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Effects of nutrient supply on photosynthesis and pigmentation in *Ulva lactuca* (Chlorophyta): responses to short-term stress

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ABSTRACT: The effects of nutrient supply on photosynthesis (estimated as chlorophyll fluorescence), chlorophyll content, biomass yield and proximate chemical composition of tank cultivated *Ulva lactuca* L. (Chlorophyta) were evaluated. To assess the effect of nutrient supply on resistance capacity against short-term stress, algae grown in high nutrient supply (HNS) fishpond effluents and in low nutrient supply (LNS) oligotrophic seawater were transferred to small vessels with increased irradiance of PAR and UV radiation (PAR+UVA and PAR+UVA+UVB using cut-off filters) and increased temperature as compared to culture tanks. Electron transport rate and chlorophyll content were higher in HNS than in LNS algae. Effective quantum yield and chlorophyll content decreased after short-term exposure to high PAR irradiance. Full recovery of photosynthesis in the shade was observed under a moderately higher temperature (Δ+6°C). UVB exposure reduced the negative effect of UVA on photosynthesis and pigment accumulation under temperature stress (Δ+10°C), particularly in HNS algae. Growth under HNS appeared to accelerate acclimation of *Ulva lactuca* to short-term environmental changes, such as higher temperatures (as in heat waves) and higher UV radiation. Furthermore, nitrogen enrichment reduced the common inhibitory effects of short-term stress such as increased irradiance, UV radiation and temperature on photosynthesis.

KEY WORDS: Carbon and nitrogen content · Chlorophyll fluorescence · Nutrient supply · *Ulva lactuca* · Photosynthetic pigments · Seaweed culture

INTRODUCTION

Biofiltration of fishpond effluents by marine macroalgae (seaweeds) improves the sustainability of the coastal marine aquaculture industry (Neori et al. 2003, Neori et al. 2007). Several seaweeds, especially *Ulva* spp., can effectively remove dissolved nutrients from fishpond effluents (Jiménez del Río et al. 1996, Neori et al. 2003). This capacity is the foundation of the integrated multi-trophic aquaculture (IMTA) system, where seaweed culture balances the environmental impact of fish and shrimp culture. Seaweeds produced in IMTA systems can have many economic values (Guiry & Blunden 1991, Neori et al. 2007). They provide a good source of proteins, carbohydrates and bioactive compounds of commercial interest (Figueroa et al. 2008).

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High UV radiation (UVR; due to depletion of the earth's ozone layer) and high temperature (heat waves and climate change) can reduce the performance of seaweeds in biofilters. The light field, temperature and nitrogen supply can affect the photosynthetic activity of tank cultivated seaweeds (Figueroa et al. 2006). Chlorophyll fluorescence, i.e. effective quantum yield ($\Delta F/F'_m$) and electron transport rate (ETR) measured by pulse amplitude modulated (PAM) *in vivo* chlorophyll fluorometry, can be used reliably to estimate seaweed photosynthetic performance (Figueroa et al. 2003). PAM fluorometry data have been reported for micro- and macroalgae from natural and culture aquatic systems (Figueroa et al. 2003 and references therein), including those for *Ulva* spp. grown in effluents from seabream (*Sparus aurata*) fishponds (Figueroa et al. 2006). The PAM fluorometry quantum yield can be used, in real time and with sensitivity, to monitor physiological stress of tank grown algae under outdoor conditions (Cabello-Pasini et al. 2000, Figueroa et al. 2006) or under semi-extensive culture systems such as those for *Gracilaria chilensis* in estuarine systems (Gómez et al. 2005). Chlorophyll fluorescence can therefore be used as an indicator of algal physiological status or as a functional indicator of algal capacity to respond to stress events. On the other hand, algal pigment content and carbon:nitrogen (C:N) ratios can be analyzed to evaluate any possible damage to internal compounds that may be caused by stressors such as high irradiance, UVR and high temperature.

This study utilizes PAM fluorometry quantum yield to examine the effects of nutrient supply on photosynthesis, accumulation of internal compounds (photosynthetic pigments and C and N contents) and biomass yields of tank cultivated green macroalga *Ulva lactuca* L. (Chlorophyta). The relationship between nutrient supply (i.e. high nutrient supply, HNS, versus low nutrient supply, LNS) and algal resistance to short-term environmental stress was evaluated. *U. lactuca* previously grown under HNS or LNS were transferred to small vessels and subjected to solar irradiance and temperature that were higher than those of the culture tanks. Furthermore, specific effects of UVR, including full solar radiation (PAR+UVA+UVB), PAR+UVA and only PAR were also assessed. We hypothesize that algae grown under HNS should have a higher capacity to acclimate to short-term stress than algae grown under LNS conditions. Acclimation to stress conditions by algae grown under nutrient enrichment can be indicated not only by high photosynthetic rates but also by high accumulation of photoprotectors and repair enzymes, as well as by an increase in antioxidant activities (Litchman et al. 2002, Korbee Peinado et al. 2004, van de Poll et al. 2005, Bischof et al. 2006).

