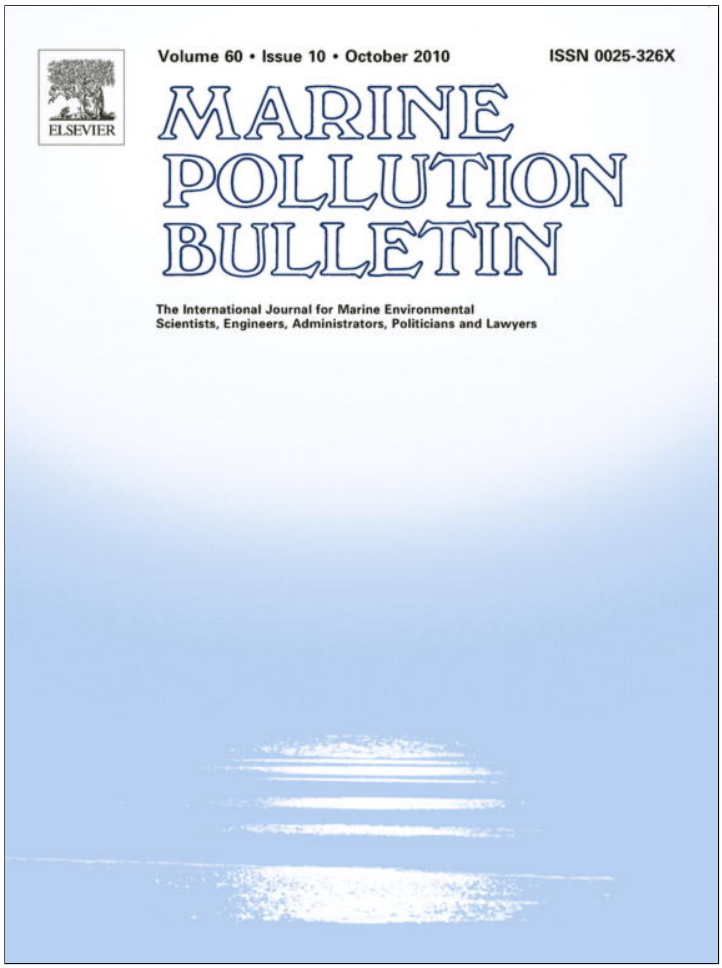

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Effect of nutrient supply on photosynthesis and pigmentation to short-term stress (UV radiation) in *Gracilaria conferta* (Rhodophyta)

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ABSTRACT

The effects of increased photosynthetic active radiation (PAR), UV radiation (UVR), and nutrient supply on photosynthetic activity, pigment content, C:N ratio and biomass yield were studied in tank cultivated *Gracilaria conferta* (Rhodophyta). Electron transport rate (ETR) and biliprotein content were higher under high nutrient supply (HNS), obtained from fishpond effluents, compared to low nutrient supply (LNS), in contrast to mycosporine-like amino acids (MAAs) dynamic. The high MAA content in LNS-algae could be explained by higher UVR penetration in the thallus and by the competition for the use of nutrients with other processes. Effective quantum yield decreased after short-term exposure to high irradiance whereas full recovery in shade was produced only under slightly heat shock. UVA radiation provoked an additional decrease in photosynthesis under high water temperature. UVB radiation reversed UVA's negative effect mainly with HNS. Results support that nutrient-sufficiency help *G. conferta* to resist environmental changes as short-term temperature increase.

1. Introduction

Awareness by scientists, industry, the public and politicians is such that technologies with uncontrolled impact are no longer considered sustainable (Chopin et al., 2001). Therefore, biofiltration of waste nutrients from fishpond effluents is key to the long-term sustainability of the aquaculture industry (Neori et al., 2003; Hernández et al., 2005; Neori et al., 2007; FAO, 2006). Seaweeds, most successfully *Ulva* spp. and *Gracilaria* spp., have been used as biofilters to remove dissolved inorganic N and P from wastewaters (Jiménez del Río et al., 1996; Msuya and Neori, 2002; Neori et al., 2003; Yang et al., 2005). Recently, the Rhodophyte *Asparagopsis armata* (=*Falkenbergia rufolanosa*) was reported to biofilter aquaculture effluents even better than *Ulva* spp. and *Gracilaria* spp., probably due to its higher yield and a higher N content (Schuenhoff et al., 2006; Mata, 2008). Increases in the levels of photosynthetically active radiation (PAR), ultraviolet radiation (UVR), and higher temperature in summer time (particularly during short-term heat events) characterize the on going global climate change. Such environmental changes can impact algal aquaculture production and, with it, waste nutrients biofiltration capacity.

The integrated multi-trophic aquaculture (IMTA) based on the integrated culturing of fed species such as finfish, inorganic extractive species such as seaweeds, and organic extractive species such as suspension- and deposit-feeders, has the promise to contribute to the sustainability of aquaculture (Chopin et al., 2001; Neori et al., 2004; FAO, 2006), based on a number of potential economic, societal and environmental benefits, including the recycling of waste nutrients from higher trophic-level species into production of lower trophic-level crops of commercial value (Troell et al., 2009). The algal biomass produced in IMTA systems is often used as food for animals, such as abalone (Troell et al., 2006). Seaweeds, such as the aforementioned *A. armata*, have been a source of commercially valuable substances, from gelling agents to cosmetics preservers (Guiry and Blunden, 1991; Moigne, 1998; Neori et al., 2007; Turan, 2009). Algae grown in N-enriched waters i.e. fishpond effluents, make a good source of useful N-compounds with commercial interest, such as biliproteins used as fluorescence markers (Luiten et al., 2003), and mycosporine-like amino acids (MAAs) (Figueroa et al., 2008). It is known that MAAs act as a passive screen, dissipating the UV energy absorbed in thermal form (Conde et al.,

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2000), but MAAs could also have a secondary photoprotective role as an antioxidant (Dunlap and Yamamoto, 1995; Nakayama et al., 1999; De la Coba et al., 2009). In fact, MAAs blocked the thymine photodimer formation in *Porphyra yezoensis* (Misonou et al., 2003).

In addition to light quality and quantity, the accumulation of both photosynthetic pigments and MAAs can be affected by N availability (Figueroa et al., 1997; Karsten and Wiencke, 1999; Litchman et al., 2002; Korbee-Peinado et al., 2004; Korbee et al., 2005a, 2006). A positive relation between N availability and MAAs accumulation has been reported in different species of the Rhodophytes *Porphyra* (Korbee-Peinado et al., 2004; Korbee et al., 2005a) and *Grateloupia* (Huovinen et al., 2006).

The light field, temperature and N supply affect the photosynthetic activity in tank-cultivated seaweeds (Figueroa et al., 2006). Photosynthesis can be estimated as chlorophyll fluorescence i.e. effective quantum yield and electron transport rate (ETR) according to Figueroa et al. (2003). Information related to *in situ* chlorophyll fluorescence measurements has increased considerably in macroalgae of different aquatic systems (Gómez and Figueroa, 1998; Gómez et al., 2005); such as algae used in aquaculture with commercial interest under laboratory conditions as *Gelidium pulchellum* (Gómez et al., 2001) and *G. sesquipedale* (Gómez and Figueroa, 1998), under out-door conditions in tank grown algae (Aguirre-von Wobeser et al., 2000; Cabello-Pasini et al., 2000; Figueroa et al., 2006) and under semi-extensive culture systems as *Gracilaria chilensis* in estuarine systems (Gómez et al., 2005) or as *Palmaria palmata* in coastal areas (Hanelt and Nultsch, 1995). Figueroa et al. (2006) used *in vivo* chlorophyll fluorescence by pulse amplitude modulated (PAM) fluorometry as indicator of photosynthetic activity in tank cultivated red algae, *G. cornea* and *A. armata* (=*F. rufolanosa*), using effluent seawater from sea bream fishponds (*Sparus aurata*). Chlorophyll fluorescence proved to be a powerful tool to detect stress situations in integrated cultivation of seaweeds using fishpond effluents. In particular, the optimal quantum yield of algae can be regularly monitored as an early warning of the physiological stress of cultures (Figueroa et al., 2006; Msuya and Neori, 2008). In fact, the use of this technique is of great interest because it could be easily monitored the early effects of climate changes on macroalgae, not only under cultured but also under natural conditions.

