 (0.02)	9.3 (1.6)	86.8 (10.6)	0.99	0.027 (0.002)
External control	0.27 (0.02)	29.7 (4.2)	91.7 (23.2)	0.99	0.026 (0.006)

Table 3 *Gelidium sesquipedale.* Net photosynthetic rates (nmol O_2 g^{-1} fresh wt min^{-1}) (Day 11 of culture) at different light spectra of algae cultured at different light qualities. Photon fluence rate was 42 $\mu mol\,m^{-2}\,s^{-1}$ for all cultures. Results of Beer and Levy (1983) for *Gracilaria* sp. exposed to 30 $\mu mol\,m^{-2}\,s^{-1}$ and different light qualities are shown in parentheses (*Underlined values* light of same colour as in culture)

Photosynthetic light spectrum	WL-grown	BL-grown	RL-grown	BL-grown: WL-grown	RL-grown: WL-grown
WL	129 ± 44 (140)	107 ± 40 (152)	146 ± 2 (158)	0.83 (1.09)	1.13 (1.13)
BL	44 ± 62 (43)	70 ± 5 (95)	91 ± 25 (105)	1.59 (1.11)	2.07 (2.44)
RL	9 ± 48 (45)	48 ± 3 (98)	70 ± 34 (120)	5.33 (2.18)	7.77 (2.67)
BL:WL ratio	0.34 (0.31)	0.65 (0.63)	0.62 (0.66)		
RL:WL ratio	0.07 (0.32)	0.45 (0.64)	0.48 (0.76)		

photosynthetic rate. The rate of photosynthesis measured in WL for WL-grown algae was higher than the rate for BL- and RL- grown algae measured in BL and RL, respectively. With respect to the different light regimes assayed, the photosynthetic rate was higher in WL than in BL and RL for algae grown in the various light treatments (WL-, BL- and RL-grown algae). However, the reduction in photosynthesis induced by PSI illumination (BL and RL) was higher for WL-grown algae than for BL- and R-grown algae.

Inorganic nitrogen

Uptake of NO_3^- was influenced by light quality and PFR. In the first phase of culture, external NO_3^- was totally depleted after 2 d in the chemostat, and maximal uptake rates were recorded (Fig. 2). Subsequently, when algae were shifted down to 40 $\mu mol\,m^{-2}\,s^{-1}$, NO_3^- uptake was drastically reduced under WL, and partially recovered through time. Under BL and RL, there was a transient enhancement of NO_3^- uptake. NO_2^- and NH_4^+ release was also affected (Fig. 3). Whereas NO_2^- was not liberated to the external medium under a moderate PFR of 100 $\mu mol\,m^{-2}\,s^{-1}$, it was released under a low PFR of WL. Under BL and RL, NO_2^- was also released, but not until the fifth day. NH_4^+ release was not detected in the first phase nor under WL of 40 $\mu mol\,m^{-2}\,s^{-1}$ (second phase). There was, however, a net NH_4^+ excretion in BL and RL cultures.

Photosynthetic pigments

The overall pigment content of *Gelidium sesquipedale* differed little between cultures grown under a low PFR of either WL, BL or RL (Table 4). Concentrations of both R-phycoerythrin (RPE) and R-phycocyanin (RPC), increased slightly in all cultures after 14 d at

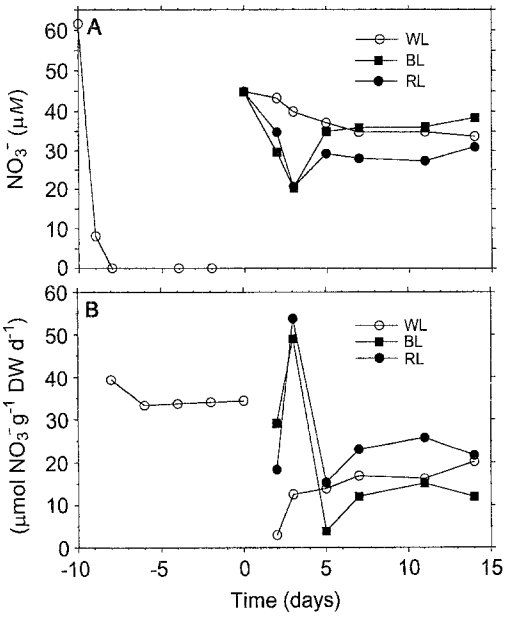

Fig. 2 *Gelidium sesquipedale.* Time-course of nitrate concentration in chemostat (**A**) and nitrate uptake of plants (**B**) grown at different spectral qualities of light of 40 μmol m^{-2} s^{-1} (*Days 0 to 14*) (*Days −10 to 0* thalli were grown at 100 μmol m^{-2} s^{-1} of WL; *DW* dry wt)

