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Nelso P. Navarro, Andrés Mansilla, Félix L. Figueroa, Nathalie Korbee, Jocelyn Jofre and Estela Plastino*

Short-term effects of solar UV radiation and NO_3^- supply on the accumulation of mycosporine-like amino acids in *Pyropia columbina* (Bangiales, Rhodophyta) under spring ozone depletion in the sub-Antarctic region, Chile

Abstract: Short-term variations of mycosporine-like amino acids (MAAs) in *Pyropia columbina* (Montage) W.A. Nelson exposed to nitrate (NO_3^-) enrichment under different outdoor light treatments during the spring ozone depletion of 2008 in Punta Arenas (Chile) were investigated. Segments of *P. columbina* thalli were cultivated under three treatments of solar radiation without or with NO_3^- supply (0.38 mmol l^{-1}): PAR (P), PAR+UVA (PA), and PAR+UVA+UVB (PAB). Samples were taken at 8:00 h (initial value), 9:30, 12:30, 15:30, and 18:00 h on November 8 and at 9:00 h on November 9 (recovery period). A complex dynamic of MAAs affected by light quality and NO_3^- supply was observed. During the light period, the highest content of MAAs was reached under PAB and NO_3^- enrichment, whereas MAAs increased during the recovery period in P and PAB with no NO_3^- supply. Five MAAs were identified: porphyra-334, shinorine, asterina-330, palythine, and mycosporine-glycine. The hourly accumulation of each MAA varied mainly according to the time of exposure and NO_3^- supply. In general, the percentage of porphyra-334 increased, whereas the other MAAs decreased during the exposure period. These results suggest that MAA content in *P. columbina* varied in the short-term (hours) and the changes were related to the solar irradiance received and NO_3^- availability.

Keywords: mycosporine-like amino acids; nitrate; *Pyropia columbina*; short-term effects; UVB radiation.

***Corresponding author: Estela Plastino,** Instituto de Biociências, Universidade de São Paulo, Rua do Matão 277, Cidade Universitária, 05508-090, São Paulo, São Paulo, Brazil, e-mail: emplasti@ib.usp.br
Nelso P. Navarro, Andrés Mansilla and Jocelyn Jofre: Facultad de Ciencias, Universidad de Magallanes, Casilla 113-D, Punta Arenas, Chile
Félix L. Figueroa and Nathalie Korbee: Departamento de Ecología, Facultad de Ciencias, Universidad de Málaga, Campus Universitario de Teatinos s/n, Málaga 29071, Spain

Introduction

One of the most recognized atmospheric changes during the last few decades has been the thinning of the stratospheric ozone layer (Kirchhoff et al. 1997). This phenomenon has resulted in increasing levels of ultraviolet B radiation (UVB: 280–315 nm) (Seckmeyer and McKenzie 1992). Even though the concentration of ozone-depleting substances in the atmosphere is decreasing, the full recovery of the ozone layer to the 1980s levels is still far from complete. There is greater uncertainty about the future levels of UVB radiation as these levels will be additionally influenced by climate change (McKenzie et al. 2007). Physical models have predicted that the stratosphere to troposphere ozone flux will increase by 23% between 1965 and 2095 due to the climate change, a much larger effect than the recovery of stratospheric ozone and, consequently, the UV index will increase (up to 20%) in southern high latitudes in spring and summer (Hegglin and Shepherd 2009).

In macroalgae, adverse UVB effects have been reported at different organization levels, including DNA (van de Poll et al. 2001, Roleda et al. 2004), photosynthesis, enzyme activities, pigments (Figueroa et al. 1997, Gómez et al. 1998, Figueroa and Gómez 2001), growth (reviewed in Franklin and Forster 1997, Navarro et al. 2008), and vertical zonation on the coast (see review in Bischof et al. 2006). Most algae that are frequently exposed to solar radiation possess various repair and protective mechanisms to minimize the damage caused by UVB. Mechanisms include photorepair of DNA mediated by PAR and ultraviolet radiation (UVR) (Mitchell and Karentz 1993, Pakker et al. 2000), the accumulation of antioxidant substances and antioxidant enzymes (Cockell and Knowland 1999, Aguilera et al. 2002, Dummermuth et al. 2003, Connan et al. 2006), and dynamic photoinhibition (Figueroa and