MATERIALS AND METHODS

Cultivation conditions and experimental design. *Ulva lactuca* was cultivated for 2 wk in square opaque outdoor tanks (1 m^2 surface area) containing ~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 & Neori 1991). Two nutrient conditions were applied in duplicates: (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 being slowly dripped in at a rate of 0.1 m^3 $tank^{-1}$ d^{-1} (see Table 1). Algal density at the beginning of the experiment was 1.67 g FW (fresh weight) l^{-1}. Light penetration through the seawater in the tanks was low, i.e. the irradiance was 70 and 35% of the incident surface light (E_o) at 5 and 10 cm depth respectively. Following 2 wk of cultivation under the conditions described above, only pristine Red Sea water (i.e. no dripping of fishpond effluent) was added to the LNS tanks, whereas no change was made in conditions for the HNS tanks. On Day 2 (2 April 2008) and Day 5 (5 April 2008) under these new conditions, algal samples from each of the 4 tanks were taken out and incubated for 1.5 h during midday (12:30–14:00 h) in 20 × 30 × 5 cm aluminum vessels filled with seawater under 3 different light qualities. These light conditions were defined by using cut-off foils as filters as described by Figueroa et al. (1997) and Villafañe et al. (2003) as follows: (1) PAB (PAR+UVA+UVB), under an Ultraphan 295 foil (Digefra), (2) PA (PAR+UVA), under a Folex 320 foil (Folex), and (3) P (PAR), under an Ultraphan 395 foil (Digefra). Due to the spectral characteristics of these filters, and given the different known accepted ranges of UVA and UVB radiation, the irradiances present according to the Commission Internationale de L'Eclairage (CIE) ranges would be UVB (280–315 nm) and UVA (315–400 nm) and in addition, other ranges used by photobiologists, i.e. UVB (280–320 nm) and UVA (320–400 nm) are presented.

Chlorophyll PAM fluorescence of the algal samples in the vessels was determined *in situ* at 12:30, 13:00 and 14:00 h (local time). After the exposure period, the vessels were transferred to the shade (20 to 25% of full sunlight) and fluorescence was again determined at 15:00, 15:30, 16:30 and 17:30 h on 2 April (Day 2) and at 15:00, 16:00 and 17:00 h on 5 April (Day 5). To serve as controls, chlorophyll fluorescence of the algal samples left in the original square opaque outdoor tanks was also determined at the same time intervals in both days.

Light and temperature measurements. Irradiance reaching the water surface of the tanks and the foil-covered aluminum vessels in the PAR, UVA and UVB wavelengths was recorded on each day (2 and 5 April)

at 10:00, 11:00, 12:30, 16:00, 17:00 and 18:00 h using a multiphotodiode spectroradiometer (SMS-500, Sphere Optics; 5 nm half bandwidth). Each value was the average of 3 measurements at 4 s intervals. Air and water temperatures in the tanks and in the aluminum vessels were measured several times throughout the 2 d using a mercury thermometer.

Nutrients. Water samples for total ammonia-N (TAN), nitrite-N, nitrate-N and orthophosphate-P were taken from each tank on 3 separate days (1, 5 and 6 April) during the experiment. Nutrient samples were also taken from coastal waters of the Red Sea. Samples (8 ml) passing through 0.45 µm disposable filters (Supatop Syringe Filter CA 0.45µm, 33 mm; Anachem) were collected and immediately frozen at −20°C. Nutrient analysis was done using a semi-continuous flow autoanalyzer (SAN ++, SKALAR, Breda) following standard protocols (Grasshoff et al. 1983).

Photosynthesis as chlorophyll fluorescence. *In vivo* PAM chlorophyll fluorescence was recorded each time from 8 algal samples vessel^{-1} using 2 cross-calibrated Diving-PAMs (Waltz) to reduce the time needed to complete the measurements. $\Delta F/F'_m$ was calculated according to Schreiber et al. (1995).

Rapid light curves (RLC: ETR versus irradiance) were measured with a PAM-2000 fluorometer using red light emitting diodes immediately after removal of the seaweeds from the tanks (i.e. before beginning the experimental exposure at 12:30 h local time), and after a dark adaptation period of 10 min. Each RLC was performed with a 15 s exposure to each of the following eleven irradiances: 11, 18, 28, 37, 52, 72, 104, 149, 218, 318 and 483 µmol photons m^{-2} s^{-1}.

ETRs were then calculated by multiplying the $\Delta F/F'_m$ by the incident solar radiation (E), the absorptance (A) and the fraction of chl *a* in PSII that is associated with light harvesting complex II, LHCII (F_{II}). The latter is related to the quanta absorbed by PSII (400–700 nm) (Schreiber et al. 1995). The F_{II} value used was 0.5 for Chlorophyta (Grzymski et al. 1997).

$$ETR = \Delta F/F'_m \times E \times A \times F_{II} \text{ (µmol electrons m}^{-2}\text{ s}^{-1}\text{)}$$

The absorptance in the PAR region of the solar radiation spectra was determined from light transmission through algal pieces placed on a cosine-corrected sensor that was connected to the multiphotodiode spectroradiometer (Beer et al. 2000).

Chlorophyll content. Seaweed samples (0.02–0.05 g FW) for measurements of photosynthetic pigments were taken in duplicate from each tank at the onset of the experiment, and after Days 2 and 5. On 5 April (Day 5), such samples were also taken after the short-term exposure experiment (at 14:00 h) and after recovery in the shade (at 17:30 h). All seaweed samples for pigments were stored at −70°C for several weeks. Chl *a* and *b* concentrations were determined in duplicates, after extraction in 3 ml of N,N-dimethylformamide at 4°C for 24 h in the dark (Moran 1982).

Biomass carbon and nitrogen content. Duplicate seaweed samples for proximate chemical analysis were taken from each tank at the onset of the experiment (after the 2-wk cultivation period), and after Days 2 and 5. Samples were frozen, freeze-dried and kept desiccated until analyses. Total internal C and N contents on a dry weight (DW) basis were determined using an element analyzer (CNHS LECO-932).

Biomass yield. Biomass was determined a day before and on the last day of the week of the experiment. The entire biomass of each tank was drained into mesh bags (0.1 mm mesh) and spun for a full cycle at 600 rpm in a household spinner. Biomass yield (DW) was determined by weighing subsamples of the harvested algae, drying them for 48 h at 60°C and reweighing them after cooling down in a silica desiccator. The average DW/FW ratio was 0.22. N yield as an indicator of the biofiltration capacity for inorganic N was calculated as the product of biomass yield and total internal N content.

Statistics. Hierarchical ANOVA was applied to test for differences in chlorophylls, C and N contents and C/N ratio in the algal fronds from the 2 nutrient treatments (factor 1: nutrient, and factor 2: time). Minor variations in conditions between tanks may, to some extent, affect the pigment composition and C and N contents of the algae. To test for this effect, 2 tanks per nutrient treatment were set (see 'Cultivation conditions and experimental design'); thus, an additional third factor (tank, nested in nutrient) was included in the ANOVA.