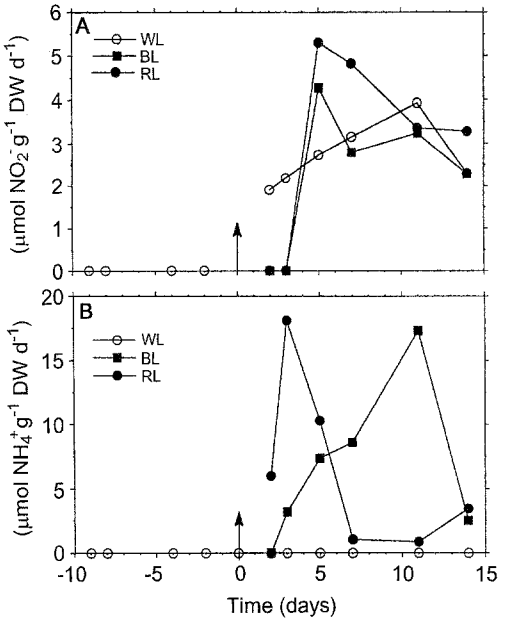

Fig. 3 *Gelidium sesquipedale.* Release rates of nitrite (**A**) and ammonia (**B**) in algae grown in different spectral qualities of light of 40 μmol m^{-2} s^{-1} (*Days 0 to 14*) (*Days −10 to 0* thalli were grown at 100 μmol m^{-2} s^{-1} of WL)

40 μmol m^{-2} s^{-1} (second phase) compared to the values of RPE and RPC at the end of the first phase. Subsequently, when the algae were shifted to 100 μmol m^{-2} s^{-1} (third phase), there was a selective increase of phycobiliproteins in previously BL- and RL- grown algae, but not in WL-grown algae. Chlorophyll *a* was affected neither by light quality nor by PFR. Thus, the ratio of RPE : chlorophyll *a* was slightly higher in RL- and BL- than in WL-grown algae (Table 4). This ratio increased markedly at the end of the third phase, due to the selective increase of phycobiliproteins for algae grown previously in BL and RL.

Proteins and amino acids

Whereas the total protein content was not affected by light quality at low PFR, the partitioning of proteins between soluble and insoluble compartments showed drastic changes (Table 4). Minimum values of soluble proteins and maximum values of insoluble proteins were recorded under BL compared with WL and RL. In the third phase, soluble but not insoluble proteins were further affected by the previous light treatments. Whereas similar levels of insoluble proteins were observed for all three light treatments, soluble protein

Table 4 *Gelidium sesquipedale.* Time-course of concentration of photosynthetic pigments (mg g^{-1} dry wt), proteins (mg g^{-1} dry wt) and amino acids (μg g^{-1} dry wt) throughout experiment. Only results at end of each phase of culture are shown, for simplicity. Standard deviations are in parentheses (*PBP* phycobiliproteins; *nd* no data)

Variables	Moderate PFR, WL (100 μmol m^{-2} s^{-1})		Low PFR (40 μmol m^{-2} s^{-1})			Moderate PFR, WL (100 μmol m^{-2} s^{-1})		
	initial (Day −10)	end (Day 0)	WL	BL (Day 14)	RL	Previous WL	Previous BL (Day 28)	Previous RL
R-phycoerythrin	4.36 (0.53)	4.18 (0.30)	5.76 (1.00)	6.10 (0.20)	5.95 (0.87)	5.67 (0.48)	8.87 (0.91)	8.87 (0.66)
R-phycocyanin	0.37 (0.06)	0.39 (0.06)	0.29 (0.07)	0.43 (0.11)	0.54 (0.06)	0.43 (0.09)	0.68 (0.02)	0.64 (0.13)
Chlorophyll *a*	nd	nd	1.57 (0.01)	1.58 (0.18)	1.41 (0.14)	1.46 (0.21)	1.45 (0.14)	1.51 (0.01)
RPE: chlorophyll *a*	nd	nd	3.68	3.88	4.24	3.87	6.12	5.88
Soluble protein	12.6 (1.1)	12.4 (0.6)	14.3 (3.4)	8.2 (1.7)	15.2 (3.4)	13.7 (0.9)	22.7 (1.7)	21.5 (1.2)
Insoluble protein	15.1 (1.4)	19.2 (2.4)	17.0 (0.8)	24.8 (1.2)	19.4 (1.5)	19.4 (1.2)	20.7 (2.0)	21.4 (2.3)
Total protein	27.7 (1.4)	31.6 (2.9)	31.3 (2.6)	32.9 (2.9)	34.5 (4.7)	33.1 (0.5)	43.4 (3.4)	43.0 (1.1)
Amino acids	119.1 (9.7)	117.4 (16.4)	119.5 (26.8)	176.6 (25.4)	157.3 (19.4)	98.7 (14.3)	146.9 (15.6)	169.9 (2.4)
PBP:total protein ratio (%)	16.9	14.6	19.0	19.0	18.6	18.5	22.0	22.3