In this paper, short-term effect on photosynthesis, as chlorophyll fluorescence, under increased solar irradiance i.e., PAR and UVR (exposure for 1.5 h around noon local time follow by a 3.0–3.5 h recovery phase in the shade) were evaluated in *G. conferta*, in comparison with the same species grown in tanks. Algae were grown under two nutrient conditions: (a) low nutrient supply, LNS (oligotrophic seawater collected from the Red Sea) and (b) high nutrient supply, HNS (seawater from the Red Sea enriched by effluents from an intensive mariculture ponds).

2. Materials and methods

2.1. Cultivation conditions and experimental design

G. conferta (Rhodophyta) (Schousboe ex Montagne) Montagne was cultivated for two weeks in square opaque out-door tanks (1 m^2 surface area) containing about 600 l of seawater at the National Center for Mariculture in Eilat (Israel), at a water flow of 9 m^3 $tank^{-1}$ d^{-1} (as in Cohen and Neori, 1991). Two nutrient conditions were applied in duplicated tanks: (1) high nutrient supply (HNS) – fishpond effluents mixed with an equal amount of Red Sea water; and (2) low nutrient supply (LNS) – pristine Red Sea water with fishpond effluents (*S. aurata*) being slowly dripped in at a rate of 0.1 m^3 $tank^{-1}$ d^{-1}. Concentrations of nutrients (μmol l^{-1}) in high (HNS) versus low nutrient supply (LNS) treatments were 28.4 ± 1.8 versus 5 ± 0.6 for ammonium, 64.8 ± 1.6 versus 0.02 ± 0.003 for nitrite, 239.4 ± 5.1 versus 0.28 ± 0.03 for nitrate and 16.1 ± 0.2 versus 0.13 ± 0.005 for ortophosphate, irradiance and temperature measurements were already reported in Figueroa et al. (2009). The algal density at the beginning of the experiment was 4.8 g FW (fresh weight) l^{-1}, 2.9 kg m^{-2}. The PAR light penetration at 5 cm depth was 20% of the incident surface irradiance, and no light was measured at 10 cm (virtual darkness).

Following two weeks of cultivation under the conditions described above, only pristine Red Sea water was added to the LNS tanks, whereas no change was made in conditions for the HNS tanks. At day 2 (April 2nd, 2008) and day 5 (April 5th, 2008) under these new conditions, algal samples from each of the four tanks were taken out, transferred and incubated for 1.5 h during midday (12:30–14:00) in 20 × 30 × 5 cm aluminum vessels filled with seawater under three different light treatments according to Figueroa et al. (1997) and Villafañe et al. (2003) as follows: (a) full sunlight spectrum of PAR + UVA + UVB (PAB treatment), using the long-pass cut-off foil Ultraphan 295 (Digefra GmbH, Munich, Germany); (b) PAR + UVA (PA treatment), using Folex 320 (Folex GmbH, Dreieich, Germany); (c) PAR (P treatment), using the long-pass cut-off foil Ultraphan 395 (Digefra GmbH, Munich, Germany). Photosynthesis measured as chlorophyll fluorescence was determined *in* algae growing in the culture tanks (600 l) at 10:00, 12:00, 14:00, 15:00 and 17:00 at day 5, as well as in algae cultured in aluminum vessels at 12:30, 13:00 and 14:00 (local time) both at day 2 and 5. After the exposure period, the vessels were transferred to the shade (20–25% of full sunlight) and chlorophyll fluorescence was again determined at 15:00, 15:30, 16:30 and 17:30 on 2nd April (day 2) and at 15:00, 16:00 and 17:00 on 5th April (day 5). Algal samples from the four tanks were incubated, leading to 12 total experimental units in total: four tanks (HNS and LNS in duplicates) × three light treatments.

2.2. Temperature measurements

Air and tanks and aluminum vessels water temperatures were measured throughout the days of experimentation using a mercury thermometer. Data are already published in Figueroa et al. (2009).

2.3. Photosynthesis as chlorophyll fluorescence

In vivo chlorophyll fluorescence of PSII was determined by pulse amplitude modulated technique using two Diving-PAMs (Waltz GmbH, Effeltrich, Germany). The effective quantum yield ($\Delta F/F'_m$) was calculated according to Figueroa et al. (2003). Measurements were made on eight replicate samples per experimental aluminum vessel (96 in total) at each time interval.

Rapid light curves (RLC), ETR versus irradiance, were conducted right after removal of the seaweeds from the tanks (before beginning the experimental exposure at 12:30 local time) with timing as described above. For this purpose, a Water-PAM fluorometer equipped with an EDF fiberoptic unit was used (Waltz GmbH, Effeltrich, Germany). After a dark adaptation period of 10 min followed by a 5 s far-red light pulse to ensure full oxidation of Q_A, a RLC program (WINCONTROL software) was initiated. RLC were performed with 15 s exposure duration to each of eight incremental irradiances by using red LED's (light emitting diodes) at 8, 12, 18, 27, 40, 60, 91, 136, 176, 245, 405 and 598 μmol photons m^{-2} s^{-1}. Calculations of ETR were then made by multiplying the effective quantum yield by the incident solar radiation (E), the absorptance (A) and the fraction of chlorophyll *a* (Chl*a*) in PSII associated to LHCII (F_{II}), which is related to the absorbed quanta to PSII (400–700 nm) according to Schreiber et al. (1995). F_{II} value was 0.15 according to Grzymski et al. (1997).

$$ETR = \Delta F/F'_m \cdot E \cdot A \cdot F_{II} \quad (\mu mol\ electrons\ m^{-2}\ s^{-1})$$

The absorptance in the PAR region of the spectra was determined from light transmission through algal pieces, placed on a cosine-corrected sensor connected to a multiphotodiode spectroradiometer SMS 500 (Sphere Optics, USA) according to Beer et al. (2000).

2.4. Chlorophyll and phycobiliprotein contents

Seaweed samples were stored at −70 °C for several weeks prior to extraction of photosynthetic pigments. Concentration of Chl*a* was determined from 0.02 to 0.05 g FW algal samples ($n = 4$, 2 per tank) immersed in 3 ml of *N*,*N*-dimethylformamide at 4 °C for 24 h in the dark (Moran, 1982). Levels of the phycobiliproteins, phycoerythrin (PE) and phycocyanin (PC), were measured in algal crude extracts from 0.01 to 0.02 g FW ground in phosphate buffer at pH 7.0 according to Beer and Eshel (1985). Concentrations of all pigments were expressed in mg g^{-1} dry weight (DW). Pigments were extracted from samples obtained after 2 weeks of pre-treatment (initial), and 2 and 5 days after the beginning of the experiment, as well as after the short-term light exposure-recovery experiment conducted on April 5th.