Orthogonal ANOVAs were used to evaluate statistical differences in photosynthetic pigment contents (chl *a* and *b*) of the algae in the short-term exposure experiments. The effects of light quality (P, PA, and PAB) during the exposure period and of the previous growing conditions, i.e. nutrient status of the tanks (HNS versus LNS) were tested.

Cochran's test was used to assess heterogeneity of the variances (Underwood 1997) and Student-Newman-Keuls (SNK) tests were used to discriminate the different treatments after significant *F*-tests (Underwood 1997). All tests were carried out using SPSS 11.0.1 for Windows.

RESULTS

Light and temperature

Solar irradiance of UVA and UVB, expressed in the CIE range, varied slightly between 2 and 5 April 2008

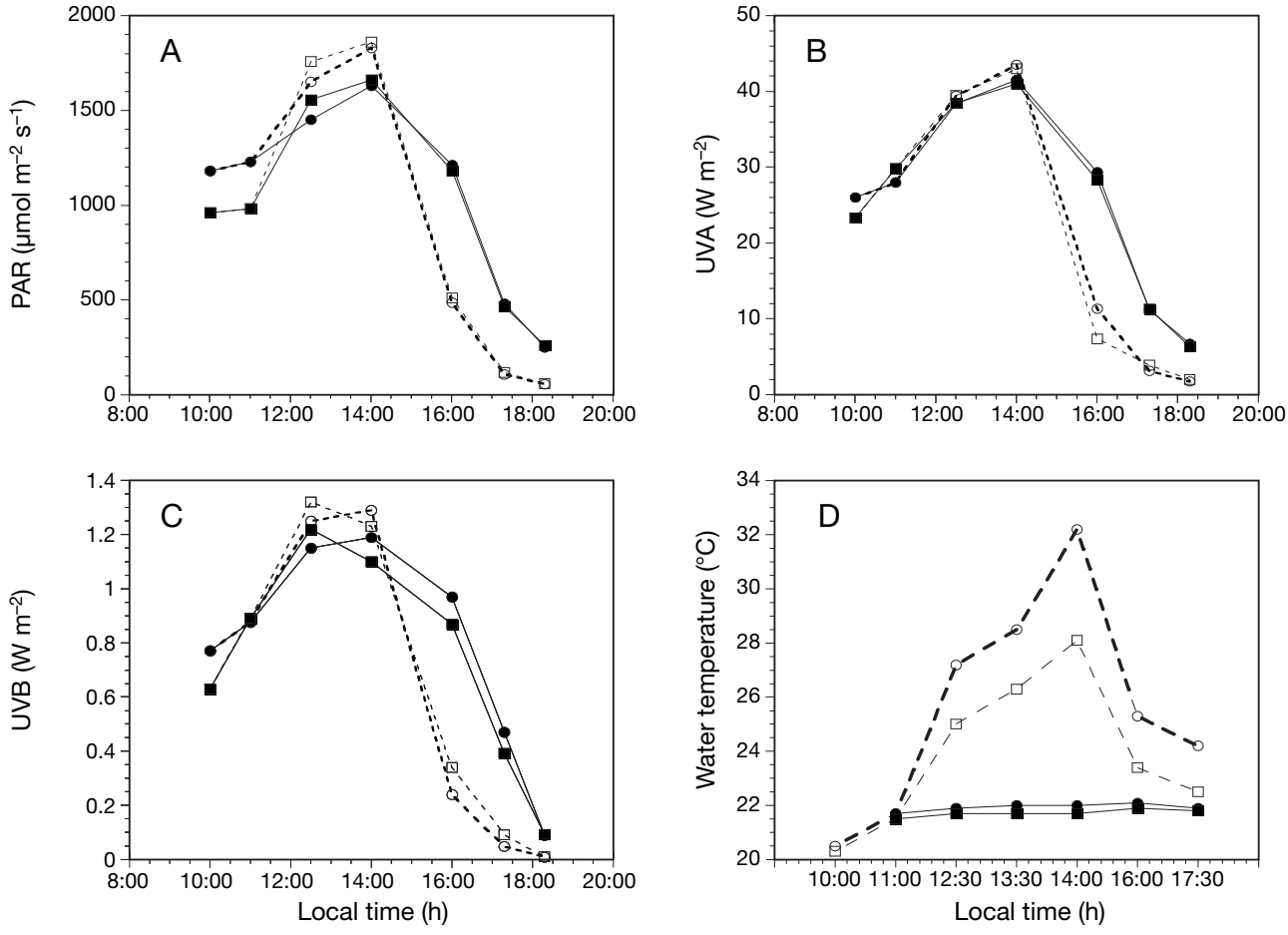

Fig. 1. Diurnal values of (A) photosynthetically active radiation (PAR, 400–700 nm), (B) UVA (315–400 nm) and (C) UVB (280–315 nm) measured in air, and (D) surface water temperature (°C) of the culture tanks (closed symbols) and the aluminum vessels (open symbols) used for the short-term exposure–recovery treatments on Day 2 (2 April 2008) (circles) and Day 5 (5 April 2008) (squares) of the experiment

(Fig. 1). The maximum solar irradiance of PAR at around 14:00 h during both dates was ~1700 µmol m^{-2} s^{-1} (Fig. 1a). UVA irradiance based on the CIE range of 315–400 nm was 43 W m^{-2} (Fig. 1b), or 42 W m^{-2} using the range of 320–400 nm. Similarly, UVB irradiance based on the CIE range of 280–315 nm was 1.29 W m^{-2} (Fig. 1c) or 2.19 W m^{-2} using the range of 280–320 nm. Irradiances above the highly reflective aluminum vessels during the 2 short exposure periods to solar radiation were 14, 5 and 8% higher for PAR, UVA and UVB respectively, than at the surface of the tanks (Fig. 1a–c). Under the PAB treatment, UVB irradiance was 172% higher in the range of 280–320 nm than in the CIE range, whereas UVA irradiance was 2% less in the range of 320–400 nm compared to that at 315–400 nm (CIE). Under the PA treatment, UVB at 280–320 nm was 45% higher than that in the CIE range, whereas UVA in the range of 320–400 nm was only 0.5% less than that in the CIE range.