concentration was higher for previously BL- and RL-grown algae than for WL-grown algae.

The concentration of amino acids under WL was maintained at similar levels in all cultures. However, amino acid content accumulated under BL and RL and was maintained in the subsequent WL phase (Table 4).

Total carbon and nitrogen

Carbon was accumulated in the tissue of algae grown at a moderate PFR of 100 μmol $m^{-2} s^{-1}$ (first and third phase), but decreased when they were exposed to a low PFR of 40 μmol $m^{-2} s^{-1}$ in the three light fields tested (Fig. 4A). Internal nitrogen content was maintained at between 3 and 4% dry wt in all cultures, and no drastic changes were observed (Fig. 4B). These levels of internal nitrogen are indicative of N-sufficient conditions for this species (Vergara et al. 1993). Internal nitrogen decreased slightly at the end of the first phase, and later was slightly higher for BL- and RL-grown algae than for WL-grown algae. As a result of the observed carbon and nitrogen dynamics, the C:N ratio was dependent on PFR. Increasing during the first phase of moderate PFR, the C:N ratio fell suddenly following the shift-down of PFR at all three light qualities tested (Fig. 4C). At the end of the first phase, the C:N ratio was slightly lower for BL- and RL-grown algae as a result of their higher nitrogen content. Thus, in this study, the C:N ratio depended primarily on carbon content of the algae. This contrasts with the typical regulation of the C:N ratio by nitrogen content that occurs when nitrogen availability constitutes the control variable of the metabolic system (Vergara et al. 1993).

Carbohydrates

In the first phase of the culture at 100 μmol $m^{-2} s^{-1}$, insoluble carbohydrate content increased parallel to an increase in the total carbon (Fig. 5A). In contrast, insoluble carbohydrate concentration decreased when algae were shifted down to 40 μmol $m^{-2} s^{-1}$. A decrease in the ratio insoluble carbohydrates:total carbon resulted (Fig. 6). Subsequently, when algae were shifted up to 100 μmol $m^{-2} s^{-1}$ of WL, insoluble carbohydrate

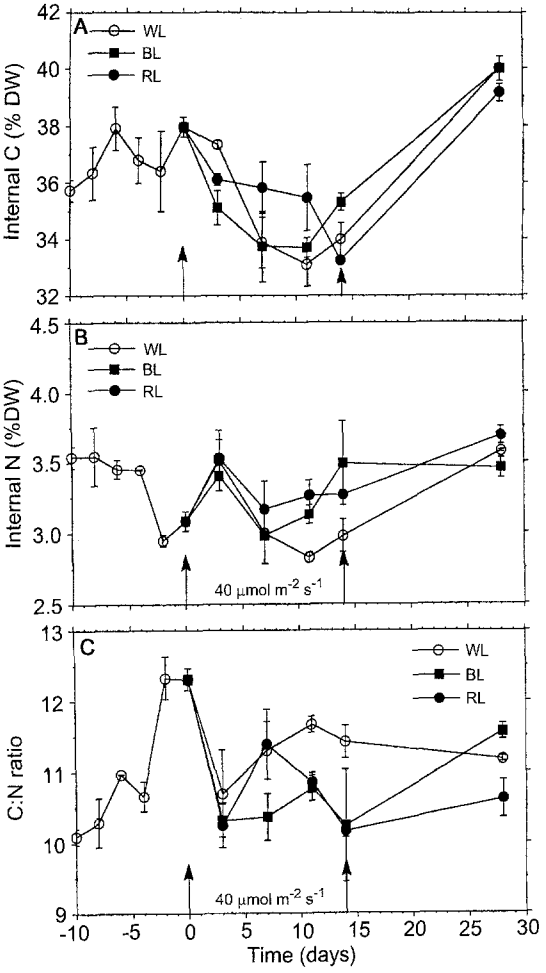

Fig. 4 *Gelidium sesquipedale.* Time-course of internal carbon (**A**), internal nitrogen concentration (**B**) and C:N atomic ratio (**C**) in algae grown in different spectral qualities of light of 40 μmol $m^{-2} s^{-1}$ (*Days 0 to 14*) [*Days −10 to 0 and 15 to 28* thalli were grown at 100 μmol $m^{-2} s^{-1}$ of WL; *bars* standard deviations ($n = 3$)]

