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The Effects of NO_3^- Supply on *Mazzaella laminarioides* (Rhodophyta, Gigartinales) from Southern Chile

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ABSTRACT

The effects of nitrate supply on growth, pigments, mycosporine-like amino acids (MAAs), C:N ratios and carrageenan yield were investigated in *Mazzaella laminarioides* cultivated under solar radiation. This species is economically important in southern Chile where an increase of nitrogen in coastal waters is expected as a consequence of salmon aquaculture activity. Apical segments were cultivated in enriched seawater with five different NO_3^- concentrations (0, 0.09, 0.18, 0.38 and 0.75 mM) during 18 days. Although phycoerythrin and phycocyanin content, as well as C:N ratios, were reduced in the control treatment (without NO_3^- supply), when compared to NO_3^- treatments, total MAA concentration, carrageenan yield and growth rates were similar in all tested conditions. Nevertheless, during the experiment, an important synthesis of mycosporine-glycine took place in a nitrate concentration-dependent manner, with accumulation being saturated around 0.18 mM of nitrate. These results indicate that exposure to high NO_3^- concentration of more than 100 times the values observed in the nature did not impair the photoprotection system, as determined by MAAs, nor did it have a deleterious effect on growth or carrageenan yield of *M. laminarioides*, a late successional species from Chile.

INTRODUCTION

Nitrogen is the most important abiotic factor limiting macroalgal growth in the marine environment (1), and it has been the focus of many studies aimed at determining the function of this critical element in the productivity of ecosystems and in all biochemical processes. Because inorganic nitrogen availability is positively correlated with protein (2–4) and pigment content (3), an increase of nitrate, nitrite and ammonium in coastal seawater could promote an increase of growth rates in macroalgae. Moreover, phycobiliproteins have been reported as nitrogen-storage under N supply and as a nitrogen source in N-limiting conditions (5–8) in order to maintain growth rate. Similarly, a positive relationship between N availability and accumulation of photoprotective compounds, such as mycosporine-like amino acids (MAAs), has been reported in different species of the Rhodophyta (6,9–12). However, a negative effect in carrageenan yield could be expected because N supply does not favor carbohydrate synthesis (8). On the other hand, a decrease in MAA content has been correlated with certain levels of N enrichment, as reported for *Asparagopsis armata* grown with fishpond effluents (13) and *Gracilaria tenuistipitata* cultivated under different nitrate concentrations (12). In high N supply, growth could be competing with MAA synthesis, resulting in a lower MAA accumulation, as reported for *A. armata* (13), which may compromise the photoprotection system, as might be determined by MAA content and composition.

An excess of nitrogen in seawater (eutrophication) promoted by urbanization, increasing human agricultural and industrial activities, as well as fish farming, could quickly stimulate the growth of such opportunistic macroalgae as Ulvaceus spp. (14), rather than the climax community or late successional species (15). While late successional species probably do not quickly change their N-status in response to ambient nitrogen, this feature is a desirable property for a bioindicator species because its N-status should reflect ambient conditions that have accumulated over the previous few days (15).

Because an increase of nitrogen in the pristine coastal waters of southern Patagonia is expected as a consequence of salmon aquaculture activity (16), it is important to study this effect on the late successional species that inhabit this region. In particular, *Mazzaella laminarioides* (Bory de Saint Vincent) Fredericq is an endemic and climax community species distributed along the continental shore of Chile from Fray Jorge National Park (30° 34′ S; 61° 06′ W; 17) to the Diego Ramírez Islands (56° 30′S; 68° 43′ W; 18). This species is also considered an important carrageenan-producing red alga, being widely harvested in southern Chile (19,20). For these reasons, *M. laminarioides* was selected as a model to assess the effect of increased nitrate. Therefore, the aim of this study was to evaluate the effects of five different NO_3^- concentrations on *M. laminarioides* cultivated under full solar radiation during 18 days in southern Chile. Growth rates, polysaccharide (carrageenan) yield and the nitrogen-containing compounds (MAA, pigments and C:N ratio) were evaluated.

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Because *M. laminarioides* inhabits in the intertidal zone, where it is exposed to a highly variable environment, we hypothesized that the species would maintain its growth rate and the nitrogen-containing compounds in nitrate supplies. However, since *M. laminarioides* is a late successional and nitrophilic species, it was expected that high levels of nitrate would neither favor photosynthetic activity nor growth rate. A decrease in carrageenan yield was also expected.

MATERIALS AND METHODS

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

UVB and UVA radiation was estimated according to Orce & Helbling (21):

$$UVB = 59.5 * E_{305} + 4.1 * E_{320}$$

$$UVA = 87.4 * E_{340} - 2.4 * E_{380},$$

with E_{305}, E_{320}, E_{340} and E_{380} measured at 305, 320, 340 and 380 nm channels, respectively. The integrated daily irradiance and dose of PAR, UVA and UVB throughout the experimental time (18 day, from 8 to 26 September 2009) were also calculated (Fig. 1; Table 1). It must be emphasized that the integrated daily irradiance and dose calculated from data obtained in the air are different from those values that algae received within the experimental cylinders, since the methacrylate used transmits different PAR percentages (90%) and UV radiation (88%). Thus, percentages of UV and PAR retained by the vessels were subtracted from the environmental values to obtain more accurate values for the daily dose to which the algae were exposed.