Irradiance during the recovery period (shade conditions) was approximately 21 (PAR), 33 (UVA) and 10–40% (UVB) of that of full sunlight. Water temperature in the tanks was slightly higher on 2 April than on 5 April (Fig. 1d). The difference in water temperature between the aluminum vessels and the seaweed tanks during the exposure was 5–10°C on 2 April and 3–6.5°C on 5 April (Fig. 1d).

Nutrients

Nitrate, nitrite, and phosphate concentrations were not significantly different either between days or between the 2 tanks within the same nutrient treatment. Hence, nutrient data were pooled to calculate their mean levels (Table 1). Similarly, TAN values in the LNS tanks were also stable during the experiment (Table 1). In HNS tanks, however, TAN levels fluctuated as

Table 1. Mean (±SE) nutrient concentrations (µmol l^{-1}) in high (HNS) and low nutrient supply (LNS) treatments. Red Sea 1: coastal waters close to the Interuniversity Institute for Marine Sciences at Eilat (Israel). Red Sea 2: concentration range in surface waters of the Red Sea close to Eilat according to Klinker et al. (1978). nd: no data

Treatment	Ammonium	Nitrite	Nitrate	Orthophosphate
HNS (n=51)	28.4 ± 1.80	64.8 ±1.60	239.4 ± 5.10	16.1 ± 0.20
LNS (n=25)	5.0 ± 0.60	0.017 ± 0.003	0.28 ± 0.03	0.128 ± 0.005
Red Sea 1 (n=9)	2.77 ± 0.30	0.095 ± 0.004	0.35 ± 0.02	0.105 ± 0.003
Red Sea 2	nd	nd	0.07 – 7.19	0.03 – 0.99

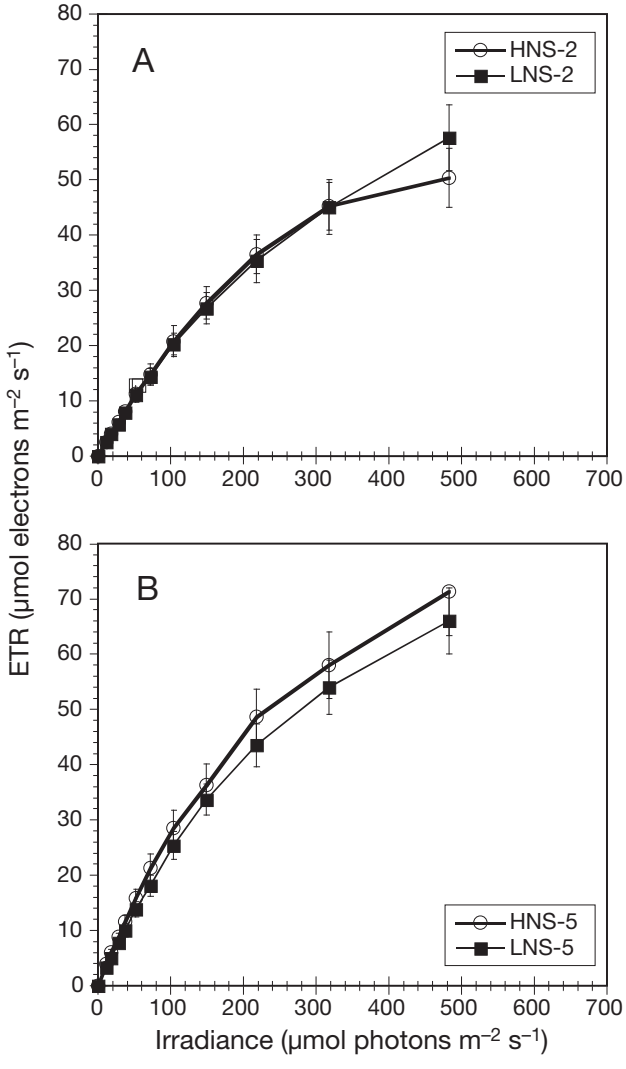

Fig. 2. *Ulva lactuca*. Mean (±SD) electron transport rate (ETR) under different irradiances just before short exposure to sunlight around noon on 2 (A) and 5 (B) April 2008. HNS-2: high nutrient supply on 2 April 2008, LNS-2: low nutrient supply on 2 April 2008, HNS-5: high nutrient supply on 5 April 2008, LNS-5: low nutrient supply on 5 April 2008

the experiment proceeded (mean ± SE: 1 April: 40.3 ± 1.0 µM, n = 24; 5 April: 25.6 ± 1.4 µM, n = 12; 6 April: 11.6 ± 0.8 µM, n = 15). The concentration of nutrients in the LNS treatment was much lower and similar to that in Red Sea waters (Table 1).

The average fluxes of TAN, orthophosphate-P and nitrate-N into each HNS tank were 16, 17 and 270 mmol h^{-1} respectively. Nutrient fluxes into each LNS tank were ~1% of these values.

ETR and Δ*F*/*F*'m

ETRs between the nutrient treatments were similar. The maximum values of ETR increased after 5 d of culture. ETR measurements did not show saturation and photoinhibition (Fig. 2a,b).