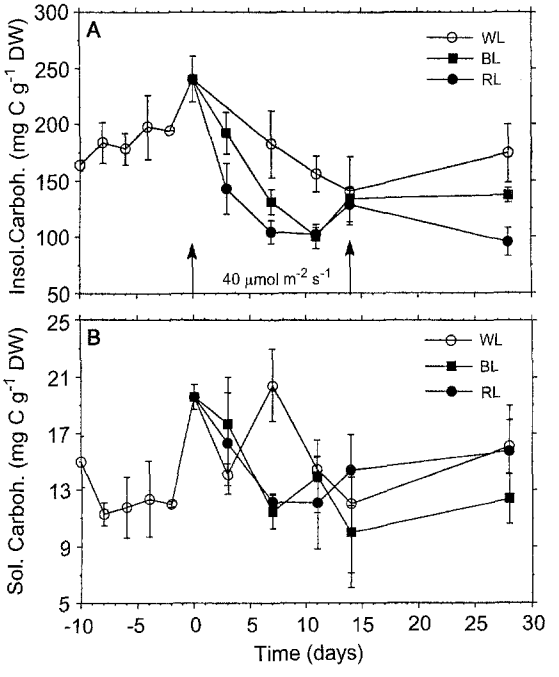

Fig. 5 *Gelidium sesquipedale.* Time-course changes of insoluble carbohydrate (**A**) and soluble carbohydrate concentration (**B**) in algae grown at different spectral qualities of light of 40 μmol $m^{-2} s^{-1}$ (*Days 0 to 14*) [*Days −10 to 0 and 15 to 28* thalli were grown at 100 μmol $m^{-2} s^{-1}$ of WL; *bars* standard deviations ($n = 3$)]

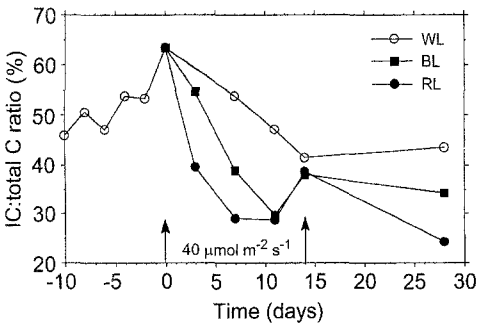

Fig. 6 *Gelidium sesquipedale.* Time-course of insoluble carbohydrates (*IC*):total carbon ratio in algae grown at different spectral qualities of light of 40 $\mu mol\,m^{-2}\,s^{-1}$ (*Days 0 to 14*) (*Days −10 to 0 and 15 to 28* thalli were grown at 100 $\mu mol\,m^{-2}\,s^{-1}$ of WL)

content increased only slightly for WL-cultured algae. In algae previously cultured in BL and RL, insoluble carbohydrate concentration was maintained, or even decreased, indicating that the accumulation of carbon in the tissue was directed not towards carbohydrates, but towards other carbon compounds such as proteins. Although more variable, the changes in the concentration of soluble carbohydrates (Fig. 5B) paralleled those of insoluble carbohydrates.

Discussion

The results reveal that light quality affects growth, photosynthetic performance and, to some extent, biochemical composition of *Gelidium sesquipedale.*

Light-regulation of nitrogen metabolism

Both the quantity and spectral distribution of light affected the uptake of NO_3^- as well as the release of inorganic nitrogen compounds such as NO_2^- and NH_4^+. The transport of NO_3^- into the cell as well as nitrate reductase activity (the subsequent step of inorganic nitrogen assimilation), are regulated by light in higher plants (Duke and Duke 1984; Lillo 1994) and in algae (Azuara and Aparicio 1985; López-Figueroa and Rüdiger 1991; Corzo and Niell 1992), since the metabolic pathways of carbon and nitrogen are highly coordinated (Turpin 1991; Foyer et al. 1994). NO_3^- entrance into the cell is mediated by active transport involving ATP consumption (Turpin 1991; Lara et al. 1993). The drastic reduction in net NO_3^- uptake following a shift-down of PFR under WL may involve energetic limitation. Under BL and RL, however, there was a transient enhancement in net NO_3^- uptake.