Biological material. Reproductive fronds of *Mazzaella laminarioides* tetrasporophytes were collected from the intertidal zone of Punta Santa (53° 37′ S; 70° 59′ W) in the Strait of Magellan on September 6th, 2009, during low tide and transported to the UMAG Marine Biology Laboratory.

Because *Mazzaella laminarioides* has a triphasic life history with isomorphic haploid male and female gametophytes and a diploid tetrasporophyte, it is difficult to distinguish between gametophyte and tetrasporophyte vegetative fronds. For this reason, we decided to collect only reproductive ones. Reproductive tetrasporophytes are easily distinguished from vegetative and reproductive gametophyte fronds. Both tetrasporangial sori (in tetrasporophytes) and cystocarps (in female gametophytes) appear as small dark-colored stains on the central part of the fronds. Cystocarps are bigger and more protruded than the tetrasporangial sori. In the laboratory, apical portions (3–4 cm in length) without sori were cut and placed in glass vessels filled with filtered seawater (32 psu salinity) without enriched medium. Vessels were placed in a controlled room at 8°C with artificial irradiation of 55 μmol photons m^2 s^{-1} PAR (provided by Philips TLT 20W/54 daylight fluorescent tubes) on a 12:12 h light:dark cycle for 2 days.

Experimental design. A total of 15 UV-transparent methacrylate cylinders were filled with 1.5 L filtered seawater enriched with Von Stosch (VS) solution (22) without N and two grams of fresh weight (fw) of apical portions (~3 cm each) of *Mazzaella laminarioides*. Five treatments run in triplicate were conducted: no addition of NO_3^- (assigned as control) and addition of 0.09, 0.18, 0.38 and 0.75 mm of NO_3^- (Table 2). The seawater used in the experiment came from the same place where *M. laminarioides* was collected. Nitrate concentration, the main inorganic nitrogen ion in Magallanes coastal waters, is rather uniform at about 0.005 mm (23). VS solutions (without and with different NO_3^- concentrations) were renewed at 6, 12 and 18 days of culture. Each cylinder received an alternating 30-min aeration period.

The cylinders were put inside a water bath (150 L) to avoid increases in water temperature. Bath water was renewed every 3 h, and the temperature varied between 5 and 8°C during the experiment.

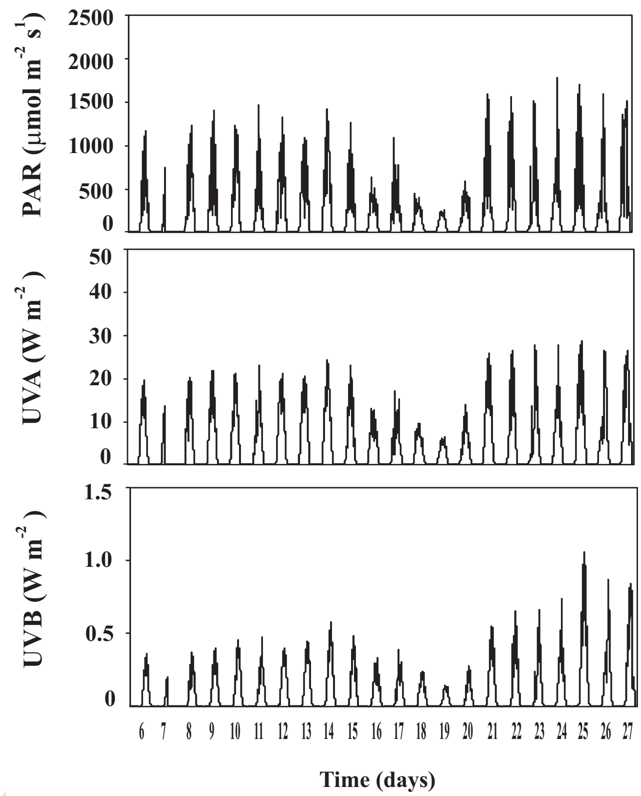

Figure 1. Diurnal values of irradiances of PAR (=400–700 nm) expressed as μmol photon m^{-2} s^{-1} and UVA (=320–400 nm) and UVB (=280–320 nm) expressed as W m^{-2} recorded from 8 to 26 September 2009 in Punta Arenas, Chile.

Table 1. UVB, UVA and PAR (KJ m^{-2}) doses recorded over Punta Arenas City, Chile (53°10′ 01″ S 70° 56′ 01″ W) during the experiment (between 8 and 26 September 2009). UVB, UVA and PAR unweighted doses transmitted by methacrylate cylinders are also shown.

	Unweighted dose			Unweighted dose transmitted by methacrylate vessel		
	UVB	UVA	PAR	UVB	UVA	PAR
From 8 to 13 September	43	1756	26 526	28	1202	20 075
From 14 to 20 September	37	1292	17 332	23	885	13 118
From 21 to 26 September	69	2040	29 226	45	1396	22 118

Three apical portions from each cylinder were removed at the beginning and after 6, 12 and 18 days of cultivation around midday. These three apical portions were divided to obtain biomass for pigments (phycobiliproteins and chlorophyll a), MAAs, carbon and nitrogen. Pigment and MAA contents were assessed at the beginning and at 6, 12 and 18 days of culture, while carbon and nitrogen contents were assessed initially and after 6 and 18 days of culture. In the case of carrageenan yield, samples were obtained at the beginning and at the end of 18 days. After weighing, samples of algae were frozen or dried according to the substance to be analyzed. These analyses are detailed below. Additionally, growth rates were assessed at 6, 12 and 18 days.