The initial fluorescence yield of *Ulva lactuca* was significantly higher on 2 April than on 5 April (ANOVA, $F_{1,56}$ = 67.57, p < 0.001), although no significant differences were observed between HNS and LNS algae ($F_{1,56}$ = 1.02, p = 0.316) (Fig. 3). The largest decrease in fluorescence yield, after exposure of both HNS and LNS algae to full sunlight in the aluminum vessels, was observed in the PA treatment (85 and 86.5% respectively) on 2 April. However, $\Delta F/F'_m$ remained high (0.60–0.77) in algae grown in both the HNS and LNS tanks over time (Table 2), with no significant differences being observed between them (Table 2, $F_{4,120}$ = 1.97, p = 0.104). This is in contrast to algae under short-term incubation in small vessels ($F_{2,94}$ = 4.19, p < 0.05). Yield recovery was higher under PAB (87% in HNS and 78% in LNS) than in other light quality treatments (HNS: 50% in both P and PA; LNS: 63% in P and 34.5% in PA) (Fig. 3a,b). On 5 April, full recovery (100%) in the shade was observed in all 6 treatment combinations (Fig. 3c,d). Thus, the largest decrease in fluorescence yield and the smallest recovery were associated with the highest water temperature on 2 April in LNS algae subjected to PAR+UVA treatment (Fig. 1). UVB radiation reduced algal sensitivity to UVA light (Fig. 3).

Photosynthetic pigments

The concentration of photosynthetic pigments was significantly affected by nutrient supply (Fig. 4; chl *a*: $F_{1,2}$ = 1620.81, p < 0.001; chl *b*: $F_{1,2}$ = 5243.01, p < 0.001). The differences in pigment concentrations between HNS and LNS algae after 5 d were 412 (chl *a*) and 407% (chl *b*). No significant variation through

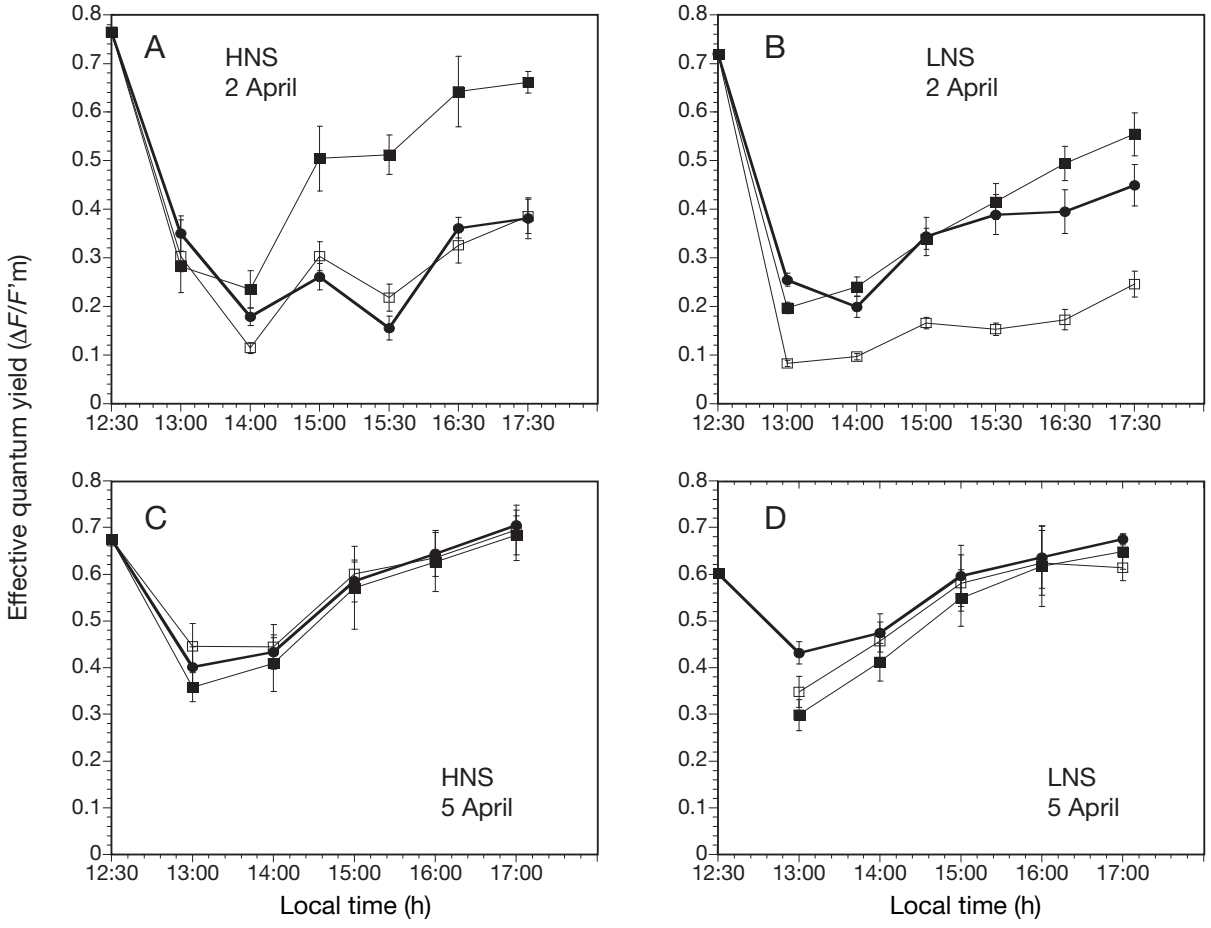

Fig. 3. *Ulva lactuca*. Changes in mean (±SD) effective quantum yield (Δ*F*/*F*'m) on 2 (A,B) and 5 (C,D) April 2008, during exposure (12:30–14:00 h local time) to different qualities of sunlight (P, PA and PAB) and during recovery (14:00 to 17:30 h) under low irradiance. The algae were previously grown at high (HNS) (A,C) and low nutrient supply (LNS) conditions (B,D). (●) Photosynthetically active radiation, PAR; (□) PAR+UVA; and (■) PAR+UVA+UVB

Table 2. *Ulva lactuca*. Daily variations in mean (±SE) effective quantum yield (Δ*F*/*F*'m) of algae grown in tanks on 2 and 5 April 2008 under high nutrient (HNS) and low nutrient supply (LNS). n = 8

Time	2 April		5 April	
(h)	HNS	LNS	HNS	LNS
10:00	0.77±0.03	0.69±0.03	0.68±0.02	0.60±0.03
12:00	0.76±0.03	0.68±0.03	0.68±0.02	0.63±0.03
14:00	0.70±0.04	0.62±0.04	0.71±0.03	0.65±0.02
16:00	0.73±0.04	0.67±0.03	0.73±0.04	0.72±0.05
17:30	0.71±0.03	0.70±0.02	0.75±0.03	0.72±0.03

time was observed for any pigment (chl *a*: $F_{2,4}$ = 3.10, p = 0.154; chl *b*: $F_{2,4}$ = 3.90, p = 0.115). Chl *b*:*a* ratio in *Ulva lactuca* was similar for both treatments and did not change through time (data not shown).