2.5. Mycosporine-like amino acids (MAAs)

MAAs were extracted in 20% aqueous methanol (v/v) from four samples of dried algal samples, two per tank. Samples were analyzed with a Waters HPLC system (Waters 600) as was described by Korbee-Peinado et al. (2004). Quantification was made using published extinction coefficients (Bandaranayake, 1998). Results of the HPLC analysis are expressed as mg g^{-1} DW. MAAs were extracted for initial samples (after 2 weeks of pre-treatment), and 2 and 5 days after beginning of the experiment, as well as for light short-term experiments conducted on both April 2nd and 5th.

2.6. Internal carbon and nitrogen contents

For C and N determinations, samples were kept desiccated until the analysis. Total internal C and N were determined by combustion using a CNHS LECO-932 (Michigan, USA) elemental analyzer. Four replicates were used for each treatment (two per tank). Analyses were made in samples from the initial (after 2 weeks pre-treatment), and 2 and 5 days after beginning of the experiment.

2.7. Biomass yield

Biomass yield was determined once a week (in this article only the results of the last week are presented), harvesting each tank by mesh bags (0.1 mm mesh) and draining the biomass to constant FW at 2800 rpm in a domestic washing machine centrifuge. Biomass yield (Y) was calculated by the equation Y (g DW m^{-2} d^{-1}) = $[(Nt - N_0)/t \cdot (DW/FW)]/A$; as modified after DeBoer and Ryther (1977), where Nt is the final FW, N_0 is the initial FW, $t = 7$ d, DW/FW the ratio of DW to centrifuged FW and A is the area of the tank (1 m^2). DW was determined from fresh algae dried for 48 h at 60 °C and weighed after cooling down in a silica-desiccator. The average ratio DW/FW in *G. conferta* was 0.175. N yield as indicator of the biofiltration capacity of inorganic N was calculated following Mata (2008) as biomass yield multiplied by N content.

2.8. Statistical analysis

A hierarchical Analysis of Variance (ANOVAs) tested for differences in Chl*a*, PE, PC, MAAs, C, N, and C:N in fronds cultivated inside the tanks under two nutrient treatments (factor 1: *Nutrient*). A second factor, *Time*, was also included in the analyses since different samples were taken at different dates as the experiment proceeded. Minor variations in conditions between tanks may affect to some extent the pigment composition of the algae, to test for this effect, two tanks per nutrient treatment were set and an additional factor, *Tank* (nested in *Nutrient)*, was included in the ANOVAs.

Orthogonal ANOVAs were used to test for differences in content of photosynthetic pigments (Chl*a*, PE and PC) after the short-term exposure experiments on April 5th. The effect of *Light conditions* (PAR, PAR + UVA, PAR + UVA + UVB) during the exposure period, and the effect of the *Nutrient* conditions in the tanks (HNS versus LNS) were tested. One additional factor, *Tank* (nested in *Nutrient,* thus hierarchical design in this case), was included in the ANOVAs to test the differences in MAAs content after the short-term exposure experiments on April 2nd and 5th, since it was known from which tank each algae sample came.

Cochran's test was used to test for heterogeneity of variances (Underwood, 1997). Student–Newman–Keuls (SNK) tests were used to discriminate among different treatments after significant *F*-tests, and significantly different means were denoted by lower case letters in the figures when applicable (Underwood, 1997). All tests were done with SPSS (11.0.1) for Windows.

3. Results

3.1. Water temperature

Water temperature in the tanks was slightly higher on 2nd April than on 5th April (Figueroa et al., 2009). However, the difference in water temperature throughout the day between culture tanks and aluminium vessels was 5–10 °C higher in the latter one on April 2nd and 3–6.5 °C on April 5th (Figueroa et al., 2009).

3.2. Electron transport rate (ETR)

ETR values on 2nd April were higher in HNS than in LNS grown algae (70%, Fig. 1a). Three days later (April 5th), these differences became insignificant (Fig. 1b), while, photoinhibition i.e., decrease of maximal ETR, became evident (Fig. 1b).

3.3. Effective quantum yield ($\Delta F/F'_m$)

The average effective quantum yield ($\Delta F/F'_m$) of algae growing in tanks on 2nd April, just before beginning the exposure experiment, was higher in HNS (0.61 ± 0.05, $n = 16$) than that in LNS (0.55 ± 0.05, $n = 16$) grown algae (Fig. 2a and b). Whereas the initial values of yield were similar in both nutrient treatments on 5th April i.e., 0.65 ± 0.07 ($n = 16$) in HNS and 0.68 ± 0.03 ($n = 16$) in LNS (Fig. 2c and d). In the exposure experiments conducted on 2nd April (Fig. 2a and b), the yield decreased drastically (approx. 70%) after 1 h exposure in the aluminum vessels and in some light treatments the decay of the yield progressed in the next hour of exposure (13:00–14:00 local time) (Fig. 2a and b). This decrease was larger in LNS than in HNS algae. In the latter, the decrease was larger following exposures to PAR and PAR + UVA than to PAR + UVA + UVB (Fig. 2a). However, the largest decrease on this day was observed following exposure to PAR + UVA in LNS algae. On 5th April, the yield in the three light treatments decreased sharply in the first 30 min of exposure and then stabilized in HNS algae (Fig. 2c) and even begun recovery in LNS algae (Fig. 2d) while still exposed. On 2nd April the largest recovery in the shade was measured in the HNS algae under a full spectrum radiation (PAR + UVA + UVB), reaching about 62% of the initial values after 3.5 h. The PAR and PAR + UVA treated HNS algae recovered in the same time

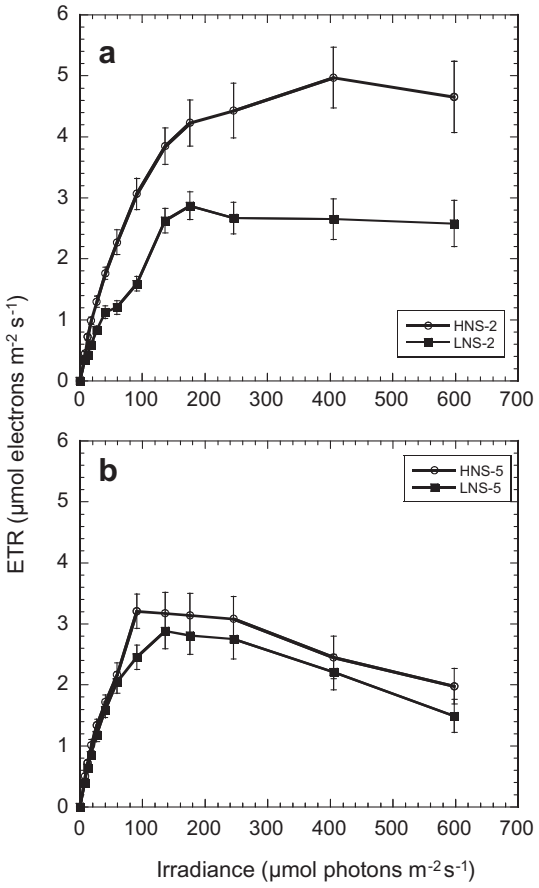

Fig. 1. Electron transport rate (ETR, µmol electrons m^{-2} s^{-1}) at different irradiances (µmol photons m^{-2} s^{-1}) in *Gracilaria conferta*, grown in tanks. The measurements were taken in midday just before short exposure experiments on April 2nd (a) and 5th (b). HNS-2: high nutrient supply algae on April 2nd, LNS-2: low nutrient supply algae on April 2nd, HNS-5: high nutrient supply algae on April 5th, LNS-5: low nutrient supply algae on April 5th. Error bars represent ±1 SD.

less than 30% of the initial values (Fig. 2a). LNS grown algae, however, showed similar recovery for PAR and PAR + UVA + UVB treatments (about 48%) i.e., smaller recovery than in HNS algae in PAR + UVA + UVB but larger than in PAR (Fig. 2b). In the set of experiments conducted on 5th April, algae exposed to the three light treatments responded rather similarly. Almost full recovery was observed in HNS treated algae (93% of the initial values in PAR + UVA + UVB and 88% in both PAR and PAR + UVA) (Fig. 2c). In LNS algae on this day, the recovery was slightly lower than in HNS algae i.e., 87% in PAR + UVA, 85% in PAR and 80% in PAR + UVA + UVB (Fig. 2d).