Growth rates (GR). Algal samples were dried with absorbent paper and weighed on an analytical balance to obtain the initial and final values of fresh weight. Values were obtained at the beginning and after 6, 12

Table 2. Treatments with different concentrations of NO_3^- used in the experiment. VS, Von Stosch medium.

Treatment	Description
0 (control)	Seawater + VS without NO_3^-
0.09	Seawater + VS with 0.09 mM NO_3^-
0.18	Seawater + VS with 0.18 mM NO_3^-
0.38	Seawater + VS with 0.38 mM NO_3^-
0.75	Seawater + VS with 0.75 mM NO_3^-

and 18 days of the experimental period. GRs were estimated according to Lignell and Pedersén (24):

$$GR = ((\ln fw_f - \ln fw_i) * t^{-1}) * 100,$$

where fw_i is the initial weight and fw_f is the final weight of *M. laminarioides* tetrasporophytes after t days of culture under different treatments of NO_3^- supply. GR units were percentages of fresh weight per day (% fw d^{-1}). Once all apical portions from each cylinder were weighed, it is important to note that three of them were removed in order to perform biochemical analysis. The remaining apical portions were weighed again, and this measurement was used as the initial mass for the next growth period.

Pigments. For Chl a extraction, 50 mg of algae (fresh mass) were inserted in 1.5 mL of dimethylformamide (DMF) in darkness at 4°C for 12 h. After this period, the extracts were tested in a spectrophotometer (Spectronic Genesys 10S, Thermo Scientific), and absorbance values based on the equation of Wellburn (25) were used to determine the total pigment concentration.

Phycobiliproteins were extracted from 50 mg of frozen samples ground in a mortar with liquid nitrogen, following the method of Beer & Eshel (26). Part of the supernatant was used for phycobiliprotein determinations in the spectrophotometer, applying the dichroic equations of these authors to determine the amounts of phycoerythrin (PE) and phycocyanin (PC).

Chlorophyll a, PE and PC concentrations were expressed as mg of photosynthetic pigment per g of dry weight (dw).

MAA content. MAAs were extracted in 1 mL of 20% aqueous methanol (v/v) for 2 h at 45°C from dry algal mass (10–20 mg per replicate). After extraction, 600 μL of the supernatant were evaporated in rotavapor under vacuum until dry. Dried extracts were redissolved in 600 μL of 100% methanol, followed by filtration through a 0.2 μm membrane filter. MAAs were determined by injecting 30 μL of each sample into a Spheroclone C8 column (Aschaffenburg, Germany) with a precolumn (5 μm packing; 250 × 4 mm I.D.) coupled to a Waters High Performance Liquid Chromatography (HPLC) system (Barcelona, Spain), according to Karsten *et al.* (27), and modified by Korbee-Peinado *et al.* (6). MAAs were detected with a Waters Photodiode Array Detector (Model 996, Barcelona, Spain) at a wavelength of 330 nm. Absorption spectra were recorded between 290 and 400 nm. Quantification followed the method described by Korbee-Peinado *et al.* (6).

Internal carbon and nitrogen. Elemental analyses were performed with 2 mg of dry weight powder. Total carbon and nitrogen (units: mg g dw^{-1}) were measured in an elemental analyzer (Model CHNS-932, LECO Corporation, St. Joseph, Michigan), and C:N ratio was obtained.

Extraction of carrageenan. Carrageenan extraction was done according to Matsuhiro *et al.* (28). Approximately 1 g of dry algae was put in distilled water at 90°C for 2 h, under agitation. The extract was centri-
4 fuged at 8500 rpm for 10 min. The supernatant was collected and submitted to dialysis using semipermeable membranes (Spectra/Por Membrane MWCO: 3500 Daltons, Spectrum Laboratories Inc., Rancho Domínguez, CA) in deionized water (12 h) and distilled water (12 h). The solution was concentrated in a vacuum rotary evaporator (R110, Büchi, Glatthalde, Switzerland), and carrageenan was subsequently precipitated in ethanol (5:1). The precipitate was dissolved in distilled water, then frozen and finally freeze-dried (Lyovac GT 2, Leybold-Heraeus, Old Greenwich, CT).

Finally, carrageenan yield was calculated as a percentage of seaweed dry mass:

$$Yield = (dw\ carrageenan/dw\ algae) \times 100.$$

Carrageenans were analyzed by Fourier Transform infrared spectroscopy (FT-IR) in order to verify the presence of lambda-type carrageenan, a polysaccharide synthesized by tetrasporophytes, but not by gametophytes.

Data analysis. To assess the effect of NO_3^- supply, incubation time, and the interaction between them on all data (MAAs, pigments, elemental contents and growth rates), a multifactorial ANOVA was performed. Because carrageenan yield was only assessed at the end of the experiment, a one way ANOVA was performed. Homogeneity of variances and normality were evaluated before ANOVA analysis. A posteriori Newman–Keuls test was used to establish statistical differences. Probability for type I errors was set to $\alpha = 0.05$. Dependent variables (C, N, PE, PC, Chl a, GR, Carrageenan yield and MAAs) were submitted to Pearson correlation analysis. Correlation coefficient between −1 and 1 enabled the determination of positive, negative and noncorrelated samples, and they were considered significant at $P < 0.05$. All data were statistically evaluated using the STATISTICA 7.0 software (Copyright © Statsoft, Inc.).