Gómez 2001) that can help the organisms deal with and potentially adapt to high UVR conditions. Other protective mechanisms are the synthesis and accumulation of UV-absorbing compounds, especially mycosporine-like amino acids (MAAs). These compounds are suggested to function as natural sunscreens in red macroalgae and other marine organisms, mitigating UV damage as they mainly absorb in the UV region at wavelengths ranging from 310 to 360 nm (Karentz et al. 1991, Dunlap and Shick 1998), thereby preventing other cellular structures from being affected by this radiation (Karentz et al. 1991). The occurrence of MAAs in macroalgae has been reported at different water depths (Hoyer et al. 2001) and geographical areas, ranging from polar to tropical regions (Karsten et al. 1998a) and including the Antarctic region (Hoyer et al. 2001). In terms of MAA concentrations, Antarctic algae were separated into three physiologically different groups: (i) species with no capability for MAA biosynthesis; (ii) species with a constant relatively high concentration and constitutive MAA composition irrespective of environmental conditions; and (iii) species with a basic MAA concentration that is adjusted according to environmental conditions (Hoyer et al. 2001). In the latter group, synthesis of MAAs could be influenced by changes in quality and quantity of environmental radiation (UVA, UVB, and/or PAR) (Shick et al. 1999, Franklin et al. 2001, Hoyer et al. 2002, Korbee et al. 2005b) and by other environmental variables such as salinity, temperature, and desiccation (Karsten et al. 2003, Jiang et al. 2008). Recently, a relationship between MAA concentration and N availability has also been found in *Porphyra* spp. (Korbee-Peinado et al. 2004, Korbee et al. 2005a), *Grateloupia lanceola* (J. Agardh) J. Agardh (Huovinen et al. 2006), and *Asparagopsis armata* Harvey (Figueroa et al. 2008) cultivated in ammonium as a source of N. Two more recent studies also found a positive effect of nitrate (NO_3^-) in the accumulation of MAAs in *Gracilaria tenuistipitata* var. *liui* Zhang and Xia (Bonomi-Barufi et al. 2011) and in dinoflagellates (Korbee et al. 2010). The accumulation of MAAs with an increase of N availability may indicate that these substances function as an N reservoir (Korbee-Peinado et al. 2004, Korbee et al. 2005a), which can be used when N sources are reduced, as has also been suggested for phycobiliproteins (Talarico and Maranzana 2000). Although MAAs are nitrogenous compounds, there is still little information about the effect of N availability on their synthesis and accumulation (Korbee et al. 2005a), especially considering NO_3^- as an N source. NO_3^- is very important in the marine environment as it constitutes the predominant available N source for macroalgae, and for this reason it was chosen rather than ammonium in this study.

Pyropia columbina (Montagne) W.A. Nelson (Sutherland et al. 2011), formerly known as *Porphyra columbina* Montagne, is an economically important red alga in Chile and, consequently, has been extensively investigated, including research on UVB effects on MAA synthesis. This species contained porphyra-334 as the main MAA and lower amounts of shinorine, asterina-330, palythine, and mycosporine-glycine (Huovinen et al. 2004, Korbee-Peinado et al. 2004). Some of these studies have focused on the effect of light quality and N on the accumulation of MAAs at different time scales. Helbling et al. (2004) reported a decrease in UV-absorbing compounds in cultures without N supply during 1 day of exposure to PAR and PAR+UV solar radiation, whereas Korbee-Peinado et al. (2004) found that both N (as ammonium) and UVA stimulated the accumulation of MAAs after a 6-day exposure in laboratory conditions. Conversely, it had been suggested that there are no consistent MAA induction patterns, even for individual MAAs, indicating that induction, formation, and accumulation of MAAs in some species are highly flexible and often a species-specific mechanism (Hoyer et al. 2002). The aim of this study was to assess the short-term effect of distinct combinations of solar radiation (PAR, PAR+UVA, and PAR+UVA+UVB) and the influence of N supply (as NO_3^-) on the accumulation of different MAAs in *P. columbina*. The experiment was conducted during one austral spring day (November, 2008), when a decrease in ozone level and consequent increase in UVB radiation were observed over the Strait of Magellan on the southern Chilean coast.

Materials and methods

Measurement of solar radiation

Continuous monitoring of solar radiation conditions was conducted using a GUV-511 multichannel radiometer (Biospherical Instruments, Inc., San Diego, USA) placed on the roof of the Science Faculty at the University of Magallanes (UMAG), Punta Arenas, Chile. This radiometer recorded UV and PAR irradiances every 1 min in four narrow spectral bands (305, 320, 340, and 380 nm) and one broad band (400–700 nm).

The biologically weighted irradiance (BWI: kJ m^{-2}) was calculated as the area under the curve that resulted from multiplying a weighting function, that is, the action spectrum for the inhibition of photosynthesis in isolated chloroplasts (Jones and Kok 1966) and DNA damage (Setlow 1974) by the incident spectral irradiance (Rundel 1983).

$$BWI = \int_{280\ nm}^{400\ nm} E(\lambda)\varepsilon(\lambda)d(\lambda)$$

where E (λ) is the irradiance at λ (nm) and ε (λ) is the biological response at λ (nm) defined by the action spectrum. Time integration (from initial, t0 to final tf, the time period of exposure) of this quantity gives the biologically weighted dose (BWD: kJ m^{-2}):

$$BWID = \int_{t0}^{tf}\int_{280\ nm}^{400\ nm} E(\lambda)\varepsilon(\lambda)d(\lambda)dt$$

The UVB (280–320 nm) and UVA (320–400 nm) radiation were estimated according to Orce and Helbling (1997):

$$UVB = 59.5 * E_{305} + 4.1 * E_{320}; UVA = 87.4 * E_{340} - 2.4 * E_{380}$$

where E_{305}, E_{320}, E_{340}, and E_{380} are the energies measured in the 305, 320, 340, and 380 nm channels, respectively.

Biological materials

Fronds of *Pyropia columbina* were collected from the intertidal zone of Mansa Bay (53° 37′ 34″ S, 70° 55′ 13″ W) in the Strait of Magellan on November 7, 2008 during low tide and transported to the UMAG Marine Biology Laboratory, where the experiment was conducted. In the laboratory, segments (2×3 cm) from the central part of *P. columbina* thalli were selected and cultivated in a 50 l tank filled with filtered seawater (32 psu salinity) during one night before the experiment. The tank was located in a temperature-controlled room at 8±1°C (close to the natural temperature).

