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AMMONIUM AND UV RADIATION STIMULATE THE ACCUMULATION OF MYCOSPORINE-LIKE AMINO ACIDS IN *PORPHYRA COLUMBINA* (RHODOPHYTA) FROM PATAGONIA, ARGENTINA[1]

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The combined effects of ammonium concentration and UV radiation on the red alga *Porphyra columbina* (Montagne) from the Patagonian coast (Chubut, Argentina) was determined using short-term (less than a week) experimentation. Discs of *P. columbina* were incubated with three ammonium concentrations (0, 50, and 300 µM NH_4Cl) in an illuminated chamber (PAR = 300 µmol photons · $m^{-2} \cdot s^{-1}$, UVA = 15 $W \cdot m^{-2}$, UVB = 0.7 $W \cdot m^{-2}$) at 15° C. Algae incubated at 300 µM ammonium showed a significant increase ($P < 0.05$) in the concentration of mycosporine-like amino acids (MAAs) compared with the initial value or with the other ammonium treatments. The increase of MAAs was, however, a function of the quality of irradiance received, with a higher increase in samples exposed to UVA compared with UVB (29% and 5% increase, respectively). However, UVB radiation was more effective in inducing MAA synthesis per unit energy received by the algae. Samples exposed to PAR only had an intermediate increase in MAA concentration of 16%. Chl *a* concentration decreased through the incubation with the greatest decrease at high ammonium concentration. Phycobiliprotein (BP) decreased through time with the smallest decrease occurring at high ammonium concentration. Photoinhibition (as a decrease of optimal quantum yield) was significantly greater under nitrogen-deprived conditions than that under replete ammonium levels. Maximal gross photosynthesis (GP_{max}), as oxygen evolution, and maximal electron transport rate (ETR_{max}), as chl fluorescence, increased with the ammonium concentration. Positive relationships between maximal GP or ETR and pigment ratios (BP/chl *a* and MAAs/chl *a*) and negative relationships with chl *a* concentration were found.

Key index words: **ammonium; biliproteins; chl fluorescence; mycosporine-like amino acids; photosynthetic activity; *Porphyra*; quantum yield; UV radiation**

Abbreviations: **a, area; A, absorbance; α, photosynthetic efficiency; BP, phycobiliprotein; DIN, dissolved inorganic nitrogen DW, dry weight; ETR, electron transport rate; F_o, initial fluorescence in the dark-adapted state (all reaction centers are open, oxidized); F_m, maximal fluorescence in the dark-adapted state (all reaction centers are closed, reduced); F_m', the same for the light-adapted state; F_v/F_m, optimal or maximal quantum yield; FW, fresh weight; GP, gross photosynthetic rate; GP_{max}, gross photosynthetic rate at light saturation; P, PAR; PA, PAR + UVA; PAB, PAR + UVA + UVB; PAM, pulse amplitude modulated fluorescence; PC, phycocyanin; PE, phycoerythrin; Φ_{PSII}, effective quantum yield as chl fluorescence; Φ_{O2}, photosynthetic quantum yield as oxygen evolution; R_d, dark-respiration rate; UVA, ultraviolet A (λ = 315–400 nm); UVB, ultraviolet B (λ = 280–315 nm); UVR, ultraviolet radiation (λ = 280–400 nm)**

Normal levels of solar UV radiation (UVR) and the increased flux due to the depletion of the stratospheric ozone layer harm many biological processes. Multiple detrimental effects of UVR on proteins, DNA, and other biologically relevant molecules of autotrophic organisms as well as chronic depression of physiological processes (photosynthesis, growth) and influences on community structures have been reported (Buma et al. 1995, 1997, Franklin and Forster 1997, Häder and Figueroa 1997, Aguilera et al. 1999b, Helbling et al. 2001, Villafañe et al. 2002). Of prime interest is the identification of repair and/or protective mechanisms that allow phototrophic organisms living in high-light habitats to survive and reproduce.

One mechanism that acts against UV damage is the biosynthesis and accumulation of UV-absorbing compounds, mainly mycosporine-like amino acids (MAAs) (Dunlap and Shick 1998). The MAAs are water-soluble low-molecular-weight molecules with high molar

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extinction coefficients ($\varepsilon = 28{,}100$–$50{,}000\ M^{-1} \cdot cm^{-1}$) and absorption bands in the UV with maxima between 310 and 360 nm (Cockell and Knowland 1999, Shick and Dunlap 2002). Mycosporine-like amino acids have been inferred as UV screen substances from their high absorptivity in the range 310–360 nm, from their increase in cellular concentration with increased UV exposure (Wood 1989, Karsten and Wiencke 1999), from the correlation with reduced photoinhibition and decreased photodamage (Lesser 1996, Bischof et al. 2000), and from the action spectra for accumulation (Sinha et al. 2002). However, this protective function against effects of UVR is not clear because there are some results in which changes in UVR or PAR might not induce MAA accumulation (Gröniger et al. 1999) and others in which MAAs do not provide complete protection against UV effects (García-Pichel et al. 1993, Neale et al. 1998, Franklin et al. 1999, Yakovleva and Titlyanov 2001). Mycosporine-like amino acids were invoked to function as passive shielding substances by dissipating the absorbed radiation energy in form of harmless heat without generating photochemical reactions (Bandaranayake 1998), but mycosporine-glycine could also have a secondary photoprotective capacity as an antioxidant (Dunlap and Yamamoto 1995). Correlations between the concentration of MAAs and irradiance, which vary seasonally (Post and Larkum 1993, Karsten et al. 1999), with microclimate (Beach and Smith 1996), or with depth (Dunlap et al. 1986, Karsten et al. 1999), have been recorded in several macroalgae and corals, leading to the proposal that they play a protective role.

In addition to solar radiation, the accumulation of MAAs can be affected by other environmental variables such as salinity, temperature, or nutrient availability (Bandanarayake 1998, Dunlap and Shick 1998, Karsten and Wiencke 1999). Despite the fact that MAAs are nitrogenous compounds, there is scarce information on the effect of nitrogen availability on the synthesis and accumulation of MAAs (Banaszak and Neale 2001, Litchman et al. 2002). Nitrogen is a critical component in the productivity of ecosystems and in all biochemical processes. Several repair mechanisms against UV effects involve N-requiring enzymes and/or protein cofactors (Roy 2000). Nitrogen limitation increased the UV sensitivity of photosynthesis in dinoflagellates, the main mechanism being less efficient repair (Litchman et al. 2002).

The objective of this study was to determine the combined effects of different concentrations of inorganic nitrogen (in the form of ammonium) and UVR on the biosynthesis and accumulation of MAAs and other nitrogenous compounds (chl and biliproteins) in the red alga *Porphyra columbina* (Montagne) collected from the Patagonian coast (Chubut, Argentina). This species grows in the eulittoral zone and can be subjected to high irradiance and desiccation during low tide. *Porphyra* spp. contain high levels of MAAs, mainly porphyra-334, and among the red macroalgae analyzed accumulate the highest concentrations (Karsten et al. 1998b, Gröniger et al. 1999, Hoyer et al. 2001). Transient ozone depletion has been reported in the region of the Patagonian coast, in addition to low cloudiness (Orce and Helbling 1997); thus, it is of great interest to establish the capacity for photoprotection of intertidal species exposed to natural high levels UVR.