RESULTS

In the context of ANOVA, elemental N, PE and MAAs are variables related to nitrogen that were influenced by incubation time and nitrate supply. In contrast, GR, Chl *a*, PC and C content were influenced a single factor, either incubation time or nitrate, but not by the interaction between incubation time and nitrate (Table 3).

Although the GR of *M. laminarioides* varied significantly throughout the experiment (Fig. 2), such variation was not caused by NO_3^- supply (Table 3). The highest GR was observed at 18 days of culture.

The highest Chl a content was observed in fronds of *Mazzaella laminarioides* cultivated in 0.09 mM treatment, while the lowest value was observed under control treatment (without NO_3^- supply) (Fig. 3). A decrease in PE and PC values was observed in all treatments throughout the experiment compared to the initial content (Fig. 3). The lowest values of both PE and PC were observed under control treatment at 12 and 18 days. At 18 days, *M. laminarioides* showed a light reddish color (data not shown).

Table 3. Multifactorial ANOVA for each dependent variable, showing simple effect results and double interaction between independent variables. These data were obtained for *Mazzaella laminarioides* cultivated at different nitrogen concentrations and exposed to solar radiation.

Source of variation	$[NO_3^-]$			Time			$[NO_3^-]$ × Time		
Variable	df	F	P	df	F	P	df	F	P
GR	4	1.0	0.443	2	48.0	0.001	8	0.7	0.692
Chla	4	7.7	0.001	2	1.4	0.265	8	1.7	0.131
PE	4	17.2	0.001	2	2.6	0.094	8	3.8	0.004
PC	4	7.1	0.001	2	4.1	0.028	8	2.2	0.057
C	4	7.2	0.001	1	0.7	0.406	4	0.4	0.787
N	4	45.7	0.001	1	3.2	0.088	4	3.3	0.032
C:N	4	116.6	0.001	1	11.3	0.003	4	10.8	0.001
MAAs	4	3.3	0.013	2	4.3	0.015	8	5.9	0.001
myc-glyc	4	18.9	0.001	2	127.3	0.001	8	6.5	0.001
shin	4	3.6	0.009	2	20.2	0.001	8	6.3	0.001
palyt	4	5.3	0.001	2	26.7	0.001	8	3.3	0.002

GR, growth rate; Chl a, chlorophyll a; PE, phycoerythrin; PC, phycocyanin; C, carbon; N, nitrogen; C:N, carbon to nitrogen ratio; MAAs, mycosporine-like amino acids; myc-glyc, mycosporine-glycine; shin, shinorine; and palyt, palythine. Bold values indicate significant differences. 5

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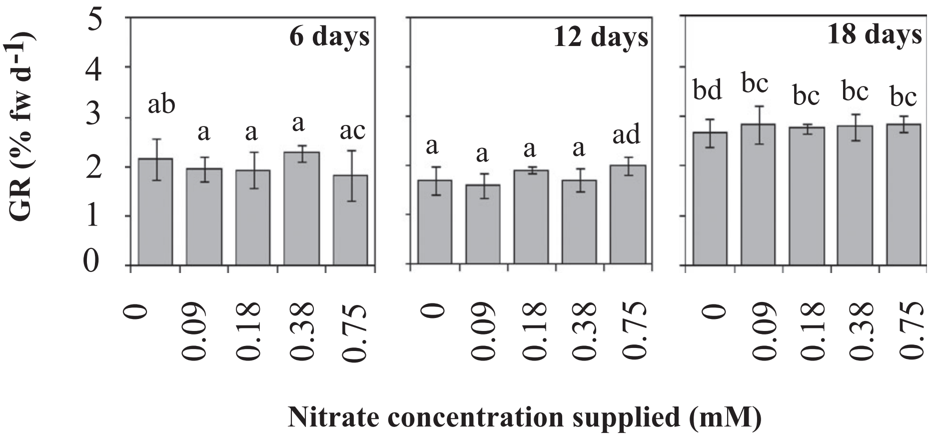

Figure 2. Growth rates (GR: % fw d^{-1}) of *Mazzaella laminarioides* tetrasporophytes cultivated under different treatments of NO_3^- supply (0, 0.09, 0.18, 0.38 and 0.75 mM) and exposed to natural solar radiation (PAR + UVR). Data are expressed as mean values ± SD (n = 3).

No differences were observed in MAA content among NO_3^- treatments after 12 and 18 days of cultivation (Fig. 4). However, differences in total MAA content among NO_3^- treatments were observed at 6 days, with higher MAA values observed in algae cultivated with 0.18 and 0.38 mM, while the lowest value was registered in control treatment.

Four different MAAs were identified in *Mazzaella laminarioides*: shinorine, palythine, mycosporine-glycine and asterina-330. The latter was detected in a few treatments and showed concentration below 0.02 mg g^{-1} dw. For this reason, asterina-330 was not considered in statistical analyses. During the experiment, mycosporine-glycine content increased significantly at 12 and 18 days of cultivation in all NO_3^- treatments. This increase was highest in fronds cultivated with 0.18 mM of NO_3^- supply. The lowest increase was observed under control condition and 0.75 mM of NO_3^- supply (Fig. 4). Palythine content was the highest under 0.18 mM of NO_3^- supply, while the lowest values were registered in control treatment (Fig. 4). In the case of shinorine content, a decrease in concentration was observed in all NO_3^- treatments during the experiment compared to values registered at the beginning of the experiment. On the other hand, the differences in shinorine concentration among NO_3^- treatments were observed at 6 and 18 days of culture, with highest values registered in 0.38 mM treatment and lowest one in control treatment.