Experimental design

Segments of *Pyropia columbina* were transferred to 24 open methacrylate containers (20×10×5 cm) filled with 1 l filtered seawater and covered with different acetate cut-off filters to expose algae to a combination of three UV treatments as reported by Villafañe et al. (2002) – PAB treatment: samples exposed to PAR+UVA+UVB, containers covered with Ultraphan-295 film (Digefra, Munich, Germany); PA treatment: samples exposed to PAR+UVA, containers covered with Folex 320 (Folex, Cologne, Germany); and P treatment: samples exposed to PAR, containers covered with Ultraphan 395 (Digefra, Munich, Germany).

Eight containers were prepared for each treatment – four of them containing seawater enriched with Von Stosch (VS) solution (Ursi and Plastino 2001) prepared without NO_3^- supply (control) and the other four containing seawater enriched with VS plus 0.38 mmol l^{-1} NO_3^-. The seawater used in the experiment was collected from the same place as the *P. columbina*. The NO_3^- concentration, as the main inorganic N ion in the Magallanes coastal waters, is rather uniform (ca. 0.005 mmol l^{-1}) and no significant variation has been reported (Aracena et al. 2011). The experimental NO_3^- concentration of 0.38 mmol l^{-1} was used as the accumulation of MAAs in other species (e.g., *Gracilaria tenuistipitata*, see Bonomi-Barufi et al. 2011) reached a maximum and NO_3^- depletion was avoided.

The containers were floating in a water bath (150 l) to avoid increases of water temperature and were exposed to natural solar radiation for 10 h from 8:00 to 18:00 h local time during one spring day (November 8, 2008) when a decrease of ozone level and consequent increase in UVB radiation were observed over the southern Chilean coast. After this period, the algae were transferred to the laboratory and maintained in the dark under constant temperature of 8±1°C until the next day (recovery period). To determine the MAA concentration, samples were taken at the following local times: 8:00 (initial value), 9:30, 12:30, 15:30, 18:00 h on November 8 and 9:00 h on November 9 (after 15 h in the dark – recovery period). The samples were blotted dry with paper tissue and then dried in silica gel using sealed plastic bags.

Mycosporine-like amino acids

Four independent replicates of algal samples were weighed (10–20 mg dw). MAAs were extracted in 1 ml of 20% aqueous methanol (v/v) for 2 h at 45°C. After extraction, 600 ml of the supernatant were evaporated under vacuum to dryness. Dried extracts were redissolved in 600 ml of 100% methanol, followed by filtration through a 0.2 mm membrane filter. MAAs were determined by injecting 30 µl of each sample into a Spheroclone C8 column (Aschaffenburg, Germany) with a pre-column (5 mm packing; 250×4 mm I.D.) coupled to a Waters (Barcelona, Spain) High Performance Liquid Chromatography (HPLC) system, according to Karsten et al. (1998b) and modified by Korbee-Peinado et al. (2004). MAAs were detected with a Waters Photodiode Array Detector (996; Barcelona, Spain) at a wavelength of 330 nm. Absorption spectra were recorded between 290 and 400 nm. The quantification followed the method described by Korbee-Peinado et al. (2004).

Statistical analyses

Mean values and standard deviations of MAA content were calculated from four independent replicates. The

influence of time and treatments (radiation and NO_3^- supply) on MAA content was assessed by three-way analysis of variance (orthogonal ANOVA). Homogeneity of variances and normality were evaluated prior to ANOVA procedures. An *a posteriori* Newman-Keuls test was used to establish statistical differences. Statistical significance was set to $p<0.05$. All data were evaluated using the software Statistica for Windows 7.0 (Statsoft, Inc., Tulsa, Oklahoma, USA, 1984–2004).

Results

Solar radiation (PAR and UV)

PAR and UV irradiance showed daily variation due to changes in cloud cover. The highest values of PAR (2399 µmol photon m^{-2} s^{-1}), UVA (46.33 W m^{-2}), and UVB (1.63 W m^{-2}) were recorded at 17:00 h. Values of unweighted UV and PAR doses and biologically weighted doses (kJ m^{-2}) are shown in Table 1.

Total MAA content

MAA content of *Pyropia columbina* under full solar radiation treatment revealed short-term variation in the daily pattern (Figure 1) with lowest MAA content (2.2±0.6 mg g^{-1} dw) observed at the beginning of the experiment (8:00 h); however, an increase was observed in all the radiation and NO_3^- supply treatments after 1.5 h of exposure (i.e., at 9:30 h). In general, the pattern of MAA accumulation differed among the radiation treatments during the experiment, independent of the NO_3^- supply treatment. In P treatment, MAA concentration increased at 12:30 h. After this time, a decrease in MAAs was observed until 18:00 h (Figure 1). In the PA treatment, fronds of *P. columbina* showed an increase in the MAA content at 9:30 h, but no significant change was observed thereafter. By contrast, in the PAB treatment there was an increase in the MAA content from 9:30 h to a maximum after the recovery period.

Three-way ANOVA showed that the time of exposure ($p<0.0001$) and the radiation treatments ($p<0.0001$) had a statistically significant effect on the MAA content, whereas the NO_3^- supply treatment did not ($p=0.332$). Interactive effects of all the treatments were also observed, with the exception of NO_3^- supply × time ($p=0.077$) (Table 2).