MATERIALS AND METHODS

Algal material. Specimens of *P. columbina* (Montagne) were collected from the intertidal zone of a rocky beach (Barrancas Blancas, Playa Unión, Rawson, Chubut, Argentina, 43°18′S, 65°03′W) during low tide in November 2001. Biological characterization of the intertidal zone of Barrancas Blancas has been previously reported (Häder et al. 2002), but the temperature of the water during our studies varied from 14 to 17° C. Irradiance conditions of the area have been published elsewhere (Barbieri et al. 2002); however, during our experiments solar radiation was $380\ W \cdot m^{-2}$ for PAR, $60\ W \cdot m^{-2}$ for UVA, and $1.7\ W \cdot m^{-2}$ for UVB. One important feature of the area is the heavy load of nutrients (mainly nitrogen) carried by the Chubut River during spring (due to wash-over of fertilizers added to the land upstream) with up to 1500 metric tons of dissolved inorganic nitrogen (DIN) discharged per year (Helbling 1989).

In all experiments, discs of approximately 1.2 cm in diameter were cut from thalli and preacclimated in an illuminated culture chamber (Sanyo MLR 350, Sanyo, Japan) at 15° C in aerated cylinders (5-L capacity UV-transparent Plexiglas) filled with artificial seawater (salinity of 34 psu, Marine Mix hw-professional, Wiegandt GmbH, Krefeld, Germany) for 14 h. During this time the algae were illuminated with fluorescent light ($40\ \mu mol\ photons \cdot m^{-2} \cdot s^{-1}$) and the water supplemented with phosphorus (Na_2HPO_4) to a concentration of 10 μM.

Experimentation. Two basic experiments were conducted as described below. Both were carried out inside the illuminated culture chamber (Sanyo MLR 350; 15° C) that was furnished with 10 daylight fluorescent tubes and five Q-Panel UVA-340 (Q-Panel Co., Cleveland, OH, USA), providing irradiances of $300\ \mu mol\ photons \cdot m^{-2} \cdot s^{-1}$ for PAR, $15\ W \cdot m^{-2}$ for UVA, and $0.7\ W \cdot m^{-2}$ for UVB.

Ammonium experiment: After the preacclimation period, discs of *P. columbina* were placed in three 5-L UV-transparent Plexiglas cylinders (100 discs per cylinder) and maintained for 6 days under three ammonium concentrations (0, 50, and 300 μM NH_4Cl). During this time the cylinders for each ammonium treatment were covered with an Ultraphan 295 filter (Digefra GmbH, Munich, Germany), and thus the algae were exposed to PAR + UVA + UVB (PAB, see irradiances above) with a 14:10-h light:dark photoperiod. Photosynthetic pigments (BP and chl *a*), MAAs, photosynthetic activity as oxygen evolution and *in vivo* chl fluorescence, and growth rate were measured after 2 and 6 days of exposure. The initial values were taken just after the preacclimation period.

Radiation experiment: Discs of *P. columbina* were maintained for 12 days in 0 μM NH_4^+ under the same radiation conditions mentioned above. After this time, the discs were sorted in three 5-L cylinders (50 discs per cylinder), and NH_4Cl was added to reach a concentration of 300 μM. Three radiation treatments were implemented by cutting off the radiation with the use of UV filters: 1) samples exposed to PAB (i.e. samples received the same radiation of the previous 12 days, see above), 2) samples exposed to PAR + UVA (PA) (cylinder covered with a Folex 320 filter, Folex GmbH, Dreieich, Germany), and 3) samples exposed to only PAR (P) (cylinder covered with an Ultraphan 395 filter, Digefra GmbH). The transmission characteristics of the filters used are shown in

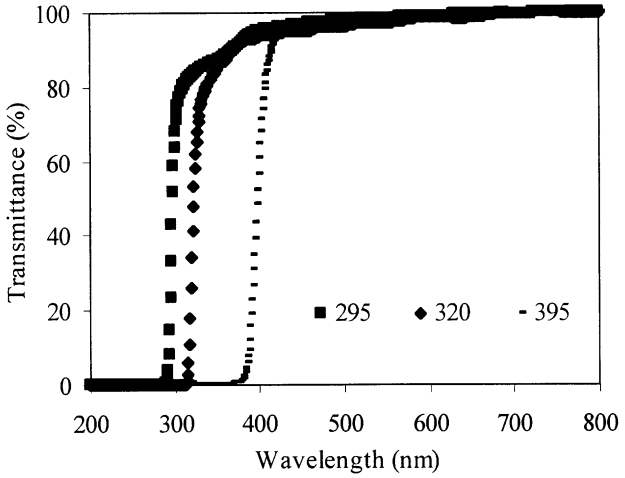

FIG. 1. Transmittance (%) of the filters used to remove UVR: Ultraphan 295, Folex 320, and Ultraphan 395.

Figure 1. With this setup, the samples (discs of *P. columbina*) were irradiated for 3 additional days and the following parameters measured in each treatment: photosynthetic pigments (chl *a*), MAAs, and photosynthetic activity as *in vivo* chl fluorescence after 1 and 3 days of exposure. The initial values were taken just before the addition of NH_4Cl.

Photosynthetic pigments. Two discs of about 10–20 mg (FW) per sample were extracted in 2 mL 100% methanol for 24 h at 4° C in darkness. After centrifugation at 5000 *g* for 10 min (Labofuge 400R, Heraeus, Kendro Laboratory Products, Langenselbold, Germany), absorption spectra between 250 and 750 nm were measured using an HP-8453 diode array spectrophotometer (Hewlett-Packard, Buenos Aires, Argentina). Chl *a* concentration was calculated from the OD using the equation given in Wellburn (1994). Triplicate samples were taken at each time from each treatment.

To determine phycobiliproteins phycoerythrin (PE) and phycocyanin (PC), samples of 30–40 mg (FW) of algal biomass were homogenized and extracted in 2.5 mL of 0.1 M phosphate buffer (pH 6.8). The extracts were centrifuged at 5000 *g* for 10 min and the concentration of phycobiliproteins in the supernatant was determined using the equations of Beer and Eshel (1985). Triplicate samples were taken at each time from each treatment (ammonium experiment).