A significant decrease in total internal N was observed in the control treatment, while the highest values were observed in 0.18 mM treatment (Table 4). Similarly, the content of C was the lowest in control treatment, but no differences were observed among NO_3^- treatments. Thus, the C:N ratio was higher in fronds cultivated in control treatment when compared to the NO_3 treatments (Table 4). The higher numerical values of carrageenan yield in control treatment were also observed (Table 4); however, ANOVA showed no significant effect of NO_3^- treatments on carrageenan yield (df = 4; F = 3.27; P = 0.058; Table 4).

Normal FT-IR spectrum of carrageenan presented characteristic signals of sulfate group at 1258.8, 835.2 and 580.8 cm^{-1}, and the second-derivative FT-IR spectrum showed that the signal at 835.2 cm^{-1} was resolved into two signals at 839.0 and at 821 cm^{-1}, usually associated with the presence of sulfate groups on C-2 of β-galactopyranosyl residue and on C-6 of α-galactopyranosyl residue, respectively.

Most variables were significantly and positively correlated (Table 5). Total N was positively correlated to PE, PC and MAA content. Furthermore, MAA content was positively correlated with PC and PE content and GR. Chl a content was correlated with PC and PE content. On the other hand, carrageenan yield showed negative correlation with all dependent variables.

DISCUSSION

Seawater of the Magellan Strait can be considered mesotrophic waters, since NO_3^- concentration is rather uniform at about 0.005 mM, and no annual significant variation has ever been reported (23). However, an increase of N could occur on account of salmon aquaculture activity (16). In this work, *Mazzaella laminarioides*, a climax community species inhabiting Patagonian areas, was exposed to a high concentration of NO_3^-, more than 100 times the values observed in the nature, thus helping to clarify the possible effects of eutrophication processes by the use of semi-intensive systems and intensive fish farms with water recirculation practices (29).

Although some parameters used to evaluate *Mazzaella laminarioides* varied throughout the experiment, mainly influenced by the interaction of NO_3^- supply and incubation time, as noted in the Results above, growth rates, an integrative parameter of all physiological processes, did not change when nitrate was added to the seawater, confirming our initial hypothesis that the model species would be able to maintain its growth rates under the stress of nitrate treatments. On the other hand, the capacity for N accumulation was not high since elemental N content appeared to be similar among treatments with NO_3^- supply, but higher in comparison to control. Irrespective of NO_3^- concentration, this result suggested that apical sections of *M. laminarioides* took up and assimilated NO_3^- in similar quantities. Furthermore, it could be concluded that the N uptake was used to maintain concentrations of such nitrogen-containing compounds as PE, PC, Chl *a* and MAAs, and, consequently, maintain growth rates. Since no discernable negative physiological effects resulted from high N supply, which did not accumulate, both growth rates and the level of internal compounds were maintained, as observed in other macroalgae-like kelp (30). Thus, high N supply under eutrophication did not result in any negative effects for this species. However, for control treatment, a

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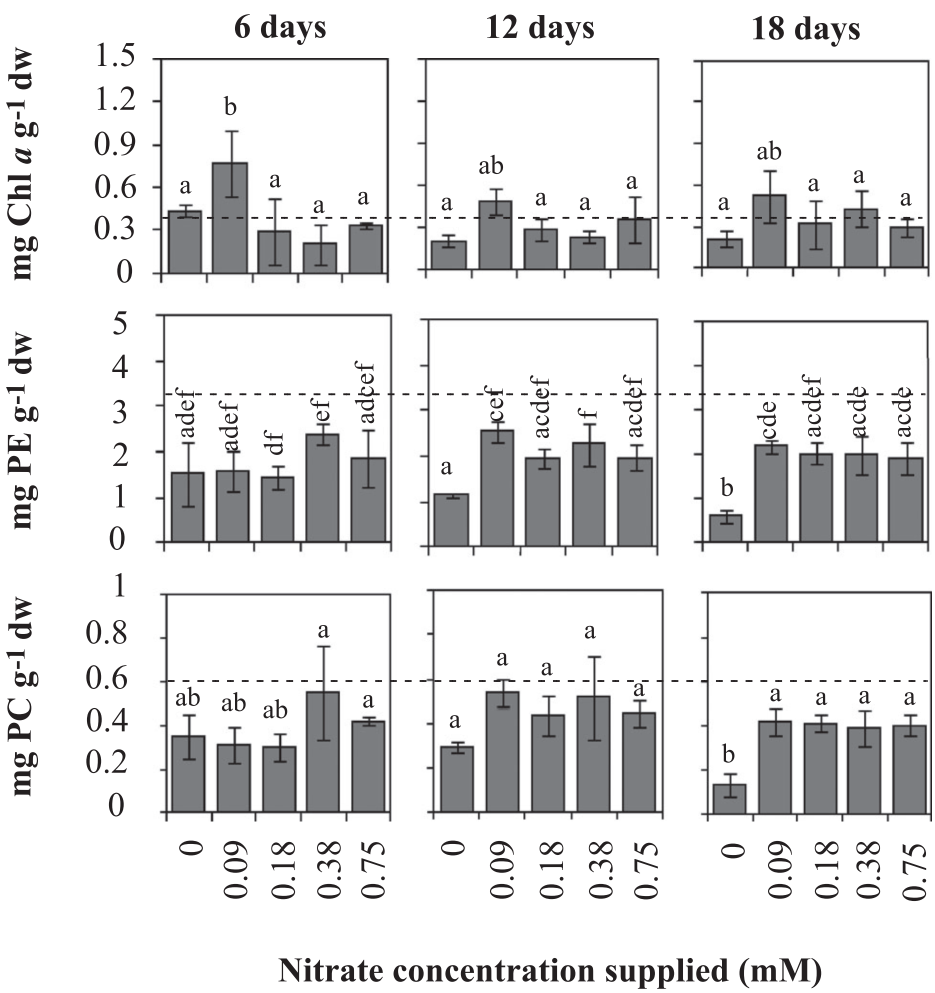