The differences in MAA content were observed during the period of exposure to solar radiation (between 12:30 and 18:00 h) and recovery period in all the radiation treatments. Meanwhile, the highest differences were observed under PAB treatment. In this treatment, the NO_3^- supply promoted the highest MAA concentration at 12:30 h (10.4±1.1 mg g^{-1} dw), which was the highest value reached during the experiment.

Detailed analysis of MAA content at each sampling time showed differences among radiation treatments. In thalli, cultivated without NO_3^- supply, differences in MAA concentration were observed at 12:30 and 18:00 h, and after a period of recovery in the dark. At 12:30 h, the content of MAAs was highest in thalli exposed to P and lowest in PAB treatment ($p=0.0001$). By contrast, at 18:00 h, the highest MAA content was observed in PAB and the lowest in P treatment ($p=0.0006$). In the case of fronds cultivated with NO_3^- supply, differences in MAA concentration among radiation treatments were observed at 9:30 ($p=0.004$) and 12:30 h ($p=0.01$), with highest values under PAB treatment and lowest in P treatment.

MAA composition

Five different MAAs were identified in *Pyropia columbina*: porphyra-334, shinorine, asterina-330, palythine,

Table 1 *Pyropia columbina*: daily integrated irradiance of UVB, UVA, and PAR (kJ m^{-2}) recorded over Punta Arenas, Chile (53°10′ 01″ S 70° 56′ 01″ W) during the experiment (November 8, 2008).

Time (h)	Unweighted dose (kJ m^{-2})			Unweighted dose (kJ m^{-2}) transmitted by filters			Biologically weighted dose (kJ m^{-2})		
							(Jones and Kok 1966)		(Setlow 1974)
	UVB	UVA	PAR	UVB	UVA	PAR	UVB	UVA	UVB
8:00–9:30	0.05	4.9	49	0.04	4.2	43	0.02	1.3	0
8:00–12:30	1.99	87.5	633	1.67	74.5	557	1.03	23.8	0.09
8:00–15:30	10.68	330.4	2662	8.85	281.4	2343	5.86	89.8	0.73
8:00–18:00	23.71	706.6	6478	19.62	601.9	5701	13.12	191.7	1.7

UVB and UVA unweighted dose transmitted by the UV cut-off filters (UVB: Ultraphan 295; UVA: Folex 320), biologically weighted dose as chloroplast photoinhibition (Jones and Kok 1966), and DNA damage (Setlow 1974) are also shown.

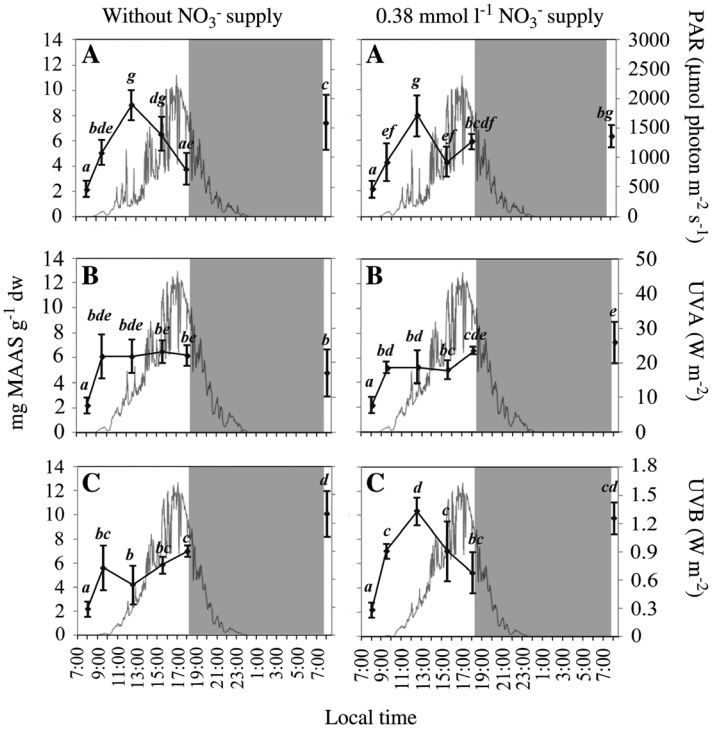

Figure 1 *Pyropia columbina*: daily variations in MAA content (mg g^{-1} dw) in fronds cultivated without and with 0.38 mmol l^{-1} of NO_3^- supply and exposed to different solar radiation treatments: (A) PAR (P); (B) PAR+UVA (PA); and (C) PAR+UVA+UVB (PAB). Data are expressed as mean values ±S.D. (n=4). Letters on the graph show results of Newman-Keuls tests $p<0.01$ different letters indicate significant differences among times and between N treatments in each radiation treatment. Before 18:00 h local time, algae were transferred to the laboratory and maintained in darkness: shaded area. Diurnal values of irradiance of PAR (µmol photon m^{-2} s^{-1}), UVA, and UVB (W m^{-2}) recorded on November 8, 2008 in Punta Arenas, Chile are also shown.

and mycosporine-glycine. The percentages at the start of the experiment were: 87.8±0.9, 6.6±0.6, 3.1±0.3, 1.8±0.4, and 0, respectively.