Determination of MAAs. Triplicate samples of dried algal samples (10–20 mg DW) were extracted for 2 h in screw-capped centrifuge vials filled with 1 mL 20% aqueous methanol (v/v) at 45° C. After centrifugation at 5000 *g* for 5 min, 700 μL of the supernatant were evaporated to dryness under vacuum (Jouan evaporator centrifuge RC 10.09, Cedex, France). Dried extracts were redissolved in 700 μL 100% methanol and mixed for 30 s. After passing through a 0.2-μm membrane filter, samples were analyzed with a Waters 600 HPLC system (Waters Cromatografia, Barcelona, Spain). The mobile phase was 2.5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at 0.5 $mL \cdot min^{-1}$. Sample volumes of 10 μL were injected into the Sphereclone C8 column (5 μm packing; 250 × 4 mm I.D., Phenomenex, Aschaffenburg, Germany) with a guard column (C8, Octyl, MOS, Phenomenex). MAAs were detected online with a Waters Photodiode Array Detector 996 at 330 nm, and absorption spectra (290–400 nm) were recorded each second directly on the HPLC-separated peaks. MAAs were identified by comparing the absorption spectra and retention times with various standards extracted from the marine macroalgae *Mastocarpus stellatus*, *Bostrychia scorpioides*, and *Porphyra yezoensis* and from fish lenses of the coral trout *Plectropomus leopardus*, kindly provided by Prof. Ulf Karsten (University of Rostock, Germany). Quantification was made using published extinction coefficients (Takano et al. 1978a,b, Tsujino et al. 1980, Dunlap et al. 1986, Gleason 1993). Results of the HPLC analysis are expressed as $mg \cdot g^{-1}$ DW.

Photosynthetic activity. To monitor changes in photosynthetic efficiency and capacity, photosynthesis versus irradiance curves were determined by oxygen evolution and *in vivo* chl fluorescence. Oxygen production rates of thallus discs from each nutrient treatment were measured using OXY M5 equipment (Real Time Computer, Erlangen, Germany) furnished with five Clark electrodes (YSI 5331, Yellow Spring, Yellow Springs; OH, USA). The equipment had five independent chambers to each of which we added two discs. Then the whole setup was incubated in the illuminated culture chamber (Sanyo MLR 350) at increasing irradiances (PAR = 0, 66, 90, 171, and 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$, 15 min each) given by a combination of 10 daylight fluorescent tubes and with the use of neutral density filters. The photosynthetic parameters were estimated by fitting a nonlinear function (Jassby and Platt 1976) to each data series:

$$GP = GP_{max} \cdot (\tanh(\alpha E/GP_{max}))$$

where GP is the gross photosynthetic rate, GP_{max} is the light saturated gross photosynthesis rate, tanh is the hyperbolic tangent function, α is the photosynthetic efficiency at low irradiance, and E is the incident irradiance. Finally, the saturation irradiance for photosynthesis (E_k) was calculated from the intercept between GP_{max} and α. Respiration (R_d) was estimated as oxygen depletion in darkness at the beginning of the incubation period.

In vivo chl fluorescence of PSII was determined with a portable pulse modulation fluorometer (PAM 2000, Waltz GmbH, Effeltrich, Germany). After 15 min in darkness to measure F_o, a saturating flash was applied to obtain the maximal fluorescence level (F_m), and the maximal quantum yield of fluorescence (F_v/F_m) was obtained (Schreiber et al. 1986). The variable fluorescence F_v is the difference between the maximal fluorescence from fully reduced PSII reaction center (F_m) and the initial fluorescence (F_o) from the antenna of fully oxidized PSII. The effective quantum yield (Φ_{PSII}) was calculated from F'_m-F_s/F'_m, where F_s is the current steady-state fluorescence in light-adapted algae, and $F'_m$is the maximal fluorescence induced with a saturating white light pulse (0.4 s, approximately 9000 μmol photons $\cdot m^{-2} \cdot s^{-1}$). For measurements of F_v/F_m and Φ_{PSII}, eight replicates were taken for each treatment and time. The electron transport rate (ETR) was calculated according to the following formula (Schreiber and Neubauer 1990):

$$ETR\ (\mu mol\ electrons \cdot m^{-2} \cdot s^{-1}) = \Phi_{PSII} \cdot E \cdot A \cdot 0.5$$

where E is the incident irradiance and A is the absorptance and the factor 0.5 comes from the quanta/electron ratio (one electron requires absorption of two quanta as two photosystem are involved). The absorptance, $A = 1 - (E_t/E_o)$, was calculated from the light transmitted through a piece of *Porphyra* placed on top of a cosine corrected PAR sensor (Licor 192 SB, Li-Cor, Lincoln, NE, USA) connected to a data-logger (Licor-1000) (E_t) and E_o, the incident irradiance in the absence of the algal piece.

The ETRs from each nutrient treatment were measured in parallel with oxygen production rates using another set of chambers illuminated in the same conditions mentioned above. The ratios ETR/GP were calculated for the initial values and after 2 and 6 days of exposure.

Growth rate. The growth rate of each disc was determined from the change disc diameter, because growth of the circular discs proceeded isodiametrically. The relative growth rate,

expressed as the percentage increase per day, was computed from the following expression (Kain 1987):

$$R = (\ln a_t - \ln a_o)/t$$

where a_t is the area measured at time (t) in days and a_o is the area at the initial time. Ten replicates were taken for each treatment.

Irradiance measurements. The irradiances of the light sources were measured with an ELDONET radiometer (Real Time Computer, Erlangen, Germany) placed on a permanent site beside the experimental system. The instrument was equipped with sensors recording wavelength bands of 400–700 nm (PAR), 315–400 nm (UVA), and 280–315 nm (UVB) at 1-min intervals and using an Ulbrich (Real Time Computer) integrating sphere to collect direct and indirect radiation.

Statistics. These were assessed by two-way analysis of variance. Where significant differences were detected, post-hoc multiple comparisons were made using the Tukey's test. Probability for type I errors was set to $\alpha = 0.05$. Computations were done with SPSS for Windows (SPSS, Chicago, IL, USA), version 10.0.

RESULTS

Effect of ammonium concentration on the synthesis of MAAs and photosynthetic pigments. Results from the ammonium experiments (i.e. three concentrations: 0, 50, and 300 μM) on the synthesis of MAAs and photosynthetic pigments (chl *a*, PE, and PC) at days 2 and 6 are shown in Figure 2. No significant increase in the concentration of MAAs was found during the 6 days of exposure at 0 and 50 μM NH_4^+. Samples cultivated at 300 μM NH_4^+, however, showed a significant increase ($P < 0.05$) compared with the initial value and other treatments at day 6 (Fig. 2a). Five MAAs were identified in *P. columbina*: mycosporine-glycine, shinorine, porphyra-334, palythine, and asterina. Porphyra-334 was the most abundant MAA (about 80% of the total concentration) and was always present. Shinorine was also present in high concentrations (about 20%), whereas the remaining