Figure 3. Pigment (Chl a: Chlorophyll a, PE: phycoerythrin, PC: phycocyanin) content (mg g^{-1} dw) in *Mazzaella laminarioides* tetrasporophytes cultivated under different treatments of NO_3^- supply (0, 0.09, 0.18, 0.38 and 0.75 mM) and exposed to natural solar radiation (PAR + UVR). Data are expressed as mean values ± SD (n = 3). Letters on the graph show the result of the Newman–Keuls test (P < 0.05); different letters refer to significant differences between mean values; dotted line shows the initial value of each pigment.

decrease in PE and PC content was observed during the experiment. The degradation of PE and PC could promote decreases in photosynthesis rates, and a decrease in growth rates could, therefore, be expected (8). Nevertheless, no differences were observed in the GR of *M. laminarioides* after 18 days of cultivation in all treatments. Thus, fronds cultivated under control condition could have reallocated N from PE and PC degradation among different nitrogenous compounds like MAAs without affecting growth rates. This reallocation is important considering that *M. laminarioides* was exposed to high irradiance, a condition in which PE and PC accessory pigments in great quantity would not be necessary and MAAs could play an important photoprotective and antioxidant roles. The positive correlation between N content and nitrogen-containing compounds, such as PE, PC and MAAs, as observed in *M. laminarioides*, has also been reported for other species (6,7,10,12), showing the importance of N availability for photoprotection.

The C:N ratios of *Mazzaella laminarioides* under treatments with NO_3^- supply ranged between 8.8 and 9.9 at 6 days of incubation. These values were higher than the classical Redfield ratio of 6.6 (31), which might suggest N deficiency in culture medium. Nevertheless, although the most commonly used indicator of nutritional status in marine algae is the C:N ratio (32), this ratio could vary according to the species and the conditions to which algae are submitted (33,34). In *Gracilaria tenuistipitata*, only values of C:N ratios higher than 15 indicated a deficiency of N (35), whereas the addition of NO_3^- (0.05 mM) promoted values ranging between 7 and 10 (12). In our experiment, N deficiency was only observed in *M. laminarioides* cultivated under control treatment (without NO_3^- supply), with C:N ratio reaching 14.

The highest C:N ratio was observed in control condition. As such, an increase in carrageenan yield in these algae was expected when compared to the algae submitted to NO_3^- treatments. It will be recalled that our hypothesis holds that carrageenan yield would decrease upon addition of nitrate to seawater, but this was not confirmed by the level of significance used in statistical analysis, *i.e.* P = 0.058. Otherwise, in these experiments, we used only tetrasporophytes confirmed by the presence of lambda carrageenan-type polysaccharide (36), but evidence did show that gametophytes had higher carrageenan yield than tetrasporohytes (Navarro *et al.*, data not shown). Thus,