NO_2^- can not be stored in the cell. Thus, when NO_3^- and NO_2^- reduction are uncoupled, any accumulation of NO_2^- is released to the external medium to avoid toxic effects (Vennesland and Guerrero 1979; Azuara and Aparicio 1985; Weger and Turpin 1989; Corzo and Niell 1994). Under WL of low PFR, NO_2^- release correlated well with NO_3^- uptake, indicating that NO_3^- and NO_2^- reduction are partially uncoupled under these conditions. Under BL and RL, however, it is interesting that NO_2^- release was delayed until the fifth day and, overall, represented a lower fraction of NO_3^- uptake than under WL (Table 5).

Unlike WL, NH_4^+ was released under BL and RL (Table 5), suggesting that NH_4^+ assimilation (either through enzymatic glutamine synthetase–glutamate synthase activity and/or the availability of carbon skeletons) may be limited. Alternatively, excess NH_4^+ could result from enhanced protein turnover. Concomitant with NO_2^- and NH_4^+ release under BL and RL, NO_3^- uptake was restricted, indicating a feedback-control of the NH_4^+ assimilation process during NO_3^- transport (Lara et al. 1987).

Photosynthesis

The light-saturated rate of photosynthesis was higher for algae grown under BL and RL than for those grown under WL. The limiting steps for V_{max} are electron-transport components, PSII density (involving oxygen evolution) and RuBisCO (ribulose-1,5 biphostate carboxylase oxygenase) activity (Wilhem 1993). Although pigment composition was little affected by light quality, differences in V_{max} could be attributable to changes in the stoichiometry between the two photosystems. If the PSII : PSI ratio increased under BL and RL, as stated by other authors (Fujita et al. 1987; Chow et al. 1990;

Table 5 *Gelidium sesquipedale.* Integrated mean values of NO_3^- uptake, NO_2^- release, NH_4^+ release, net NO_3^- uptake (minus NO_2^- and NH_4^+ release) and ratios NO_2^- release:NO_3^- uptake and NH_4^+ release:NO_3^- uptake in cultures. Values are µmol NO_3^-, NO_2^- or NH_4^+ g^{-1} dry wt d^{-1}

	NO_3^- uptake	NO_2^- release	NO_4^+ release	net NO_3^- uptake	$NO_2^-:NO_3^+$	$NO_4^+:NO_3^-$
100 $\mu mol\,m^{-2}\,s^{-1}$						
WL	35	0	0	35	0	0
40 $\mu mol\,m^{-2}\,s^{-1}$						
WL	14.67	2.88	0	11.79	0.20	0
BL	16.81	2.42	8.01	6.38	0.14	0.48
RL	23.91	3.11	4.77	16.03	0.13	0.20

Cunningham et al. 1990), an enhancement of the light-saturated rate of photosynthesis would be expected, especially for red algae in which the PSII:PSI ratio is low.

The initial slope (α) of the PI curves was affected by PFR. It is known that a is dependent on acclimation PFR (Dubinsky et al. 1986; Herzig and Dubinsky 1993), as there is less necessity of capturing light under moderate PFRs than under limiting PFRs. Following a shift-up of PFR, the reduction of α was lower for RL-grown algae, followed by BL- and WL-grown algae. As α decreased, despite the increase of accessory pigments recorded for previously BL- and RL-grown algae, a "package effect" may be operating to modify the in vivo absorption of light (Berner et al. 1989).