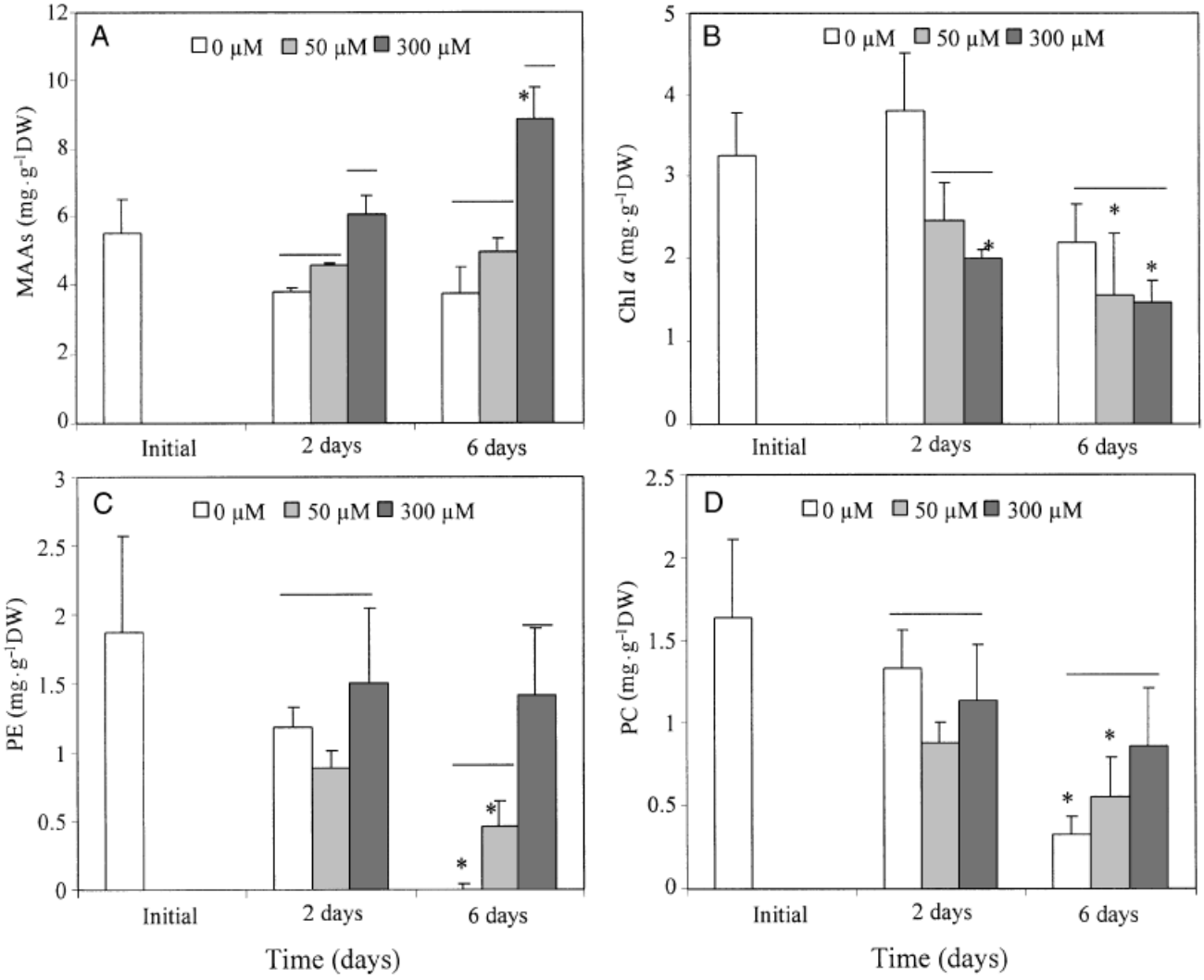

FIG. 2. Concentration of total MAAs (a), chl *a* (b), phycoerythrin (PE) (c), and phycocyanin (PC) (d) expressed as $mg \cdot g^{-1}$ DW in *Porphyra columbina* grown for 2 and 6 days under 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of PAR, 15 $W \cdot m^{-2}$ of UVA, and 0.7 $W \cdot m^{-2}$ of UVB and different NH_4Cl concentrations (0, 50, and 300 μM). Data are expressed as mean value ± SD ($n = 3$). The horizontal lines over the bars indicate the differences among treatments, whereas the asterisks are the differences among days of experimentation.

MAAs occurred at much lower concentrations. All MAAs identified had the same pattern during the experiment period (data not shown), so we do not present the behavior of each one but rather the total concentration.

Chl *a* and phycobiliprotein contents were significantly affected by the NH_4^+ concentration (Fig. 2, b–d). The concentration of chl *a* in the 50- and 300-μM NH_4^+ treatments decreased continuously from 3.2 to 1.5 and 1.46 $mg \cdot g^{-1}$DW, respectively, after 6 days. There was no significant difference, however, in the concentration of chl *a* in the 0-μM NH_4^+ treatment during the incubation period (Fig. 2b). Phycobiliprotein content decreased significantly after 6 days of exposure to 0 and 50 μM NH_4^+, PE decreasing from 1.9 to 0 and 0.45 $mg \cdot g^{-1}$ DW, respectively, and PC decreasing from 1.6 to 0.33 and 0.56 $mg \cdot g^{-1}$ DW, respectively. There were no significant differences in PE and PC concentration in 300 μM NH_4^+ during the experiment.

The ratios PE/PC and BP/chl *a* (Fig. 3, a and b) increased through the incubation time in the 300-μM NH_4^+ treatment. In the 0- and 50-μM NH_4^+ treatments, however, these ratios decreased and after 6 days of exposure were lower than the initial values (Fig. 3, a and b). The ratio MAAs/chl *a* increased with time and with ammonium concentration, reaching the highest values after 6 days in 300 μM NH_4^+ (Fig. 3c). The ratio between the two nitrogenous compounds (MAAs/BP) increased throughout the experiment, but this increase was negatively correlated to ammonium concentration, reaching the highest value in the absence of ammonium (Fig. 3d).

Effects of ammonium concentration on photosynthetic activity and growth. Both optimal quantum yield (F_v/F_m) (Fig. 4a) and effective quantum yield (Φ_{PSII}) (Fig. 4b) decreased throughout time under PAB compared with the initial values. These declines in photosynthetic activity were significantly greater in the treatment that had 0 μM NH_4^+.

Ammonium supply had little effect on the photosynthetic quantum yield (Φ_{O2}); GP_{max}, however, increased significantly at higher ammonium concentrations (Fig. 5, Table 1). Plants grown in 300 μM NH_4^+ had a higher GP_{max} and R_d than plants grown in 0 or 50 μM NH_4^+. There were no differences, however, in the initial slopes (Fig. 5, Table 1). GP_{max} and R_d also increased significantly from the day 2 to day 6 of culture in the 50- and 300-μM ammonium treatments (Table 1). The slopes increased after 2 days in all the treatments.

The ETR under different absorbed irradiances did not differ significantly after 2 days of culture (Fig. 6a). At day 6, however, maximal ETR was significantly higher in the 300-μM than in the 0-μM NH_4^+ treatment (Fig. 6b). The maximal ETR at all irradiances, however, was observed in the initial values, suggesting a decrease of ETR with both ammonium concentration and time of experimentation.