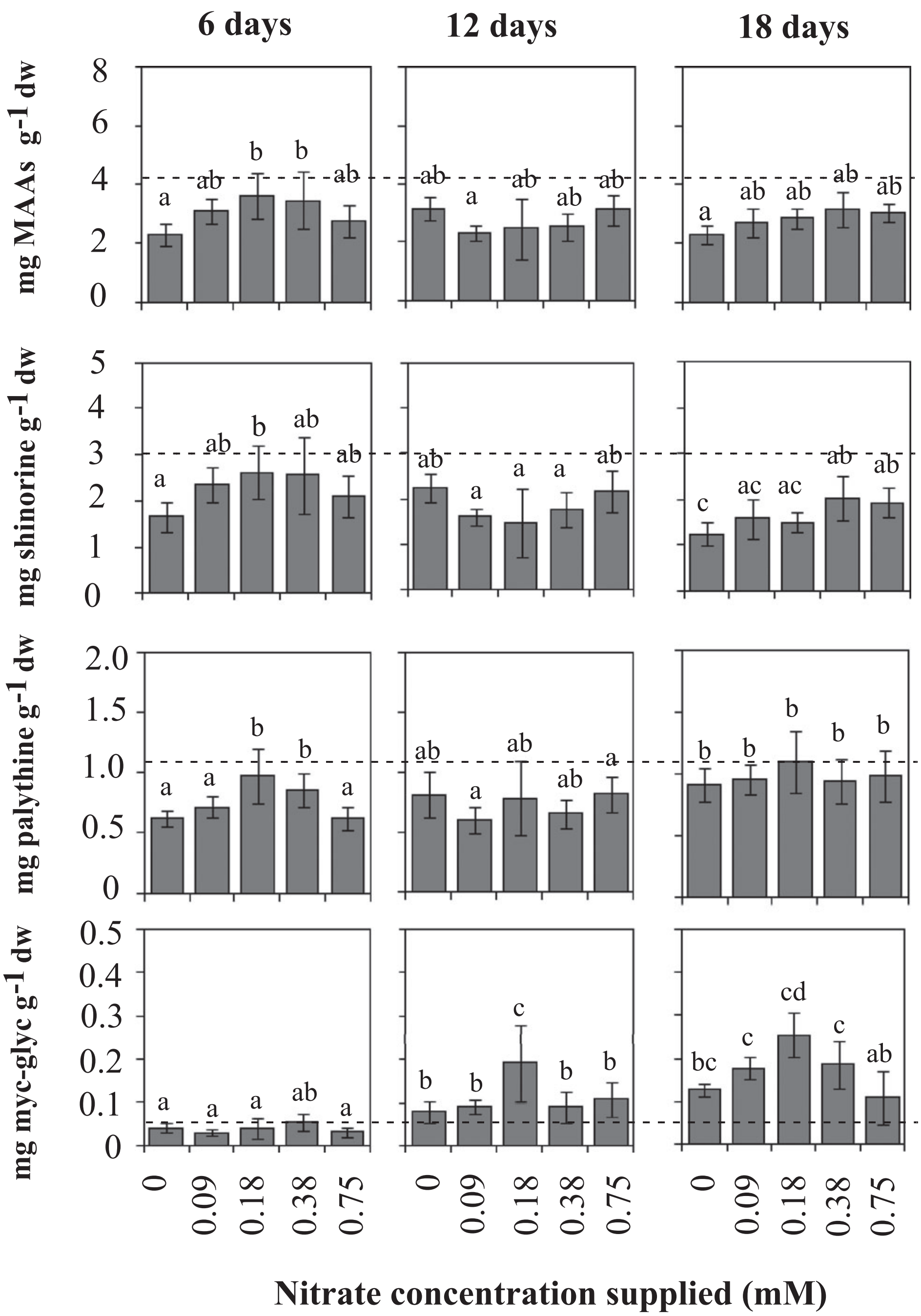

Figure 4. Total MAAs and MAA composition content (mg g^{-1} dw) in *Mazzaella laminarioides* tetrasporophytes cultivated under different treatments of NO_3^- supply (0, 0.09, 0.18, 0.38 and 0.75 mM) and exposed to natural solar radiation (PAR + UVR). Data are expressed as mean values ± SD (n = 3). Letters on the graph represent results of the Newman–Keuls test ($P < 0.05$); different letters refer to significant differences between mean values; dotted line shows the initial value of each MAA.

if gametophytes had been used in the experiments, it is possible that a significantly higher carrageenan yield would have been observed.

The effect of NO_3^- on *Mazzaella laminarioides* was evident by the preferential accumulation of some types of MAAs, rather than total MAAs. In this context, an important synthesis of mycosporine-glycine, a UVB sunscreen (310 nm) and antioxidant substance (37–39), was observed in all NO_3^- treatments. However, the accumulation of this compound occurred in a nitrate concentration-dependent manner, with accumulation being saturated around 0.18 mM of nitrate. It has been suggested that high NO_3^- or NH_4^+ concentration in seawater could promote a decrease in MAA concentration of *Gracilaria tenuistipitata* (12), *Asparagopsis armata* (13) and *G. conferta* (40), which may compromise the photoprotection system. In the case of *M. laminarioides*, although the mycosporine-glycine concentration was lower under 0.75 mM treatment when compared to the 0.38 mM treatment, a similar concentration of total MAAs was

Table 4. Content of C and N (mg g^{-1} dw), C:N ratios and carrageenan yield in *Mazzaella laminarioides* at different incubation times, under different treatments of NO_3^- supply (0, 0.09, 0.18, 0.38 and 0.75 mM), and exposed to natural solar radiation (PAR + UVR). Data are expressed as mean values ± SD (n = 3). Letters show the result of the Newman–Keuls test ($P < 0.05$).

	NO_3^- supply treatments	C	N	C/N	Carrageenan yield
Initial		236 ± 2.8	23.9 ± 3.0	10.0 ± 1.2	27.2 ± 1.4
6 days	0 (control)	243 ± 0.8 a	20.0 ± 0.8 a	12.2 ± 0.5 a	
	0.09	242 ± 2.1 a	24.3 ± 0.6 b,d,f	10.0 ± 0.2 b,e,f	
	0.18	252 ± 0.5 b	28.6 ± 0.4 c	8.8 ± 0.1 c,e	
	0.38	244 ± 7.0 a	26.4 ± 1.8 c,d	9.3 ± 0.4 b,c,f	
	0.75	244 ± 0.6 a	24.4 ± 0.1 b,d,f	10.0 ± 0.1 c,f	
18 days	0 (control)	239 ± 2.1 a	16.7 ± 0.2 e	14.3 ± 0.1 d	34.3 ± 5.3 a
	0.09	243 ± 4.4 a	25.1 ± 1.4 f,d	9.7 ± 0.3 c,e	26.4 ± 2.6 a
	0.18	251 ± 5.5 b	26.6 ± 1.6 f,c	9.4 ± 0.4 e,f	29.4 ± 1.2 a
	0.38	244 ± 2.3 a	25.5 ± 1.6 f,c	9.6 ± 0.5 e	28.6 ± 1.1 a
	0.75	244 ± 4.6 a	25.6 ± 2.1 f,d	9.6 ± 0.6 c,e	33.2 ± 3.5 a