The effect of light quality (UVR) on algal pigment content was analyzed on 5 April, following exposure to full solar radiation and recovery in the shade. Chl *a* concentration in HNS and LNS algae did not change, compared to that of the tank algae (Fig. 5a). In HNS algae, the concentrations of chl *a* and chl *b* were respectively 3 to 4 times higher than those of LNS algae (Fig. 5; chl *a*: $F_{1,6}$ = 379.48, p < 0.001; chl *b*: $F_{1,6}$ = 248.17, p < 0.001). The presence of UVR did not produce any significant differences (chl *a*: $F_{2,6}$ = 1.50, p = 0.296; chl *b*: $F_{2,6}$ = 0.95, p = 0.437) in the pigment content of the algae (Fig. 5).

Biomass C and N content

C content was similar in HNS and LNS algae ($F_{1,2}$ = 1.61, p = 0.332) and decreased from the initial time to the 2nd day of incubation (Fig. 6a; $F_{2,4}$ = 43.63,

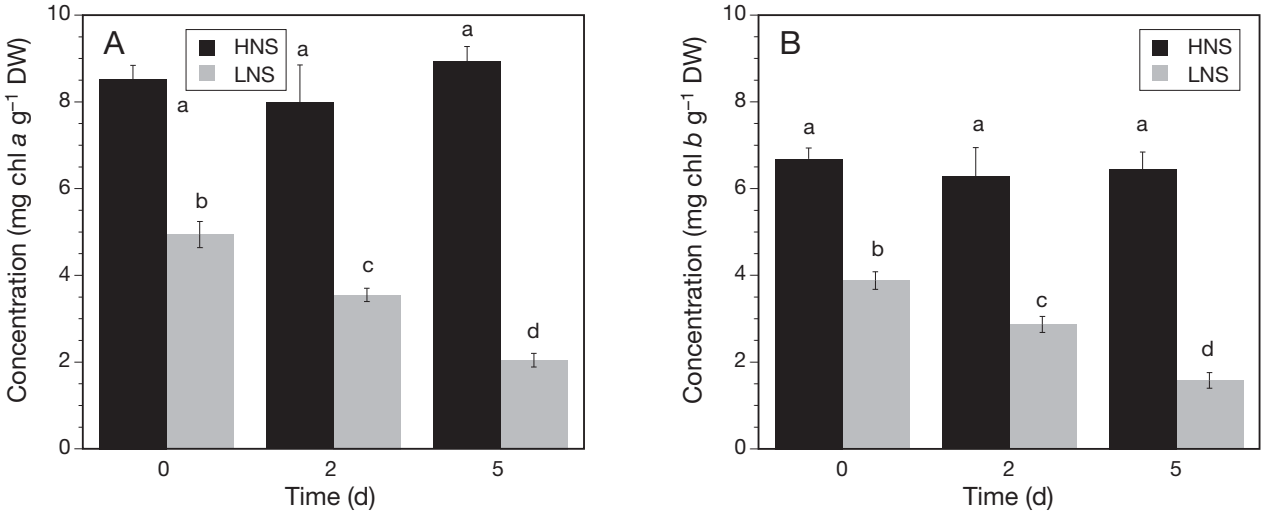

Fig. 4. *Ulva lactuca*. Mean (±SD) concentration (mg g^{-1} DW) of (A) chl *a*, and (B) chl *b* at the initial time and after 2 and 5 d of culture under high (HNS) and low nutrient supplies (LNS). Data indicated with the same letters are not significantly different (SNK test, $p > 0.05$). DW: dry weight