With the data presented in Figures 5 and 6, we calculated the ratio ETR/GP, which varied for the initial values from 6.4 ± 2.2 at 66 μmol photons $\cdot m^{-2} \cdot s^{-1}$ to

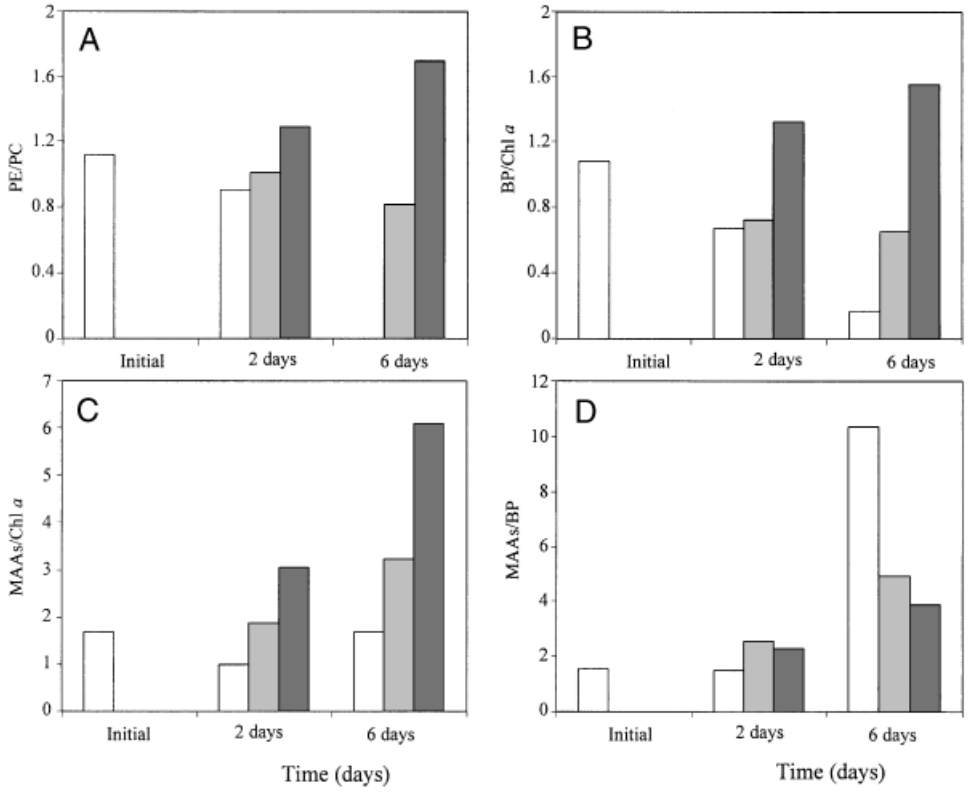

FIG. 3. PE/PC (a), BP/chl *a* (b), MAAs/chl *a* (c), and MAAs/BP (d) in *Porphyra columbina* grown for 2 and 6 days under 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of PAR, 15 $W \cdot m^{-2}$ of UVA, and 0.7 $W \cdot m^{-2}$ of UVB and different NH_4Cl concentrations (0, 50, and 300 μM). Data are expressed as mean value ± SD ($n = 3$).

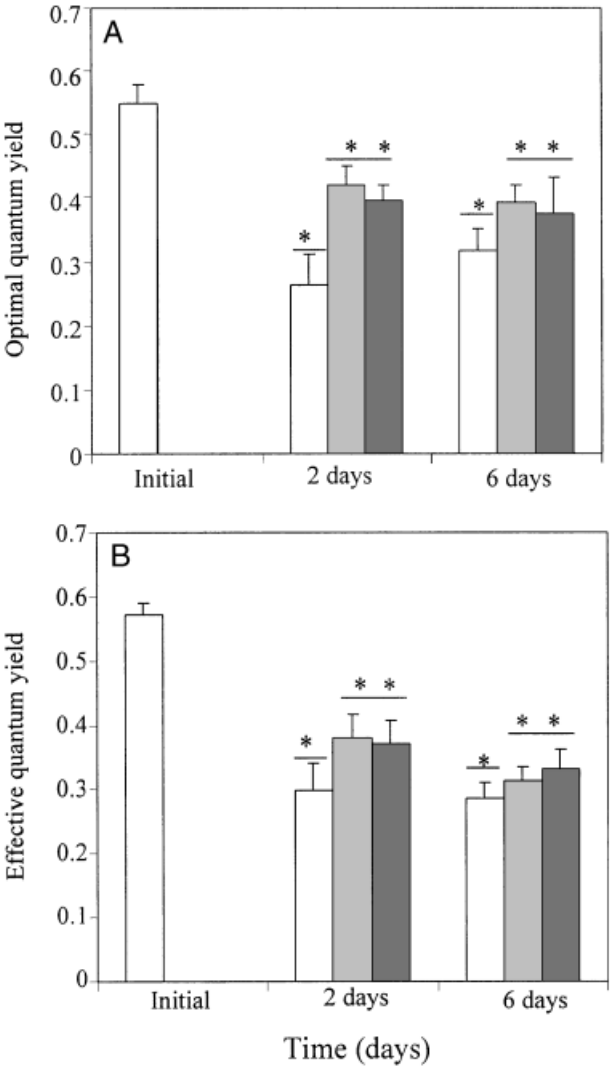

FIG. 4. Optimal quantum yield (F_v/F_m) (a) and effective quantum yield (Φ_{PSII}) (b) in *Porphyra columbina* grown for 2 and 6 days under 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of PAR, 15 W $\cdot m^{-2}$ of UVA, and 0.7 W $\cdot m^{-2}$ of UVB and different NH_4Cl concentrations (0, 50, and 300 μM). Data are expressed as mean value ± SD ($n = 8$). The horizontal lines over the bars indicate the differences among treatments, whereas the asterisks are the differences among days of experimentation.

10.1 ± 1.4 at 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$. After 2 days exposure the ratio increased from 5.4 ± 0.9 to 16.0 ± 2.3 in the 0-μM NH_4^+ treatment, from 5.6 ± 1.0 to 12.5 ± 1.4 in the 50-μM NH_4^+ treatment, and from 4.7 ± 0.9 to 7.6 ± 1.0 in the 300-μM NH_4^+ treatment. After 6 days exposure, even though the ratio still showed a relative increase with irradiance within each NH_4^+ treatment (i.e. from 4.5 ± 0.8 to 11.4 ± 1.9, from 4 ± 0.7 to 8.3 ± 1, and from 4.1 ± 0.9 to 5.9 ± 1.2 in the 0-, 50-, and 300-μM NH_4^+ treatments, respectively), ETR/GP was lower at high irradiance than that after 2 days. This indicates that fewer electrons were required to produce 1 oxygen molecule. GP and ETR functions did not follow a linear relationship but a hyperbolic function (data not shown); the maximal values of GP versus ETR function increased at 50- and 300-μM NH_4^+ concentrations.

The growth rate of *P. columbina* in the different ammonium treatments was calculated after the second and last day of incubation. There was a significant increase in the disc area and thus in the relative growth rate in the 300- and 50-μM NH_4^+ treatments compared with the 0-μM NH_4^+ treatment after 6 days of incubation. The relative growth rates were 1.89 ± 1.22, 7.21 ± 2.06, and 10.61 ± 2.16 in the 0-, 50-, and 300-μM NH_4^+ treatments, respectively.