Three-way ANOVA showed that the time of exposure had a statistically significant effect on the percentage of each MAA, whereas NO_3^- supply treatment had an effect only on the percentage of mycosporine-glycine, shinorine, and porphyra-334. Although the radiation treatments, as an isolated factor, did not have a significant effect on any of the individual MAAs, interactive effects among all the treatments were observed (Table 2).

In general, the percentage of porphyra-334 increased and percentages of asterina-330, palythine, and shinorine decreased when compared with the initial values (Figure 2).

The mycosporine-glycine level was very low (0%–0.15%) during the experiment. This compound was recorded mainly between 12:30 and 18:00 h in all the radiation treatments with NO_3^- supply. The maximum concentration was found at 12:30 h in P[0]A treatment (Figure 2).

The decrease of porphyra-334 percentage in fronds exposed to PAB treatment without NO_3^- supply at 12:30 h was correlated with an increase in shinorine, asterina-330, and palythine (Figure 2). The reduction in porphyra-334 percentage produced a lower total MAA content in PAB when compared with P and PA treatments in this period (Figure 1) as the porphyra-334 was the most abundant MAA in *P. columbina*. The reduction in porphyra-334 percentage also promoted an increase in shinorine/porphyra-334, palythine/porphyra-334, and asterina-330/porphyra-334 ratios at 12:30 h under PAB treatment (Figure 3). A decrease in these ratios was observed in fronds cultivated with NO_3^- supply at 12:30 h under PAB when compared with values obtained at 9:30 h (Figure 3), whereas an increase in those ratios was observed at 18:00 h due to a decrease in porphyra-334 (Figure 2). By contrast, fronds cultivated without the NO_3^- supply showed a decreasing trend of shinorine/porphyra-334, palythine/porphyra-334, and asterina-330/porphyra-334 ratios under PAB treatment at 18:00 h (Figure 3).

Discussion

An increase of UVB irradiance on the day of the experiment (November 8, 2008) was related to a decrease of

Table 2 *Pyropia columbina*: results from three-way analysis of variance for the effect of NO_3^- supply (A), radiation treatments (PAR, PAR+UVA, and PAR+UVA+UVB; B), time (C), and interaction effects on MAA content.

Source of variation	Df	F	p-Value
Total MAAs			
NO_3^- supply (A)	1	0.9	0.332
Radiation (B)	2	10.1	**0.001**
Time (C)	5	52.7	**0.001**
A×B	2	5.1	**0.008**
A×C	5	2.1	0.077
B×C	10	5.4	**0.001**
A×B×C	10	5.9	**0.001**
Mycosporine-Glycine			
NO_3^- supply (A)	1	8.3	**0.005**
Radiation (B)	2	0.8	0.469
Time (C)	5	2.9	**0.017**
A×B	2	1.1	0.340
A×C	5	3.8	**0.003**
B×C	10	1.8	0.078
A×B×C	10	1.4	0.201
Shinorine			
NO_3^- supply (A)	1	34.4	**0.001**
Radiation (B)	2	0.3	0.709
Time (C)	5	35.9	**0.001**
A×B	2	2.1	0.125
A×C	5	6.9	**0.001**
B×C	10	2.5	**0.010**
A×B×C	10	1.3	0.217
Porphyra-334			
NO_3^- supply (A)	1	11.2	**0.001**
Radiation (B)	2	0.7	0.512
Time (C)	5	66.1	**0.001**
A×B	2	4.1	**0.020**
A×C	5	3.8	**0.004**
B×C	10	2.0	**0.041**
A×B×C	10	3.5	**0.001**
Palythine			
NO_3^- supply (A)	1	0.2	0.622
Radiation (B)	2	0.2	0.983
Time (c)	5	32.0	**0.001**
A×B	2	1.9	0.149
A×C	5	2.6	**0.030**
B×C	10	1.4	0.200
A×B×C	10	1.2	0.271
Asterina-330			
NO_3^- supply (A)	1	0.04	0.834
Radiation (B)	2	1.9	0.155
Time (C)	5	51.7	**0.001**
A×B	2	3.5	**0.035**
A×C	5	3.7	**0.004**
B×C	10	2.9	**0.003**
A×B×C	10	5.4	**0.001**

Bold values indicate significant differences at $p<0.05$.

ozone concentration in southern Chile [316, 299, and 364 Dobson units (DU) on November 7, 8 and 9, respectively; Goddart Space Flight Center – http://jwocky.gsfc.nasa.gov)]. This event promoted a substantial increase of UVB daily integrated irradiance, including biologically effective irradiance for DNA damage and photoinhibition (Table 1).

This study constitutes the first report of short-term variations in MAAs (<2–3 h, during a period of 1 day) in red macroalgae exposed to solar radiation in a day with ozone layer depletion. Previous reports of the dynamics of the accumulation of MAAs were based on studies over days or up to 48 h (Korbee-Peinado et al. 2004). Helbling et al. (2004) reported variations over 3–5 h periods of UV-absorbing compounds determined as optical density at 330 nm in *Pyropia columbina* (as *Porphyra columbina*) but MAAs were not identified.