On April 5th, in addition to the exposure and recovery in the aluminum vessels, the effective quantum yield was measured at the same time in algae growing in tanks exposed to natural sunlight (PAR + UVA + UVB) (Fig. 3). Effective quantum yield decreased until 15:00, with a partial recovery at 17:00 local time in LNS algae (Fig. 3). The yield was higher in HNS algae from 10:00 to 15:00 local time than in LNS algae, whereas at 17:00 both treatments converged to similar values (Fig. 3).

3.4. Photosynthetic pigments

Phycobiliprotein content in the algae was affected by nutrient supply (Fig. 4b and c; $F_{1,2} = 2223.86$, $F_{1,2} = 733.93$ for PE and PC, respectively, $p < 0.01$), meanwhile the content of Chl*a* was the same for both nutrient treatments (Fig. 4a; $F_{1,2} = 9.14$, $p = 0.094$). The difference in phycobiliprotein content between HNS and LNS grown algae was 249% and 255% for PE and PC, respectively, after 5 days (Fig. 4). No significant variation through time was observed ($F_{2,4} = 2.43$, $p = 0.204$ [Chl*a*], $F_{2,4} = 0.42$, $p = 0.683$ [PE], $F_{2,4} = 6.06$, $p = 0.062$ [PC]) and the interaction term (nutrients and time) was also not significant (not shown). After 5 days, the ratio PE:Chl*a* and (PE + PC):Chl*a* was about 2.7 times higher under HNS than under LNS (data not shown).

The effect of light spectrum exposure on pigment content was analyzed in the last set of experiments (5th April), after recovery in the shade. Pigment concentration, particulary PE, decreased in all three light treatments (Fig. 5) compared to algae in the tanks (Fig. 4). The pigment contents were significantly lower in LNS than in HNS grown algae ($F_{1,6} = 9.10$ [Chl*a*], $p < 0.05$; $F_{1,6} = 43.14$ [PE] and $F_{1,6} = 138.74$ [PC], both $p < 0.01$). The differences in pigment content among light treatments were not significant ($p = 0.76$ and $F_{2,6} = 0.287$ [Chl*a*], $p = 0.91$ and $F_{2,6} = 0.102$ [PE], $p = 0.80$ and $F_{2,6} = 0.227$ [PC]) (Fig. 5) and the interaction term (nutrients and light) was not significant (not shown).

3.5. Mycosporine-like amino acids (MAAs)

MAA content was always higher in LNS than in HNS algae, sampled directly from the tanks (Fig. 6a; $F_{1,2} = 2104.72$, $p < 0.01$) and after the exposure-recovery experiments (Fig. 6b; $F_{1,6} = 6778.13$, $p < 0.01$. Fig. 6c; $F_{1,6} = 701.54$, $p < 0.01$).

MAA content did not vary through time in algae grown in the tanks ($F_{2,4} = 6.49$, $p = 0.06$) and the interaction term (nutrients and time) was not significant (not shown). MAA content was about 4.6 times larger in LNS than that in HNS grown algae (Fig. 6a). In the experiment conducted on 2nd April, MAA content decreased after exposure to solar radiation (increased irradiance) (Fig. 6b) compared to the tank values on that day (Fig. 6a), no effect of light quality was observed ($F_{2,4} = 3.83$, $p = 0.118$) and the interaction term (nutrients and light) was not significant (not shown). However at 5th April, with similar irradiances as on 2nd April but with a smaller increase in temperature, MAA content was significantly higher after PAR and PAR + UVA + UVB exposure under LNS ($F_{2,4} = 106.69$, $p < 0.01$), i.e., UVA (PAR + UVA treatment) provoked a significantly decreased on MAA content whereas UVB (PAR + UVA + UVB treatment) reversed the negative effect of UVA radiation in the low nutrient treatment (Fig. 6c; SNK test lower case letters in Fig. 6c). Corresponding the interaction term (nutrients and light) was significant ($F_{2,4} = 83.55$, $p < 0.01$).

3.6. Internal carbon and nitrogen contents

The biomass content of C remained constant throughout the experiment ($F_{2,4} = 1.94$, $p = 0.257$) and between nutrient treatments ($F_{1,2} = 3.24$, $p = 0.214$) (Fig. 7a). The interaction term (nutrients and time) was not significant (not shown). N content was significantly higher in HNS than in LNS algae and C:N significantly lower in HNS than in LNS, in addition N content decreased and the C:N ratio increased at day 5 in LNS algae (SNK test lower case letters in Fig. 7b and c). The interaction term (nutrients and time) was thus significant in both cases ($F_{2,4} = 8.60$, $p < 0.05$ [for N content] and $F_{2,4} = 10.28$, $p < 0.05$ [for C:N ratio]).

3.7. Biomass yield

Biomass yield was slightly higher in LNS compared to HNS algae. However, due to the higher N content, biomass N yield was 2.45 higher in HNS than in LNS algae (Table 1).