Additional effect of UVR. Results from the radiation experiments indicated that there were no significant differences in MAA concentration in the different radiation treatments after 24 h exposure. The concentration in all treatments, however, was significantly higher than the initial value (Fig. 7a). After 3 days exposure, however, the content of MAAs was significantly lower in thalli exposed to P in comparison with the PA and PAB treatments (Fig. 7a),

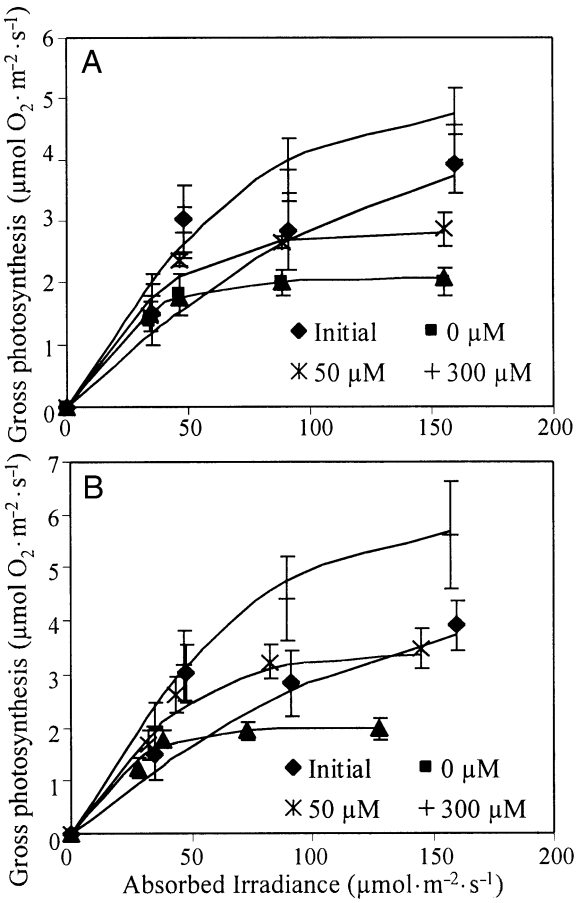

FIG. 5. Gross photosynthesis (GP) expressed as μmol $O_2 \cdot m^{-2} \cdot s^{-1}$ vs. absorbed irradiance in μmol photons $\cdot m^{-2} \cdot s^{-1}$ of *Porphyra columbina* grown for 2 (a) and 6 (b) days under 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of PAR, 15 W $\cdot m^{-2}$ of UVA, and 0.7 W $\cdot m^{-2}$ of UVB and different NH_4Cl concentrations (0, 50, and 300 μM). Data are expressed as mean value ± SD ($n = 5$).

TABLE 1. Maximal gross photosynthesis (GP_{max}), photosynthetic quantum yield (Φ_{O2}), and dark respiration (R_d) in *Porphyra columbina* after 2 and 6 days of incubation.

Time	Ammonium treatment	GP_{max} ($\mu mol\ O_2 \cdot m^{-2} \cdot s^{-1}$)	Φ_{O2} ($\mu mol\ O_2 \cdot \mu mol$ photon absorbed irradiance^{-1})	R_d ($\mu mol\ O_2 \cdot m^{-2} \cdot s^{-1}$)
Initial	—	4.46 ± 0.088	0.034 ± 0.003	0.27 ± 0.090
2 days	0 μM	2.06 ± 0.143	0.055 ± 0.003	0.93 ± 0.096
	50 μM	2.81 ± 0.200	0.058 ± 0.008	0.91 ± 0.149
	300 μM	4.94 ± 0.440	0.061 ± 0.010	0.97 ± 0.148
6 days	0 μM	2.01 ± 0.20	0.060 ± 0.009	0.44 ± 0.040
	50 μM	3.41 ± 0.19	0.063 ± 0.008	0.86 ± 0.083
	300 μM	5.93 ± 1.00	0.073 ± 0.009	1.23 ± 0.270

Values are means ± SD after fitting the data with the Jassby and Platt (1976) equation.

indicating a stimulation of MAA synthesis in the treatments exposed to UVR. Chl *a* concentration did not show any significant change during the 3 days (Fig. 7b). The ratio MAA/chl *a* increased with time of exposure in all light treatments. The MAA/chl *a* ratio was higher in the presence of UVR than under P, reflecting the increase of MAAs and the decrease of chl *a* in the P treatment. Maximum ETR was lower after 3 days under PAB (26.6) than under P (34.2) (Fig. 7c).

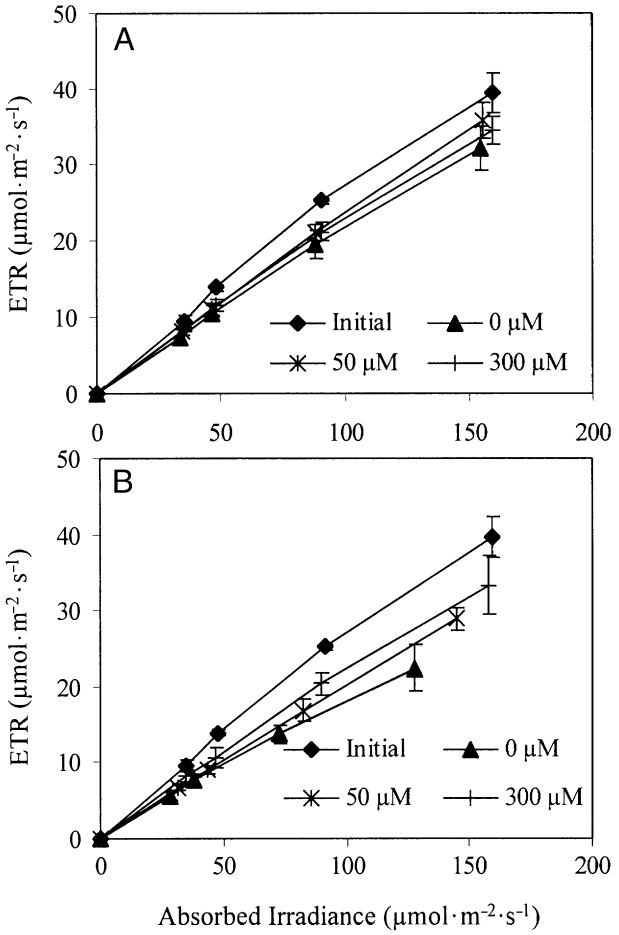

FIG. 6. Electron transport rate (ETR) expressed as μmol electrons $\cdot m^{-2} \cdot s^{-1}$ vs. absorbed irradiance in μmol photons $\cdot m^{-2} \cdot s^{-1}$ of *Porphyra columbina* grown for 2 (a) and 6 (b) days under 300 μmol photons $\cdot m^{-2} \cdot s^{-1}$ of PAR, 15 $W \cdot m^{-2}$ of UVA, and 0.7 $W \cdot m^{-2}$ of UVB and different NH_4Cl concentrations (0, 50, and 300 μM). Data are expressed as mean value ± SD ($n = 5$).