Table 5. Pearson correlation values obtained among dependent variables after cultivating *Mazzaella laminarioides* with five NO_3^- supply treatments (0, 0.09, 0.18, 0.38 and 0.75 mM) and exposed to natural solar radiation (PAR + UVR). Values range between −1 and 1. Marked bold values are significantly correlated at $P < 0.05$. Dependent variables: C, carbon; N, nitrogen; C:N, carbon to nitrogen ratio; Chl a, chlorophyll a; PE, phycoerythrin; PC, phycocyanin; MAAs, mycosporine-like amino acids; myc-glyc, mycosporine-glycine; shin, shinorine; palyt, palythine and Carrag yield, Carrageenan yield n = 15.

	N	C:N	Chla	PE	PC	MAAs	myc-glyc	shino	palyt	GR	Carrageenan yield
C	0.72	−0.60	−0.08	0.55	0.46	0.19	0.47	−0.02	0.49	0.05	−0.15
N		−0.98	0.33	0.92	0.89	0.61	0.39	0.45	0.41	0.24	−0.41
C:N			−0.41	−0.94	−0.92	−0.65	−0.37	−0.51	−0.35	−0.26	0.45
Chla				0.47	0.50	0.13	0.20	0.19	−0.25	0.06	−0.42
PE					0.98	0.61	0.28	0.50	0.29	0.20	−0.39
PC						0.65	0.31	0.54	0.31	0.22	−0.50
MAAs							0.03	0.92	0.39	0.57	−0.26
myc-glyc								−0.27	0.45	−0.01	−0.29
Sin									0.03	0.53	−0.28
Palyt										0.22	0.14
GR											−0.01

observed in all NO_3^- concentrations. On the other hand, the fact that mycosporine-glycine, but not total MAAs, was increased could have resulted from interconversions among different MAAs, as discussed for *Porphyra* species (9) and *Chondrus crispus* (41). Changes in specific MAA concentration, but not in total MAA content, were reported in other red macroalgae when exposed to different radiation treatments (10,42,43). Moreover, changes in the concentration of specific MAAs could be observed as a consequence of different stress factors, *e.g.* radiation, N availability, temperature or salinity, leading to adjustments in the response of species to conditions in the laboratory or natural environment. Thus, accumulation of mycosporine-glycine in *M. laminarioides* could be a response to the cultivation conditions used in this experiment. Under some conditions, synthesis of reactive oxygen species and, consequently, oxidative stress could occur (44–48). Under this condition, synthesis and accumulation of compounds with antioxidant activities, such as mycosporine-glycine (38,39,49), could play an important ecological role.

In conclusion, the high concentration of NO_3^- did not promote a deleterious effect on either growth or the photoprotection system, such as MAAs, in the late successional species *Mazzaella laminarioides*. Furthermore, the fact that carrageenan yield was not changed under high NO_3^- concentration is a very important result considering the economic importance of *M. laminarioides* in southern Chile and also considering that the presence of salmon aquaculture activity in Patagonian seawater is an expected important input of N.

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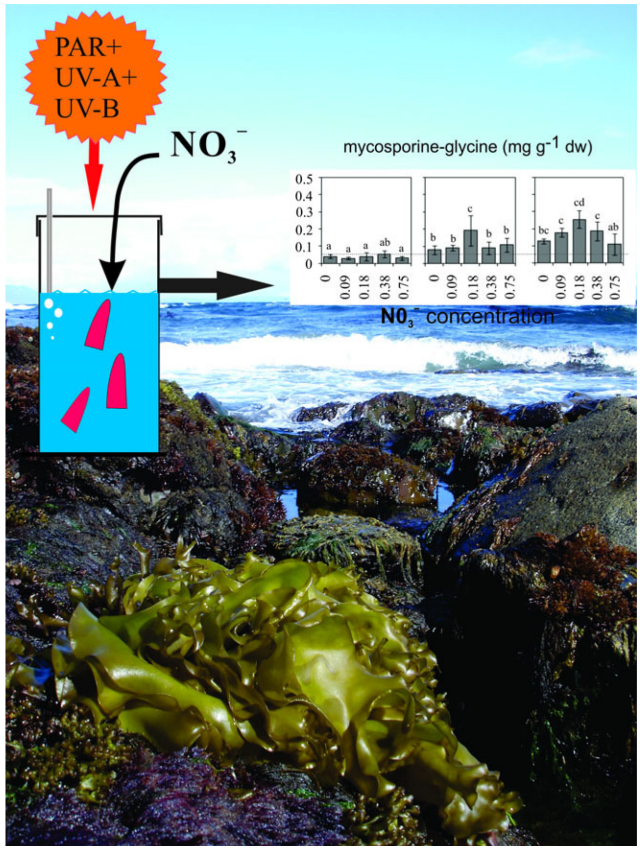

The effect of NO_3^- on *Mazzaella laminarioides* was evident by the preferential accumulation of mycosporine-glycine, rather than total MAAs. The fact that mycosporine-glycine, but not total MAAs, was increased could have resulted from interconversions among different MAAs. Synthesis and accumulation of compounds with antioxidant activities, such as mycosporine-glycine, could play an important ecological role.

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