The daily variation of the relative abundance of individual MAAs in *P. columbina* suggests that there is a rapid synthesis (on the order of hours) of these compounds. Short-term variation of biocompounds as photosynthetic pigments has usually produced controversy because the variation could be associated with technical artefacts (see for review López-Figueroa and Niell 1991, Rüdiger and López-Figueroa 1992). Meanwhile, daily variations of photosynthetic pigments under solar radiation and laboratory experiments have been reported (López-Figueroa 1992, Figueroa et al. 1997). Furthermore, short-term variations of phenolic compounds in the brown alga *Cystoseira tamariscifolia* (Hudson) Papenfuss (Abdala-Díaz et al. 2006) and trihydroxicoumarins in the green alga *Dasycladus vermicularis* (Scopoli) Krasser (Gómez et al. 1998) have also been reported. Korbee et al. (2005b) observed a similar pattern of MAA and biliprotein accumulation under different UVR and NO^{-3} treatments in several *Porphyra* species, though on a longer time scale. Thus, the short-term variation of MAAs in *P. columbina* is not an unexpected result, but rather this study supports the previous reports of daily variations of MAAs and biliproteins. MAAs as well as biliproteins increased with N supply (Bonomi-Barufi et al. 2011) but the photodegradation of biliproteins was much higher than that of MAAs (Figueroa et al. 2012).

The initial value of MAAs observed in *P. columbina* was 2.2±0.6 mg g^{-1} dw, reaching a peak of 10.4±1.1 mg g^{-1} dw throughout the day. The initial MAA concentration was lower than those observed previously in the same species (as *P. columbina*). Huovinen et al. (2004) observed 7.2 to 10.6 mg g^{-1} dw in fronds collected in Valdivia, Chile (30° S) and Korbee-Peinado et al. (2004) reported 4 mg g^{-1} dw in *P. columbina* collected from Playa Unión, Argentina (43° S). The latter value, however, was measured after