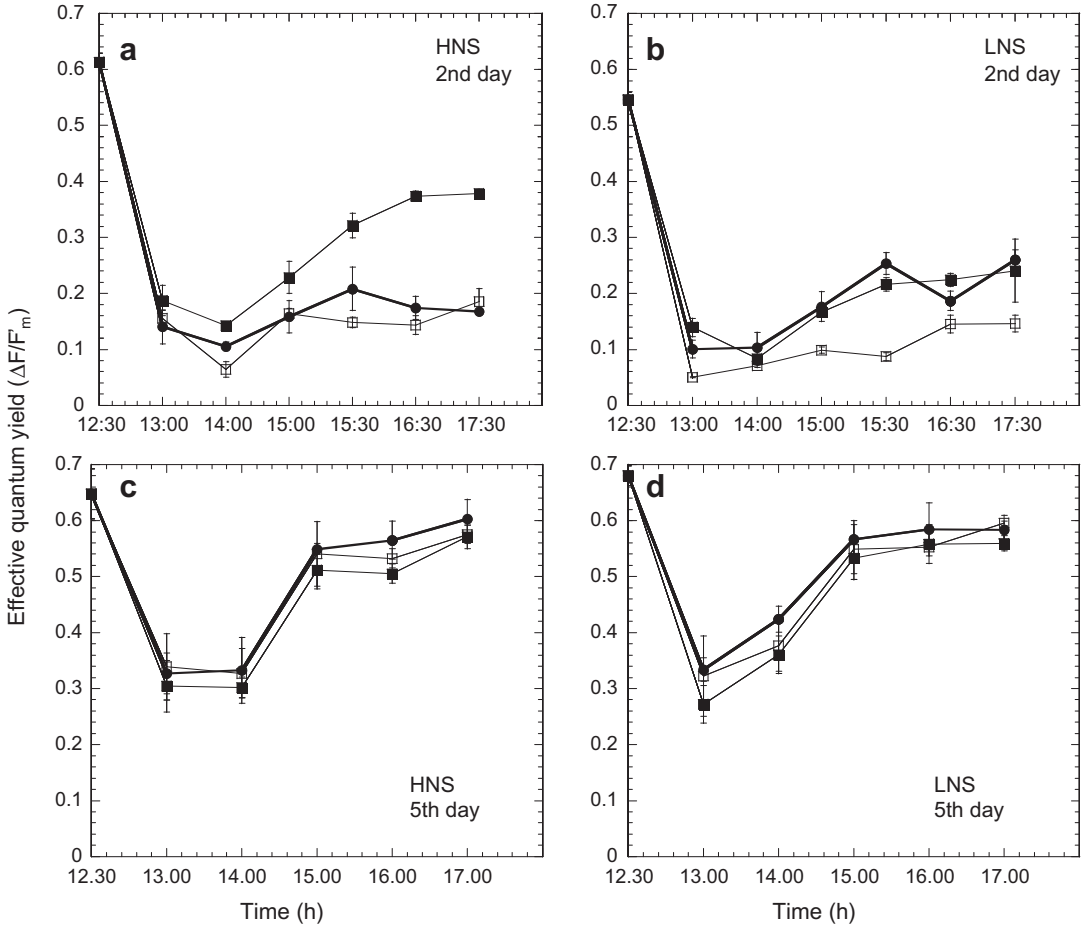

Fig. 2. Time courses of effective quantum yield ($\Delta F/F'_m$) at 11:00 h local time in *Gracilaria conferta*. High nutrient supply (HNS) algae (a and c) and low nutrient supply (LNS) algae (b and d) were exposed on April 2nd and 5th to solar radiation in aluminum vessels in midday (12:30–14:00 h local time) and then allowed to recover in the shade (14:00–17:30 h local time). Exposure treatments where PAR (closed circles), PAR + UVA (open squares) and PAR + UVA + UVB (closed squares). Error bars represent ±1 SD.

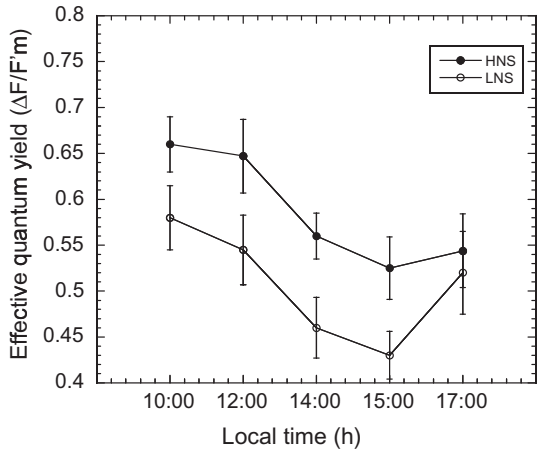

Fig. 3. Time courses of effective quantum yield (10:00–17:00 h local time) on April 5th in *Gracilaria conferta*, grown in the culture tanks (600 l) under high nutrient supply (HNS, closed circles) and low nutrient supply (LNS, open circles). Error bars represent ±1 SD.

4. Discussion

G. conferta responded to a HNS from fishpond effluents with higher content of phycobiliproteins and higher photosynthetic activity. HNS reduced the stress provoked by combinations of short exposure to high solar PAR and UVR and heat shocks. Our findings are important because macroalgae have the capacity to biofilter wastewaters from fishponds using IMTA systems, being it an effective manner to reduce the eutrophication of the coastal areas causes by traditional aquaculture, as they can eliminate effectively the dissolved inorganic nutrients. Additionally, these findings suggest that this red macroalga grown in water enriched with fishpond effluents, considering integrated multi-trophic aquaculture (IMTA) principles, can resist and get acclimated to stress conditions typical of climate change scenarios such as the increase of the frequency of days with high temperatures, and current increased UVB radiation due to the depletion of the ozone layer.

The positive effect of nutrient supply was observed in two different experiments: (1) algae growing under solar radiation in nutrient-replete and nutrient-deficient tanks, and (2) algae transferred from these tanks to small reflective vessels, where they experienced for several hours increased solar PAR and UVR.

4.1. Algae growing in the tanks

The effective quantum yield in *G. conferta* slightly decreased at noon as a consequence of the increased solar irradiance, the effective quantum yield being smaller in nutrient-deficient algae. The small decrease is probably a result of the low average light levels, due to the high algal density. Photoinhibition was observed only in the nutrient-deficient algae, where the competition for N led to low pigment content (Lapointe and Duke, 1984). Thus, algal

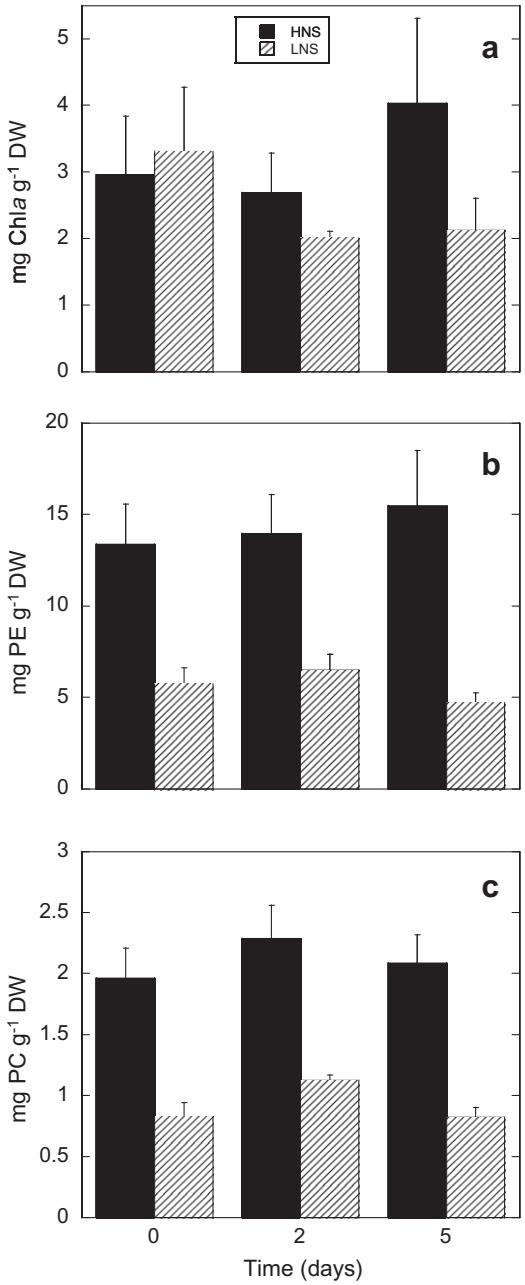

Fig. 4. Concentration of chlorophyll *a* (Chl*a*) (a), phycoerythrin (PE) (b) and phycocyanin (PC) (c) as mg g^{-1} DW at initial time, and after 2 and 5 days in *Gracilaria conferta*, grown under high (HNS, filled bars) and low (LNS, dashed bars) nutrient supply. Error bars represent ± 1 SD.