Pigment photoacclimation

The response of photosynthetic pigments appears to be compensatory (Ley and Butler 1980; Cunningham et al. 1990) rather than chromatically adaptive. The concentration of phycobiliproteins is little affected by light quality, and the ratio RPE:chlorophyll *a* (Table 4) was only slightly higher in RL, followed by BL, and then by WL. Thus, a stimulated synthesis of the pigments appears to be associated with a less-activated photosystem in terms of light. Whereas complementary chromatic adaptation has been shown to occur in cyanobacteria (Tandeau de Marsac 1977), this mechanism is not clear in red algae (Ley and Butler 1980; Cunningham et al. 1990). However, complementary chromatic adaptation has been described on a short-term scale for red algae (López-Figueroa and Niell 1990; Algarra et al. 1991; Torres et al. 1995). These two mechanisms, complementary and compensatory chromatic adaptation, although different, are not mutually exclusive. A first short-term response (hours), a complementary chromatic adaptation, may be superseded by a compensatory control of pigment synthesis on a long-term scale (days). Stoichiometric adjustments of the photosystems have been shown to be a compensatory strategy employed to correct an unbalanced absorption of light by both photosystems (Chow et al. 1990). Although in the present study the phycobiliprotein concentration was little affected by light quality, this does not imply constant photosystem stoichiometry. Although not controlled in our study, the photosynthetic performance [i.e. the higher V_{max} of BL and RL cultures (Table 2) and the higher photosynthesis of BL- and RL-grown algae compared to WL-grown algae (Table 3)] suggests a modification of the photosystem stoichiometry, increasing the PSII:PSI ratio in BL and RL cultures. In the unicellular red alga *Porphyridium cruentum*, light quality induces drastic changes in the photosystem stoichiometry (PSII:PSI:phycobilisomes), with values of 4:4:1 under RL and 2:6:1 under green light, and small changes in overall pigment composition (Cunningham et al. 1990). Recently, Sagert and Schubert (1995) observed complementary chromatic adaptation in terms of an enhanced RPE:RPC ratio under green light in the red alga *Palmaria palmata* L., with an increase of PSII emission in red light-adapted algae and a decrease in green light-adapted algae.

Long-term light-quality effects

Light treatments had repercussions on the subsequent WL phase at moderate PFR (third phase). RPE and RPC concentrations increased markedly for previously BL- and RL- grown algae, (but not for WL-grown algae), whereas chlorophyll *a* was unaffected. This indicates that the previous light treatments caused an activation and/or repression of several metabolical pathways that became fully expressed upon transfer to WL. These effects are not restricted to phycobiliproteins. While total carbon, total nitrogen and insoluble proteins were little affected by previous illumination, C- and N-partitioning displayed changes subsequent to the previous light treatments. Soluble protein and amino acid concentration was higher for previously BL- and RL-grown algae, whereas insoluble carbohydrate concentration was lower, resulting in a decrease in the ratio insoluble carbohydrates:total C (Fig. 6). This ratio is an indicator of C-partitioning between carbon compounds and organic nitrogen compounds, varying inversely to the magnitude of nitrogen supply in *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto et Foldvik (Vergara et al. 1995). In the present study, the recorded increase in nitrogen compounds (soluble proteins and amino acids) promoted a change in the C-partitioning, carbon being directed towards organic nitrogen compounds and away from carbohydrates. N-partitioning also varied. The ratio phycobiliproteins:total proteins increased for previously BL- and RL-grown algae (Table 4), indicating an increased amount of nitrogen in red pigments (García-Sánchez et al. 1993; Vergara and Niell 1993).

Our results on growth and biochemical composition of *Gelidium sesquipedale* are quite different from those obtained for the laminar red algae *Porphyra leucosticta* Thur. in Le Jolis cultured under BL and RL of the same spectral composition (Figueroa et al. 1995a, b). In *P. leucosticta*, growth was only stimulated in RL; it was limited in BL, and accumulated internal nitrogen compounds under this latter light regime. Two facts should be considered when comparing the responses of these species. First, *P. leucosticta* is an RPC-rich species whereas *G. sesquipedale* is a RPE-rich species (Dring 1981; Lüning and Dring 1985). The ratio RPE:RPC varies between 1 and 2 in *P. leucosticta* (Figueroa et al. 1995b), whereas it is 13 ± 2 in *G. sesquipedale* (present study). This means that for *P. leucosticta* the drop in the red portion of the photosynthetic action spectrum will

be lower than for *G. sesquipedale*. Thus, PSII-dependent oxygen evolution may be more efficiently achieved in *P. leucosticta* under RL. In fact, in this species the rate of photosynthesis in RL was even higher than in a WL control of the same PFR (Figueroa et al. 1995a). Second, *G. sesquipedale* is a slower-growing species than *P. leucosticta*, and accumulates carbon to a greater extent. In *P. leucosticta*, carbon degradation was also observed under BL that reached a critical level of 20% dry wt after 2 d. Therefore, *G. sesquipedale*, with a high reserve of carbon (in the form of cell-wall polysaccharides and starch) capable of mobilization, can sustain higher growth under BL than *P. leucosticta*.

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