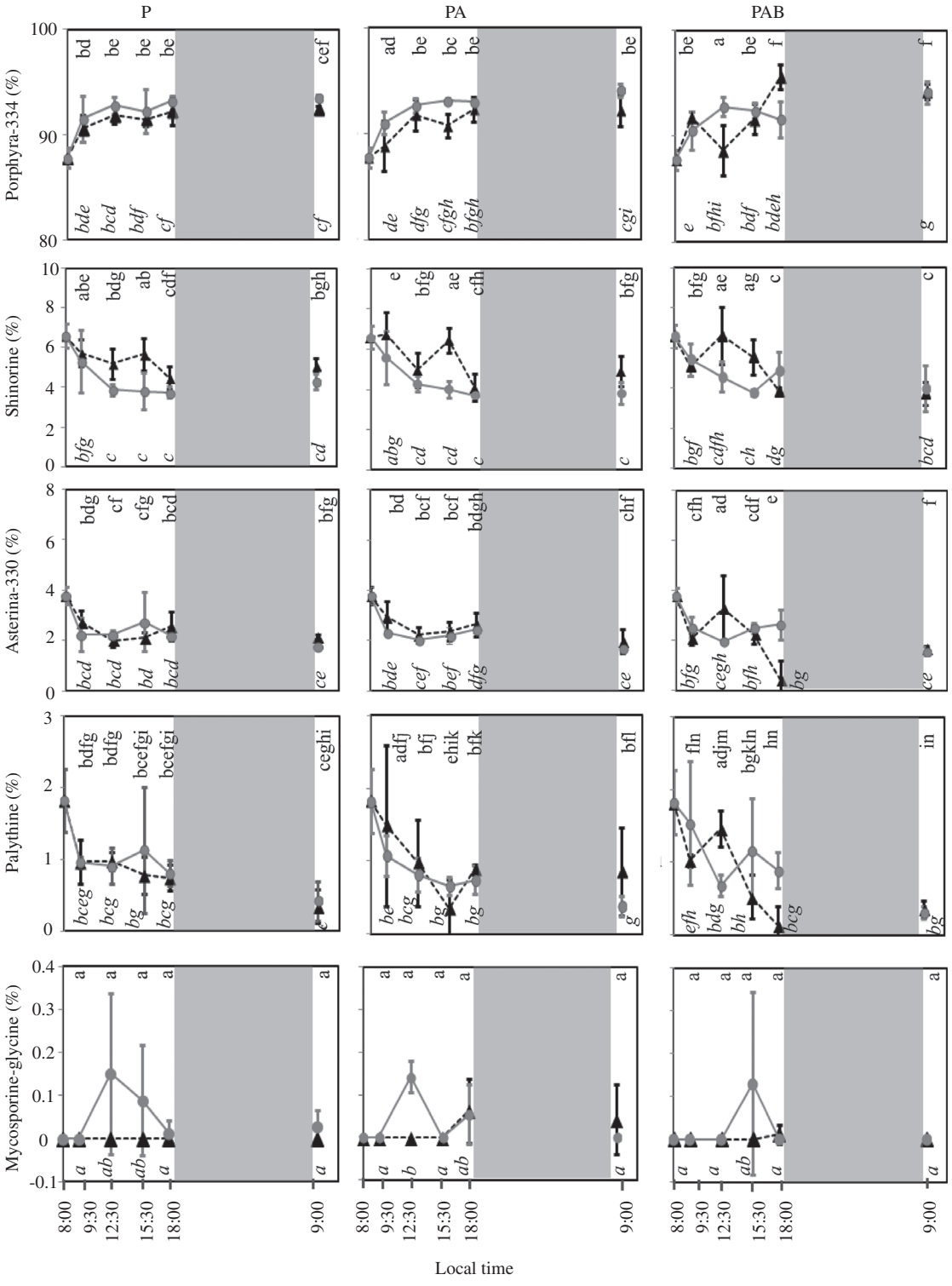

Figure 2 *Pyropia columbina*: percentages of different types of mycosporine-like amino acids (MAAs) identified in fronds cultivated without (broken black line) and with 0.38 mmol l^{-1} of NO_3^- supply (continuous gray line) and exposed to different solar radiation treatments: PAR (P), PAR+UVA (PA), and PAR+UVA+UVB (PAB). Data are shown to compare different solar radiation treatments throughout the experiment: Roman type at the top presents results of Newman-Keuls test $p<0.01$ for without NO_3^- supply treatment, whereas italic type at the bottom presents corresponding results for with NO_3^- supply treatment. In both the cases, different letters indicate significant differences between mean values (n=4). Before 18:00 h local time, algae were transferred to the laboratory and maintained in darkness: shaded area.

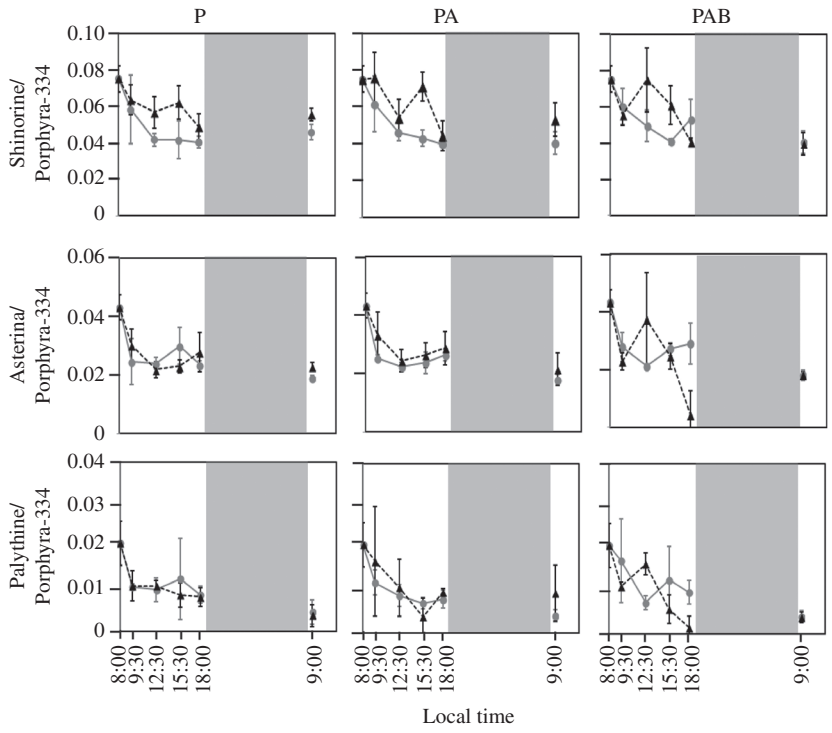

Figure 3 *Pyropia columbina*: asterina-330/porphyra-334, palythine/porphyra-334, and shinorine/porphyra-334 ratios calculated for fronds cultivated without (broken black line) and with 0.38 mmol l^{-1} of NO_3^- supply (continuous gray line) and exposed to different solar radiation treatments [PAR (P), PAR+UVA (PA), and PAR+UVA+UVB (PAB)]. Before 18:00 h local time, algae were transferred to the laboratory and maintained in darkness: shaded area.

the algae had spent 12 days in N starvation. The increase of MAAs in *P. columbina* in this study could indicate that the NO^{-3} supplied was used for the synthesis of MAAs as has been previously reported in other red macrolagae (Bonomi-Barufi et al. 2011). Nonetheless, further collections of data on pigment, C, and N content are necessary in order to reinforce this conclusion.

A complex dynamic of MAAs in response to the interaction of light quality and NO^{-3} supply was observed. The decrease of MAAs observed after 12:30 h in the P treatment could be due to their photodegradation as a consequence of increasing solar irradiance, whereas the absence of a decrease in the presence of UVA suggests a role of these wavelengths in photoprotection against photodamage. In the case of sections of fronds exposed to the PAB treatment and cultivated without NO_3^- supply, a decrease in MAA content was observed at 12:30 h, whereas when NO_3^- was supplied, there was a rapid increase of MAAs, showing the importance of an N source to synthesize these compounds when algae are exposed to high UVB levels, as has been previously reported in other *Porphyra* species (Korbee-Peinado et al. 2004), *Gracilaria tenuistipitata* (Bonomi-Barufi et al. 2011) and in *Hydropuntia cornea* (J. Agardh) M.J. Wynne (formerly *Gracilaria cornea*; Figueroa et al. 2012). Conversely, the addition of NO_3^- could reduce the positive effect of UVB photoinduction on MAA accumulation when fronds of *P. columbina* were exposed to high UVB (PAB treatment). In fact, a decrease in MAA concentration was observed after 12:30 h, which suggests that these compounds were lost, and N and energy destined for growth and MAA synthesis could have been used in other metabolic pathways associated with repair mechanisms of damage promoted by exposure to a high dose of UVB such as antioxidant enzymes.