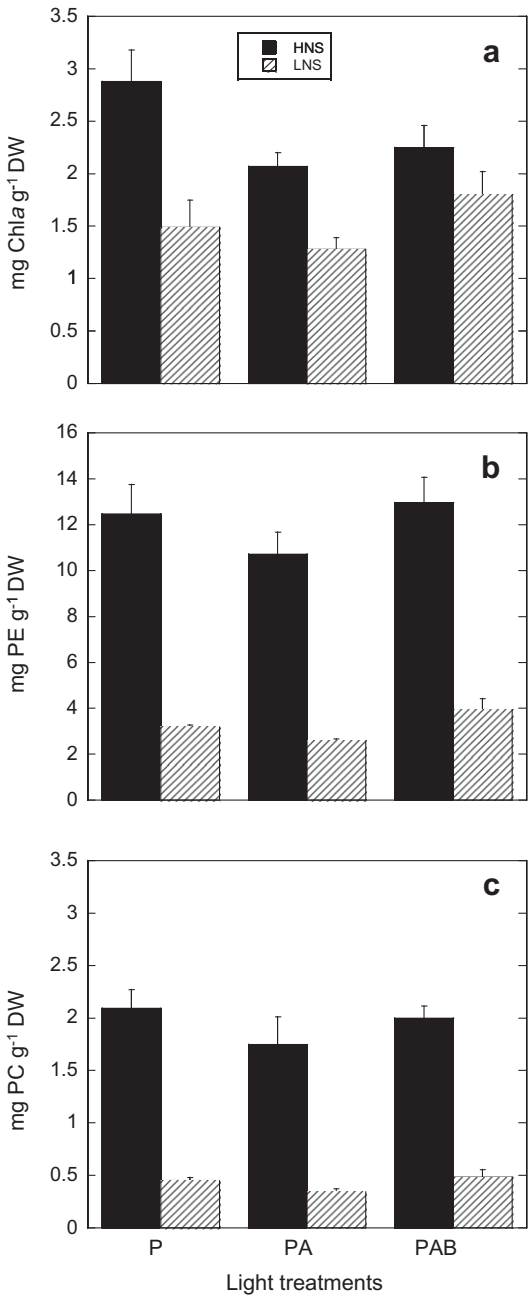

Fig. 5. Concentration of chlorophyll *a* (Chl*a*) (a), phycoerythrin (PE) (b) and phycocyanin (PC) (c) as mg g^{-1} DW, after exposure time (12:30–14:00 h local time) and recovery time (14:00–17:30 h local time) at April 5th under different light treatments (PAR, PAR + UVA, PAR + UVA + UVB) in *Gracilaria conferta*, grown previously under high (HNS, filled bars) and low (LNS, dashed bars) nutrient supply. Error bars represent ±1 SD.

photoinhibition in tanks depends also on algal density, which influences the light penetration in the tank. Mata et al. (2007) and Figueroa et al. (2008) reported that *A. armata* cultures were resistant to high irradiance, with dynamic photoinhibition (decrease of F_v/F_m) produced at noon hours only when algae are cultivated at very low densities (1.5 g FW l^{-1}) and no photoinhibition was observed at higher densities than 4 g FW l^{-1}. In other algal cultures daily photoinhibition fluctuations were observed due to high UVR penetration in the tanks (Cabello-Pasini et al., 2000; Aguirre-von Wobeser et al., 2000). Daily photoinhibition fluctuations in *Macrocystis pyrifera*, *Chondrus crispus* and *Ulva rigida* in out-door tanks with increased irradiance (Cabello-Pasini et al., 2000) were consistent with daily photoinhibition cycles observed by other species in the field (Häder and Figueroa, 1997). Maximum quantum yield and pigment content decreased drastically in *G. cornea* transferred from in-door to out-door conditions (Figueroa et al., 2006). The low photoinhibition and rapid recovery of photosynthesis of tank cultivated *A. armata* (Mata et al., 2007) and *G. conferta* in this study, could be explained by the protection conferred by N-replete conditions (Figueroa et al., 2008).

The biomass yield in the nutrient-deficient tanks did not drop, because the experiment was too short for the algae to become physiologically N-limited. During nutrient deficiency, formerly nutrient-repleted *Gracilaria* used stored N much longer (Lapointe and Duke, 1984). Similar to biomass yield, biofiltration capacity, expressed as N yield, was much lower in N-repleted *G. conferta*

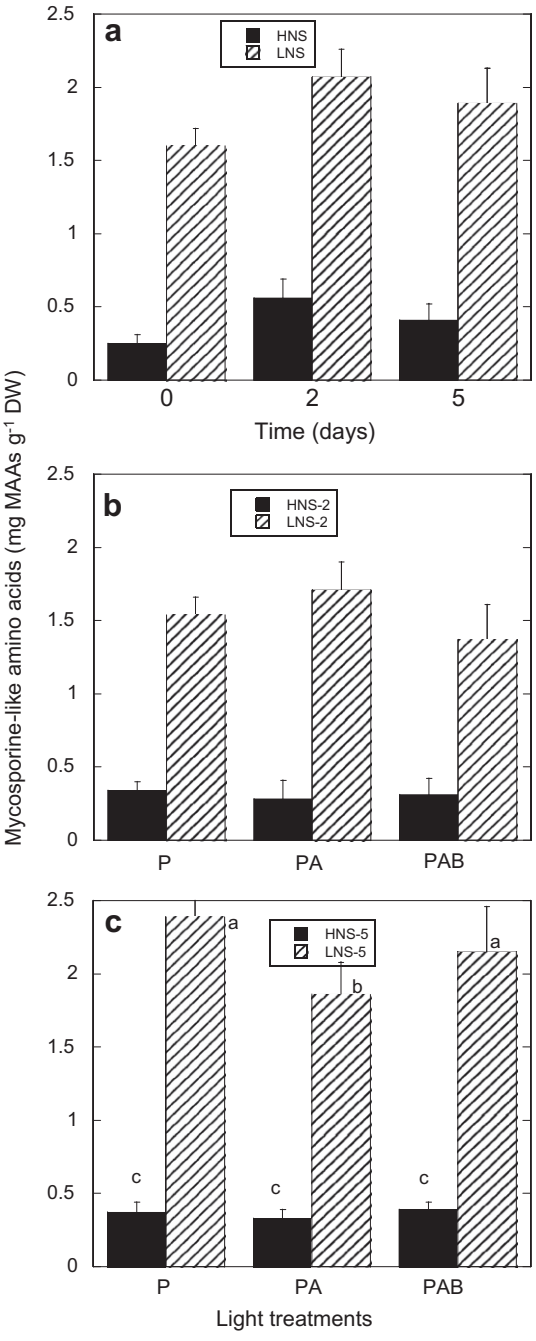

Fig. 6. Concentration of mycosporine-like amino acids (MAAs) in *Gracilaria conferta*, grown in tanks with high (HNS, filled bars) and low (LNS, dashed bars) nutrient supply. (a) algae taken straight from the tanks on the designated days of the experiment; (b) 2nd day, April 2nd, after exposure to different light treatments (PAR, PAR + UVA, PAR + UVA + UVB) and recovery in the shade, as described in Methods; (c) as in (b) but on the 5thday, April 5th. Error bars represent ±1 SD. Significantly different means (SNK test) were denoted by lower case letters in Fig. 6(c).