DISCUSSION

Mycosporine-like amino acids are mainly UVA absorbing compounds; however, studies (Marchant et al. 1991, Helbling et al. 1996) have shown that their synthesis can be stimulated by UVB radiation in a variety of algae. Other wavelengths (i.e. different energy quality) might also have an important role in inducing the synthesis of MAAs, as seen in *Chondrus crispus* (Karsten et al. 1998a). This study showed that both UVA and UVB stimulated a strong accumulation of shinorine, whereas the content of palythinol and palythine was mainly stimulated by PAR, suggesting an MAA-specific induction triggered by UVR or PAR. Nevertheless, an increase in MAA concentration is not always the case; for example, Gröniger et al. (1999) showed no increment of MAAs after UVR or PAR exposure of *Porphyra umbilicalis*. The quality of incident radiation is not the only important variable to induce MAAs synthesis, and previous studies (Karsten et al. 1998a, Franklin et al. 2001) have shown that the accumulation of MAAs was dependent of both dose and wavelength, with higher accumulation of MAAs under high daily doses (i.e. different latitudes). The location in the water column, and thus the amount of solar radiation, is also important because it was demonstrated that the accumulation of MAAs was greater in intertidal than in subtidal algae (Karsten et al. 1998b, Karsten and Wiencke 1999, Hoyer et al. 2001).

Data obtained in this study revealed that not only quality and quantity of the energy received by the algae induced MAAs synthesis, but also nutrient concentration (i.e. high ammonium concentration) significantly increased the content of MAAs in *P. columbina* after 6 days of incubation. It is interesting, however, that the stimulation of MAA synthesis for *P. columbina* under high nutrient concentration is different if the alga is exposed or not to UVR. In the presence of UVR there seems to be a faster increase of MAAs with the concentration being significantly higher even after 1 day of exposure. The increase of MAAs, however, was a function of the quality of irradiance received, with an

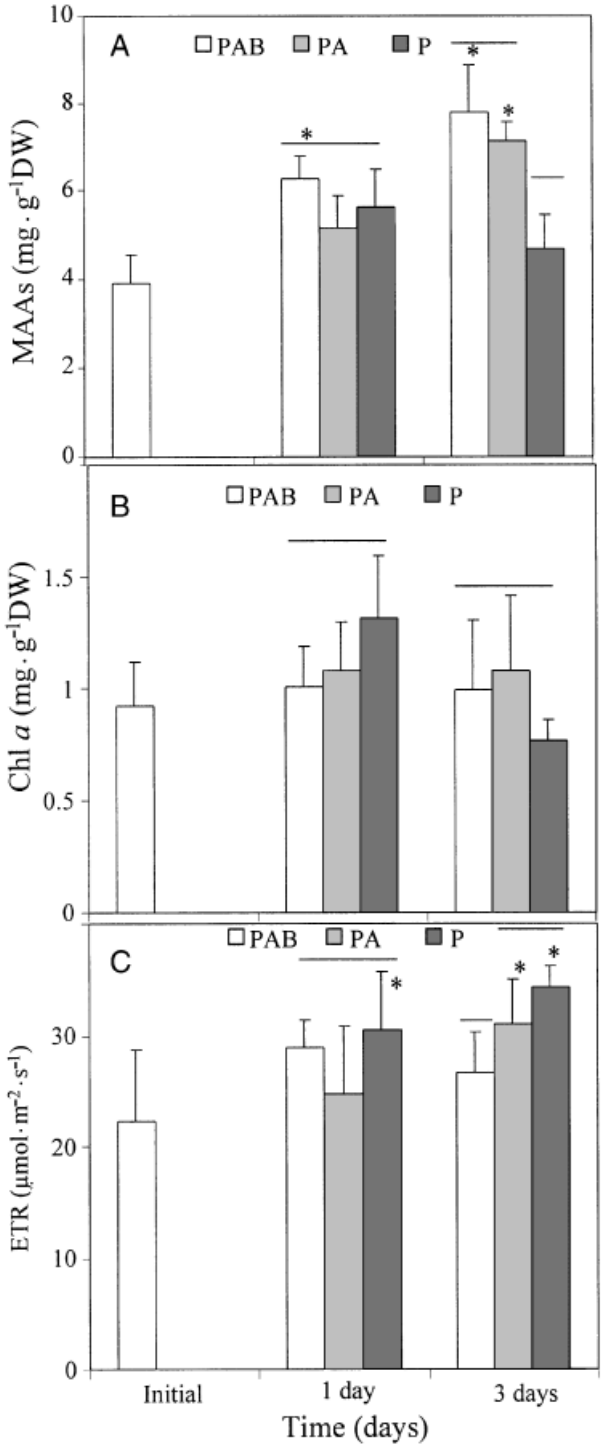

FIG. 7. Concentration of total MAAs (a), chl *a* (b) expressed as $mg \cdot g^{-1}$ DW, and ETR (c) in *Porphyra columbina* grown for 12 days in 0 µM of NH_4Cl under 300 µmol photons $\cdot m^{-2} \cdot s^{-1}$ of PAR, 15 $W \cdot m^{-2}$ of UVA, and 0.7 $W \cdot m^{-2}$ of UVB and transferred for 1 and 3 days under PAR (P), PAR + UVA (PA), and PAR + UVA + UVB (PAB) in the presence of 300 µM of NH_4Cl.

increase of 29% or 5% in samples receiving UVA or UVB, respectively. However, UVB was most effective per unit energy in inducing MAA synthesis. Samples exposed to PAR only had an intermediate increase in MAA concentration of 16%. Two considerations need to be made at this point for this latter comparison. First, because the experiments were conducted with artificial radiation (see Materials and Methods), the exposure period relates directly to the dose received by the algae. Second, because the algae were grown under no nitrogen for 12 days before NH_4Cl was added, this might have induced a faster incorporation of nitrogen into the algae. In any case, there was no significant decrease of MAAs during these 12 days, suggesting no loss of these compounds due to low nitrogen conditions. Karsten and West (2000) suggested that the lack of stimulation of MAA synthesis in many studies was due to the high initial concentration of MAAs (about 10 $mg \cdot g^{-1}$ DW) in the specimens they studied. In our case, the relatively low concentrations of MAAs in the samples might have favored the accumulation of MAAs as observed above.