The MAA content in all radiation treatments was recovered after 15 h in darkness. Thus, the induction and synthesis of MAAs could be influenced not only by specific wavelengths during the light period, as suggested for other macroalgae (Franklin et al. 2001, Hoyer et al. 2002, Korbee et al. 2005b), but also by any possible endogenous biological control. New experiments run over several days and including dark or low light controls should be set up in the future in order to check the results obtained in this study.

The presence of blue light in all the treatments could explain the MAAs accumulation in *P. columbina* during the experiment, mainly at 12:30 h, considering that the

specific wavelengths of PAR favored the accumulation of MAAs (Korbee et al. 2005b). The fact that the highest MAA concentrations in *P. columbina* cultivated without NO_3^- supply were observed in P treatment at 12:30 h could be related to the use of Ultraphan URUV filter, which transmitted low quantities of UVA (20% of UVA between 375 and 395 nm), as this radiation can stimulate MAA synthesis in *Chondrus crispus* Stackhouse (Franklin et al. 2001, Kräbs et al. 2002).

Synthesis of MAAs can be induced not only by specific wavelengths of PAR and UVA but also by UVB with differences among types of MAAs (Karsten et al. 1998b, Shick et al. 1999, Franklin et al. 1999, 2001, Hoyer et al. 2002). However, UVA and UVB can also promote cell damage and the energy available for MAA synthesis could have been used to repair the UV-induced damage. In fact, at 12:30 h, fronds of *P. columbina* exposed to PA and PAB treatments and cultivated without NO_3^- supply had synthesized less MAAs than those algae exposed to the P treatment. By contrast, at 18:00 h, an increase in MAA content was observed in *P. columbina* exposed to PAB when compared with P and PA treatments, suggesting that the presence of UVB not only affects important processes, such as photosynthesis (Figueroa et al. 1997, Figueroa and Gómez 2001, Rautenberger et al. 2009), but may also have a beneficial effect. Species from shallow water, which are adapted to high UV, were observed to recover less under the treatment with depleted solar UVB radiation (Flores-Moya et al. 1999, Hanelt and Roleda 2009).

Five different MAAs were identified in *P. columbina* that varied in concentration according to the time of exposure to solar radiation but not to the radiation treatment. The mycosporine-glycine increased at 12:30 h under PAR and PAR+UVA and at 15.30 h under PAB treatment in fronds cultivated with NO_3^- supply, although the contribution to total MAAs was low. The fact that mycosporine-glycine was recorded mainly under NO_3^- supply treatment shows the importance of N availability in its synthesis when algae are exposed to high PAR, UVA, and UVB irradiance.

In general, the percentage of porphyra-334 in *P. columbina* increased in all the treatments during the experiment, reaching its highest value after the recovery period, whereas the percentages of shinorine, asterina-330, and palythine decreased with time. However, the presence of high UVB irradiance and NO_3^- led to the opposite effect as the porphyra-334 decreased at 12:30 h, whereas palythine, shinorine, and asterina-330 increased. This tendency was more evident in the increase of asterina-330/porphyra-334, palythine/porphyra-334, and shinorine/porphyra-334 ratios. By contrast, the NO_3^- could induce the synthesis of porphyra-334 and inhibit the synthesis of palythine, shinorine, and asterina-330. Conversely, when the intensity of UVB decreased in the evening, the NO_3^- supply seemed to promote palythine, shinorine, and asterina-330 accumulation as the ratios asterina-330/porphyra-334, palythine/porphyra-334, and shinorine/porphyra-334 increased. Thus, we suggest that the effect of NO_3^- on the synthesis and accumulation of MAAs in *P. columbina* depends on the intensity of UVB radiation. Furthermore, even if there is no direct evidence, it is possible that the high variability of different MAAs observed in *P. columbina* could be the consequence of inter-conversions among different MAAs, leading to adjustments in response to the conditions the alga is submitted to, as has been discussed for dinoflagellates (Carreto et al. 1990, Callone et al. 2006), *Porphyra leucosticta* Thuret in Le Jolis (Korbee et al. 2005a), and *Chondrus crispus* (Franklin et al. 1999, 2001, Kräbs et al. 2002, 2004).

The increase of MAAs in *P. columbina* could contribute to protection against UVR and high PAR levels as these compounds absorb UVR, reduce photoinhibition and decrease photodamage (Lesser 1996, Bischof et al. 2000), and dissipate the UV energy absorbed in thermal form (Conde et al. 2004). It has also been reported that MAAs have a high antioxidant capacity inhibiting lipid peroxidation (Dunlap and Yamamoto 1995, Dunlap and Shick 1998). In this context, De la Coba et al. (2009) reported antioxidant capacity in porphyra-334 plus shinorine, asterina-330 plus palythine, and shinorine; all these MAAs were detected in this study, therefore *P. columbina* could have a high antioxidant capacity to prevent photodamage.

In conclusion, our data suggest that *P. columbina* can acclimate (at least in the short term) to the frequent changes in UVB radiation that occur as a consequence of ozone layer depletion over Punta Arenas during the spring months. Future research could be aimed at understanding the daily cycles of reactive oxygen species production and antioxidant activity and their relationship with MAA accumulation.

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