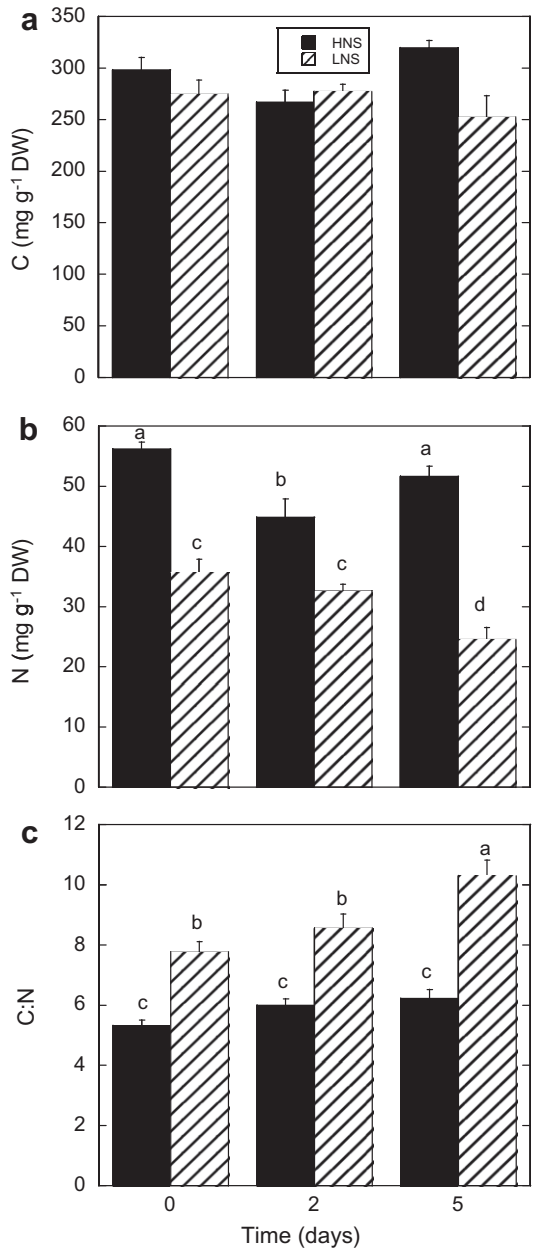

Fig. 7. Total proximate biomass content (mg g^{-1} DW) of carbon (a) and nitrogen (b), and C:N atomic ratio (c) in HNS (filled bars) and LNS (dashed bars) grown *Gracilaria conferta*, on the designated days of the experiments as in Fig. 6 (a). Error bars represent ±1 SD. Significantly different means (SNK test) were denoted by lower case letters in Fig. 7(b) and (c).

than in similarly grown *U. lactuca* (Cohen and Neori, 1991; Figueroa et al., 2009), and *U. rigida* (Jiménez del Río et al., 1996; Mata and Santos, 2003). Fishpond effluents supply algae with inorganic N and C for their photosynthesis (Mata et al., 2007). In fact, the biomass chemistry data suggest no DIC deficiency developed under any of the experiments.

The low average irradiances in the tanks stimulated the photoadaptive accumulation of phycobiliproteins, particularly in combination with the nutrient-replete tanks. Under N-replete condition, red macroalgae have been shown to incorporate much N into pigments (Figueroa et al., 1995; Figueroa et al., 2008). Sufficiency of N and other nutrients increases rate of production of the light harvesting complexes (LHC) and phycobilisomes with their associated pigments, increase production of protective substances and accelerates biochemical recovery of damaged structures. In this way it protects not only photosynthetic performance but also light sensitivity of algae (Geider et al., 1993). The increase in photosynthetic capacity, expressed as ETR, was related to the increase of photosynthetic pigments. Algae can acclimate to different light conditions by changing their photosynthetic apparatus to optimize light absorption and minimize damage to this system by different mechanisms (Henley and Ramus, 1989; Falkowski and Raven, 1997; Häder and Figueroa, 1997). Among these mechanisms, the photocontrol and nutrient control of pigment synthesis have

Table 1
Biomass yield (g DW m^{-2} d^{-1}) and biomass N yield (g N m^{-2} d^{-1}) in the last week of cultivation in high and low nutrient supply (HNS and LNS) algae, compared to data on other seaweeds cultivated in fishpond effluents. Listed are tank volumes (l), algal stocking density (g FW l^{-1}) and mean water temperature (°C). The tank walls were not opaque but with 70% light transmission in Schuenhoff et al. (2006) and in Pinchetti (personal communication).

Species/conditions	Tank volume (l)	Stocking density (g FW^{-1})	Mean water temperature (°C)	Biomass yield (g DW m^{-2} d^{-1})	N-yield (g m^{-2} d^{-1})	References
Gracilaria conferta (HNS)	600	4.8	22	15.7	0.81	This study
Gracilaria conferta (LNS)	600	4.8	22	18.6	0.46	This study
Gracilaria cornea	750	8.0	23	25.9	0.68	Pinchetti (pers. comm.)
Ulva lactuca (HNS)	600	1.0	22	46.5	1.35	Figueroa et al. (2009)
Ulva lactuca (LNS)	600	1.0	22	46.3	0.55	Figueroa et al. (2009)
Ulva lactuca	600	1.7	20	55.0	2.30	Cohen and Neori (1991)
Ulva rigida	750	2.5	24	40.0	1.80	Jiménez del Río et al. (1996)
Ulva rigida	1900	2.0	22	48.0	1.45	Mata and Santos (2003)
Asparagopsis armata	110	5.0	13	43.0	2.70	Schuenhoff et al. (2006)
	110	5.0	22	120.0	5.90	

received most attention (López-Figueroa and Niell, 1990; Talarico and Maranzana, 2000). Different photoreceptor systems control chlorophyll and biliprotein accumulation (Rüdiger and López-Figueroa, 1992) and the photoregulation of pigment synthesis is linked to the photoregulation of N metabolism, i.e., stimulation of nitrate reductase and glutamine synthetase activities (López-Figueroa and Rüdiger, 1991; Figueroa, 1996). Biliproteins, as N-compounds, have been proposed as N storage in cyanobacteria and red algae (Boussiba and Richmond, 1980; Talarico and Maranzana, 2000). The increase in internal N-compounds was confirmed by the total internal N content and an C:N ratio close to the classical Redfield ratio of 6.6 (Redfield et al., 1963). The internal N content in this alga was much higher than that in algae growing in the coastal waters whereas C content was not so different (Enríquez et al., 1995). According to the review on C:N among marine algae by Duarte (1992), *G. conferta* presents one of the highest internal N content among the algae analyzed. In the N-deficient algae, C:N exceeded the classical Redfield ratio of 6.6 due to low N content. In conclusion, N in the N-repleted algae was accumulated in phycobiliproteins and it produced a positive effect for photosynthesis.

Surprisingly the accumulation of other N-compound i.e., MAAs (UV-screen photoprotector) was inversely related to N supply. MAA content increased in different *Porphyra* species and *Grateloupia lanceola* when algae were cultivated in laboratory under enriched ammonium seawater (300 μM NH_4^+) in close culture chambers and under artificial lamps (Korbee-Peinado et al., 2004; Korbee et al., 2005a; Huovinen et al., 2006). The large MAA accumulation in the N-deficient algae probably resulted from the high average levels of PAR and UVR they experienced in the tanks, as the light absorptance of LNS grown algae was lower, and thus the need for photoprotection higher. Similar MAA stimulation by increased irradiance has been shown when algae were transferred from subtidal to uppertidal environments and higher MAA content were observed in algae in summer than that in winter (Karsten et al., 1998). By other hand, it could be also possible that growth was competing with MAA synthesis for nutrients in HNS algae, as in *A. armata* grown with fishpond effluents (Figueroa et al., 2008), resulting in a lower MAA accumulation. In fact, in the red alga *A. armata* exposed to solar radiation under open tank systems and different N fluxes, MAA content increased up to a certain level of ammonium enrichment, and then decreased (Figueroa et al. 2008).