One important point in our study was to establish if the relatively low concentration of MAAs (as compared with other studies as mentioned above) had a protective role in *P. columbina* and if the further stimulation of MAAs synthesis had an even higher protective effect. This in fact was what we observed, as the lowest photoinhibition and the highest maximal GP and ETR always occurred in algae with the highest concentration of MAAs. The improved resistance to UV stress of *P. columbina* after N addition may also be due to improved repair processes. Litchman et al. (2002) found that changes in apparent repair activity accounted for most of the difference in UV sensitivity of high versus low N grown dinoflagellates cultures. The high level of MAAs in the 300-M ammonium treatment can explain the reduced inhibition of the photosynthetic apparatus due to UVR and thus the algae can decrease the chl *a* content and still maintain the photosynthetic yield. The origin of MAAs via the shikimate pathway has been a persistent assumption (Favre-Bonvin et al. 1987, Shick et al. 1999). Because the shikimate pathway is closely coupled to photosynthesis (Bentley 1990, Carreto et al. 1990), it could be suggested that the highest photosynthetic rate under 300 µM ammonium might equate to a higher synthesis of cyclohexenone and cyclohexenimine cores for MAAs, that in the presence of high levels of ammonium allows the inclusion of the nitrogen substitute in the cores of MAAs. This process might indeed result in a positive feedback mechanism, allowing the better photosynthetic performance and growth of *P. columbina*.

In addition, a positive relationship between maximal GP and ETR with the pigment ratios (BP/chl *a* and MAAs/chl *a*) and negative relationship with chl *a* concentration were found. The mobilization of ammonium to MAAs and biliprotein at higher rates than to chl *a* are related to the maximal photosynthetic capacity. In red algae, both chl *a* and biliprotein concentrations increased with inorganic nutrient supply (López-Figueroa and Niell 1991), but the accumulation depended on the light quality (Figueroa et al.

1995) and quantity (Algarra and Niell 1990). Most studies on the pigment accumulation, however, have been conducted under artificial light (i.e. fluorescent lamps without UV) (Häder and Figueroa 1997). In our study, *P. columbina* was incubated under artificial white fluorescent lamps supplemented with artificial UV lamps, and thus the decrease of chl *a* may be related to both PAR and UVR. Chl *a* was reported to decrease by a factor of 5 when nutrient-replete cultures were shifted from an irradiance of 10 to 1000 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (Goericke and Montoya 1998). Phycoerythrin is rapidly disassembled from phycobilisomes in red algae (Aguilera et al. 1999a, Talarico and Maranzana 2000) and cyanobacteria (Sinha et al. 1995). In the natural environment, chl *a* in *P. laciniata* (López Figueroa 1992) and in *P. leucosticta* (Figueroa et al. 1997) varied daily with lower concentrations of chl *a* and biliprotein at noon time due to UVR.

Low nitrogen nutrition reduces chl and soluble protein, such as RUBISCO, in different algae (Beardall et al. 1991, Wulff et al. 2000). Biliprotein contents decreased in both cyanobacteria (Boussiba and Richmond 1980, Schenk et al. 1983) and red algae (Talarico and Maranzana 2000), and in contrast, high nutrient supply produced a rapid increase in phycobiliproteins, reaching about 30%–40% of the soluble proteins in cyanobacteria (Tandeau de Marsac and Houmard 1993). The proteolysis of phycobiliproteins, which can represent 17.3% of soluble proteins of red algae (Hernández et al. 1993), provides the cells with the amino acids and microelements necessary for their survival. When organisms are deprived of an essential nutrient, the phycobilisomes are specifically degraded (Aráoz and Häder 1997). Phycobilproteins have been suggested to have two functions, as accessory pigments and as storage proteins (Algarra and Rüdiger 1993, Tandeau de Marsac and Houmard 1993). In this study, the ratios of accessory pigments to chl *a* increased with nutrient addition, and this might indicate a nutrient limitation for the nonenriched treatment as was found by Wulff et al. (2000) in marine microphytobenthos.

In addition to radiation (quantity and quality), it is crucial to investigate if other variables such as C or N availability affect the ETR/GP relationship. In this study, calculated molar ratios ETR per O_2 evolved were close to the theoretical values (1 oxygen molecule needs 4 electrons from PSII) at low irradiances (66 μmol photons $\cdot m^{-2} \cdot s^{-1}$) and at high ammonium concentration (300 μM). However, under nitrogen limitation, increasing number of electrons were necessary per oxygen molecule. The slopes of ETR versus GP function or Φ_{PSII} versus Φ_{O2} were high in algae with high N assimilation such as *Ulva rotundata* or *Pophyra leucosticta* (Figueroa et al. 2003). Such findings were supported by high internal N contents (total N and soluble proteins) and nitrate reductase activity in these species. Although the electron pathway to N assimilation diverts at the level of ferredoxin, thus not influencing the O_2 rates but rather the CO_2 fixation rates, the extent of the electron flow depends on the electron sink (i.e. carbon but also nitrogen assimilation). Babin et al. (1996) found that the maximum quantum yield of carbon fixation roughly covaried with nitrate concentration in phytoplankton. In our study, algae were incubated in a nitrogen-enriched seawater media and in these conditions the sudden nitrogen assimilation can inhibit RUBISCO activity, whereas electrons are used for nitrate assimilation and respiratory C flow increases to provide carbon (Turpin 1991).

At moderate irradiances, ETR calculated from Φ_{PSII} closely matches gross O_2 evolution in different *Ulva* species and *Porphyra columbina* (Beer et al. 2000, Franklin and Badger 2001), but in *Ulva lactuca* at higher irradiances the relation ETR/GP increased up the theoretical value of 4 (Longstaff et al. 2002). Nitrogen levels can affect ETR/GP ratios in *U. rotundata* (Henley et al. 1991a,b). Plants growing in low nitrogen environments are limited in their production of proteins, including RUBISCO (Logan et al. 1999). Figueroa et al. (2003) found that the different ETR/GP ratios in two species of *Ulva* could be due to drastic differences not only in the absorbance and pigment content, but also in the different N assimilation rates. Nitrogen assimilation is another competing sink for electrons in addition to C fixation. However, with the exception of a study in *U. rotundata* (Henley et al. 1991a,b), a possible relation of the loss of linearity of ETR/GP ratio and nitrogen metabolism in macroalgae has not been intensively examined. Figueroa et al. (2003) observed the same pattern in algal species with higher rates of N assimilation (*U. rotundata* and *P. leucosticta*) than that of a species with low nitrogen incorporation (*Ulva olivascens*). Maximal photosynthesis decreased on an areal basis in high-light grown algae but only under N limitation (Pérez-Lloréns et al. 1996). At limited light, maximal photosynthesis in *U. rotundata* decreased not only on an areal basis but also on an N basis (Pérez-Lloréns et al. 1996). This may indicate that the electron transport chain limited light saturated photosynthetic rates (Pérez-Lloréns et al. 1996). It appeared that under strong N deficiency, cell division and expansion temporarily outpaced N-limited synthesis of RUBISCO and/or PSII repair, eventually resulting in declining maximal photosynthetic and growth rates in *U. rotundata*.

In this paper we showed that both ammonium and UVR, mainly UVA, can stimulate the short-term (days) accumulation of MAAs in the red alga *P. columbina*, contributing to the photoprotection against UVR. This is the first time that an increase in the content of MAAs has been observed under any treatment or within a seasonal cycle for the group of Bangiales.

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