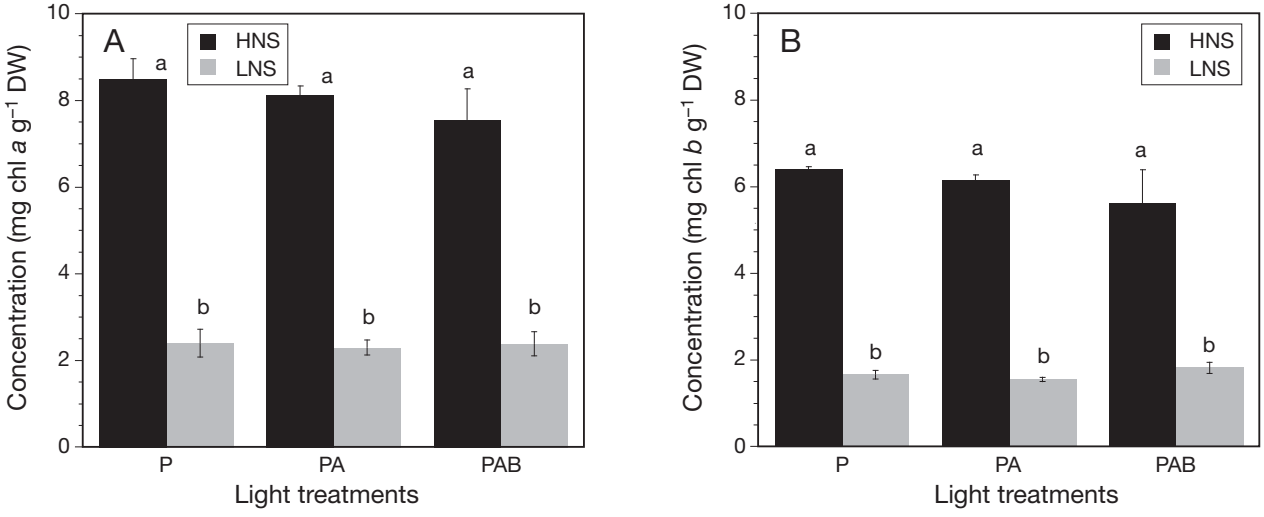

Fig. 5. *Ulva lactuca*. Mean (±SD) concentration (mg g^{-1} DW) of (A) chl *a*, and (B) chl *b* in algae grown in high (HNS) and low nutrient supplies (LNS) on 5 April 2008 (Day 5). Algae were exposed to different light treatments (P, PA, PAB) from 12:30–14:00 h and then allowed to recover in the shade from 14:00–17:30 h local time. P: Photosynthetically active radiation, PA: PAR+UVA, PAB: PAR+UVA+UVB, DW: dry weight. Data indicated with the same letters are not significantly different (SNK test, $p > 0.05$)

$p < 0.01$). Tissue N was ~2× higher in HNS than in LNS algae ($F_{1,2} = 132.75$; $p < 0.01$; Fig. 6b) and decreased after 2 d of incubation (Fig. 6b; $F_{2,4} = 72.57$, $p < 0.001$). C:N ratio remained constant in HNS algae and increased in LNS algae (Fig. 6c; $F_{2,4} = 584.17$, $p < 0.0001$), being highest in LNS algae ($F_{1,2} = 180.68$, $p < 0.01$).

Biomass yield

Biomass yield was similar in both nutrient treatments but N yield was 2.45 times higher in HNS than in LNS algae (Table 3) because of the difference in N content. Biomass yield and N yield of HNS *Ulva lactuca* in this study were similar to results in other reports (Table 3).

DISCUSSION

High nutrient supply appears to aid *Ulva lactuca* in resisting short-term stress caused by combinations of high PAR and UVR and increased temperature. This is a combination of stresses that are typically expected to occur at longer-term scales due to current trends in climate change and ozone depletion (Häder et al. 2007). However, this study was initially designed to evaluate

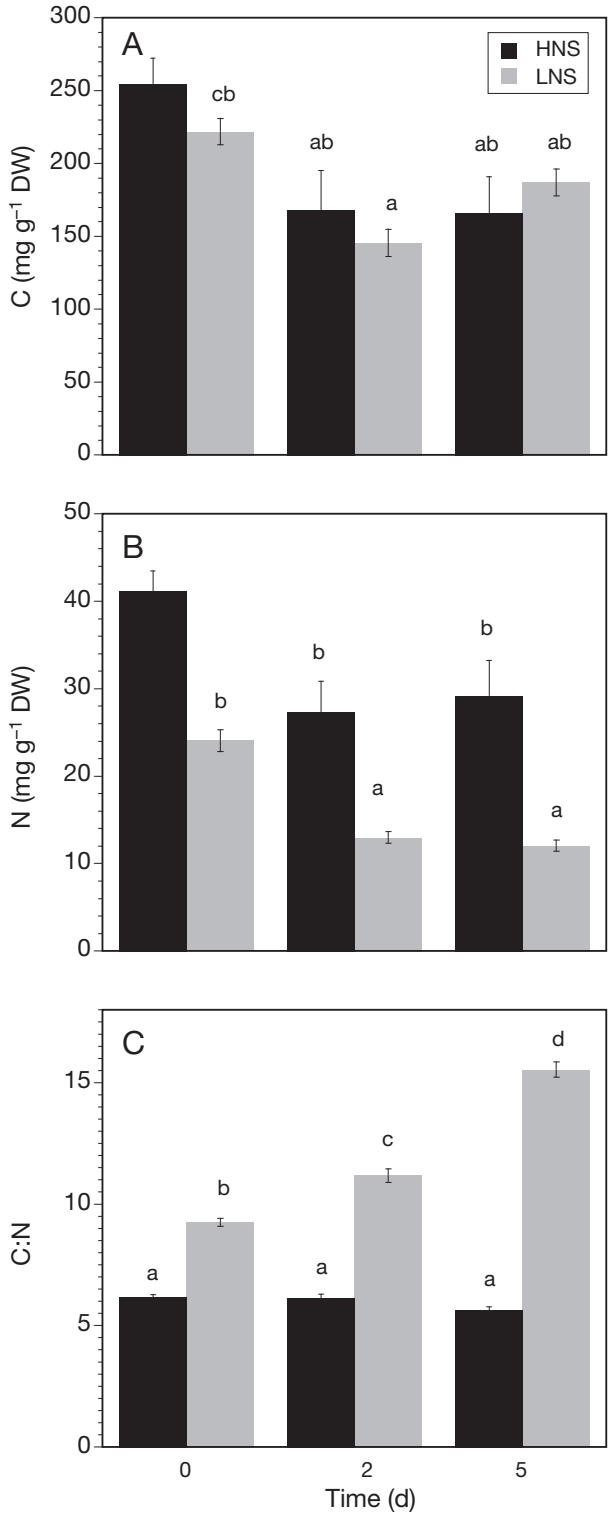

Fig. 6. *Ulva lactuca*. Mean (±SD) (A) total intracellular carbon, (B) nitrogen content and (C) C:N atomic ratio in algae grown under high (HNS) and low nutrient supplies (LNS) at the initial time (Day 0), Day 2 (2 April 2008) and Day 5 (5 April 2008) of the short-term exposure–recovery experiment. Data indicated with the same letters are not significantly different (SNK test, $p > 0.05$). DW: dry weight

the physiological response of a seaweed to different scales of short-term stresses, with combining effects anticipated in the longer term. It has been documented that photosynthetic parameters respond to environmental changes faster than the C and N content of algae. Photosynthesis was therefore determined at different scales. ETR was determined on different days (Days 2 and 5), as were the pigment concentrations and C:N ratios. On the other hand, photosynthesis was also determined on a shorter term on these 2 different days, i.e. on an hourly basis after exposure of the algae to increased irradiance and temperature followed by shaded conditions (recovery phase), as well as in terms of the daily variation in $\Delta F/F'_m$ of the tank grown algae.