4.2. Short-term exposure to increased irradiance (under PAR, PAR + UVA, PAR + UVA + UVB)

Effective quantum yield ($\Delta F/F'_m$) decreased in both species drastically after a short exposure to high solar irradiance and consequently to a heat shock. The decrease was not related to the UVR. However, the extent of recovery seemed to depend on light quality but also on vessels water temperature i.e., the highest recovery was produced in presence of UVB (PAR + UVA + UVB treatment) on April the 2nd, when the water temperature of the aluminum vessels was 2–4 °C higher than on 5th April. Replete N supply assisted the recovery of *G. conferta* at first, but in algae cultivated for 5 days the recovery was faster in the N-depleted algae, possibly due to the MAAs accumulated in them.

Nutrient limitation has been associated with increased vulnerability to photoinhibition by PAR and UVR in macroalgae (Döhler et al., 1995; Korbee-Peinado et al., 2004; Korbee et al., 2005a, 2005b; Van de Poll et al., 2005). On the other hand, N supply exerts a positive effect on photosynthesis and pigmentation and it is advantageous against both stress factors, high radiation and temperature. Moreover, photosynthesis and respiration can be also affected by temperature and UVR (Lobban et al., 1985) by the extent of oxidative damage as it has been reported in other algae (Lesser, 1996; Aguilera et al., 2002). In presence of UVB radiation, respiration is stimulated in several algae and cyanobacteria reducing the net photosynthetic rate and C store (Beardall et al., 1997; Aguilera et al., 1999). Thus, a complex interaction between light quality, temperature and nutrients on recovery process might be produced. Several species have been shown to growth effectively at high temperatures around 25 or 30 °C under open tanks (Lapointe et al., 1984; Hanisak, 1987) or laboratory conditions (Raikar et al., 2001). However, these are the temperatures found in their natural habitats at which they are acclimated. In our study, an increase of water temperature in the aluminum vessels was found i.e., heat shock and it is known that photosynthesis is heat sensitive, with the primary site of thermal damage considered to be associated with PSII (Berry and Björkman, 1980; Rokka et al., 2000), the repair of which can be impeded by oxidative stress (Nishiyama et al., 2001). Small increases of temperature could damage photosynthesis but also other physiological proccesses. For example, *Macrocystis* spp. submitted to ambient temperatures, ambient −4 °C and ambient +4 °C, showed that high water temperatures (the latest treatment) provoked rapid degradation of *Macrocystis* rafts (Rothausler et al., 2009).

UVB radiation reversed the negative effect of UVA radiation on photosynthesis under additional stress (higher heat shocks), mainly under N-repleted algae. The involvement of UVB in the protection against UVR and repair of UVR damage has been already reported in the Phaeophyta *Dictyota dichotoma* (Flores-Moya et al., 1999), in the Chlorophyta *U. pertusa* (Han and Han, 2005) and in the marine angiosperm *Posidonia oceanica* (Figueroa et al., 2002). Flores-Moya et al. (1999) suggested that UVA radiation exerts the

main role in the photoinhibition while UVB may be involved both in the impairment and the recovery of photosynthesis. Hanelt et al. (2006) showed that photosynthesis of some aquatic plants from New Zealand lakes showed increased inhibition when UVB was filtered out of the simulated sun spectrum, indicating a supporting effect of short UVR wavelengths range against photoinhibition. Our data confirm that UVB does not only cause damage on photosynthesis, but also supports recovery processes in algae adapted to high UVR environments in contrast to habitats with lower natural UVR exposure as the Polar Regions or North Sea (Bischof et al., 2006). The mechanism involved in protection and repair by UVB radiation is still unknown, but it could be related to the induction of the accumulation of UV-screen substances (mycosporine-like amino acids and phenols) as it has been shown in other macroalgae (Karsten et al., 1998; Han and Han, 2005).

N-depleted *G. conferta,* in spite of the presence of MAAs, suffered photoinhibition after short exposure to UVR as in other species analyzed of this genus, *G. chilensis* (Gómez et al., 2005) and *G. cornea*, when the photosynthetic capacity drastically decreased after algae were transferred from in-door to out-door growing conditions (Figueroa et al., 2006). A high content of MAAs was not sufficient to protect photosynthesis against UVR in contrast to other red algae species such as *Porphyra* (Korbee et al., 2005a, b). The concentration of MAAs in *G. conferta* was about 1.8 mg g^{-1} DW under LNS and 0.4 mg g^{-1} DW under HNS, in both cases the composition of MAAs was the same (about 95% shinorine and 5% porphyra-334). The concentration under LNS was similar to that of *G. chilensis* growing under natural environment i.e., 1.75 mg g^{-1} DW but with other composition, 70% porphyra-334, 20% shinorine and the rest asterina-330 and palythine (Huovinen et al., 2004; Gómez et al., 2005). In *Gracilaria* species MAA content is much lower than in other red macroalgae with lower photoinhibition due to UVR i.e., *Porphyra* (5–10 mg g^{-1} DW) species (Korbee et al., 2005a, 2005b). MAAs present antioxidant capacity, as mycosporine-gly or usujirene (Dunlap and Yamamoto, 1995; Nakayama et al., 1999), porphyra-334, asterina-330 or shinorine (De la Coba et al., 2009). The 95% of all MAAs in *G. conferta* was shinorine, the MAA with the smallest antioxidant activity according to results shown by De la Coba et al. (2009). This fact could explain the low photoprotection capacity of MAAs in *G. conferta* compared to other Rhodophytes containing high active antioxidant MAAs as *Gelidium corneum* (asterina-330) and *Porphyra rosengurttii* (porphyra-334) or the marine lichen *Lichina pygmaea* (mycosporine-gly) (De la Coba et al., 2009).

In summary, N supply exerted a positive effect on photosynthesis against the stress factors of increased irradiance and temperature. N-repletion stimulates the accumulation of phycobiliproteins. UVB reversed the negative effect of UVA on photosynthesis and pigment accumulation under additional stress (higher heat shock), particularly in N-repleted algae. Therefore, these algae present a stronger tolerance to environmental changes, i.e., short-term increases in irradiance and temperature, when compared to N-deficient algae. The LNS treatment showed nutrient concentrations similar to those found in oligotrophic waters of the Red Sea (Klinker et al., 1978). It is expected that sudden or short-term increases in temperature, UVR and increased number of eutrophication processes, predicted during a climate change scenario, may damage algae species in the Red Sea, influencing the structure of algal communities (Bischof et al., 2006). A possible concurrent increase in the nutrient supply, usually considered negative to the coral reef (Lapointe, 1997), may help some algal species to resist the environmental stress. Under these conditions, algae species growing close to fish cages in an integrated multi-trophic marine system, such as *Gracilaria* spp. (Troell et al., 1997), might present a better tolerance to increased stress conditions (i.e., UVR and heat shocks) compared to algae growing under such conditions in oligotrophic waters.

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