The lack of photoinhibition at noon in tank-cultivated algae differs from results reported in several other studies. Photoinhibition was observed in *Macrocystis pyrifera*, *Chondrus crispus*, and *Ulva rigida* in outdoor tanks (Cabello-Pasini et al. 2000) and in other species in the field (Häder & Figueroa 1997). Optimum quantum yield and pigment content decreased drastically in *Gracilaria cornea* that was transferred from indoor to outdoor conditions (Figueroa et al. 2006). However, *Asparagopsis armata* that was grown in fishpond effluents was resistant to high irradiance (Schuenhoff et al. 2006). Dynamic photoinhibition (decrease in F_v/F_m) in this species was observed at noon hours only when algae were cultivated at very low density (1.5 g FW l^{-1}) but no photoinhibition was observed at higher densities (Figueroa et al. 2008). The low photoinhibition and rapid recovery of photosynthesis in tank-cultivated *Asparagopsis armata* (Schuenhoff et al. 2006) and in *U. lactuca* (this study) could be explained by the protection stimulated by nutrient-rich conditions in fishpond effluents (Figueroa et al. 2008). N supply, particularly reduced TAN, has a pronounced effect on the production of LHC and their associated pigments (Geider et al. 1993). N supply affects not only photosynthetic performance but also light sensitivity by reducing average exposure to light (Geider et al. 1993). In addition, N supply increases the production of some protective substances and accelerates the biochemical recovery of damaged structures (Korbee Peinado et al. 2004).

Given the short duration of the experiment (5 d), no competition for N between growth and accumulation of pigments developed in HNS algae, as has been shown in other experiments with lower levels of nutrient supply (Figueroa et al. 1995, Figueroa et al. 2008). For *Ulva lactuca* in this study, both the pigment content and the maximum level of photosynthesis increased under HNS; thus, a similar increase in the number of reaction centers is expected. On the other hand, with the high algal density and the dark background of the culture tanks, a shaded environment might have been

Table 3. Conditions in the last week of cultivation in fishpond effluents in this study as compared to cultivation conditions of *Ulva* spp. in other reports. HNS: high nutrient supply; LNS: low nutrient supply; FW: fresh weight; DW: dry weight

Species	Conditions						Source
	Tank volume (l)	Nutrient supply	Stocking density (g FW l^{-1})	Mean water temperature (°C)	Biomass yield (g DW m^{-2} d^{-1})	N yield (g m^{-2} d^{-1})	
U. lactuca	600	HNS	1.0	22	46.5	1.35	This study
	600	LNS	1.0	22	46.3	0.55	This study
	600	HNS	1.7	20	55	2.3	Cohen & Neori (1991)
U. rigida	750	HNS	2.5	24	40	1.8	Jiménez del Río et al. (1996)
	1900	HNS	2.0	22	48	1.45	Mata & Santos (2003)

created such that the increase in pigment contents could be attributed to acclimation to low irradiance, which is likely mediated by inorganic N, as has been previously reported (López-Figueroa & Niell 1990). Low irradiance stimulates changes in the photosynthetic apparatus to optimize light absorption and minimize damage to this system (Henley & Ramus 1989, Falkowski & Raven 1997). Different photoacclimation mechanisms have been reported in macroalgae (Häder & Figueroa 1997, Talarico & Maranzana 2000). Among these mechanisms, photocontrol of pigment synthesis is one of the most analyzed in both microalgae (e.g. Senger 1987) and macroalgae (López-Figueroa & Niell 1990). The increase in the amount of internal N compounds is reflected in the total internal N content. Under HNS conditions in this study, C:N ratios were close to the classical Redfield ratio of 6.6 (Redfield et al. 1963). However, C:N ratios of the algae under LNS ranged between 9 and 15. *Ulva* spp. have a low capacity to store N (Cohen & Neori 1991, Jiménez del Río et al. 1996). In Mediterranean macrophytes, the internal N content in HNS algae that were incubated with fishpond effluents was much higher than that of algae from coastal waters, whereas C content was not so different (Enríquez et al. 1995). It is therefore not surprising that the average C:N ratios of *U. lactuca* remain close to those of several other marine algae despite being grown under HNS conditions (Duarte 1992).

$\Delta F/F'_m$ decreased drastically after 1.5 h of exposure to high irradiance and temperature, particularly in LNS algae. Nutrient limitation has been associated with increased vulnerability to photoinhibition by PAR and UVR in microalgae (Litchman et al. 2002, Shelly et al. 2002, van de Poll et al. 2005) and macroalgae (Döhler et al. 1995, Korbee Peinado et al. 2004, Korbee et al. 2005). The decrease in $\Delta F/F'_m$ was similar in all light treatments, i.e. there were no additional negative effects of UVR. However, the extent of quantum yield recovery depended on both light quality and temperature. Our results indicate that at least in the span of a day, UVB radiation aids recovery of photosynthetic performance that is damaged by high light and UVA when accompanied by increased temperatures.

The effect of temperature may be related to increased respiration and an increased photorepair process as suggested for other seaweeds (Altamirano et al. 2000). In the presence of UVB radiation, respiration is stimulated in several algae and cyanobacteria, reducing the net photosynthetic rate and the C stored (Beardall et al. 1997, Aguilera et al. 1999). The negative effect of increased temperature and UVB (Aguilera et al. 2002) may be related to oxidative damage as reported in several algae by Lesser (1996). The involvement of UVB in the protection against UV light damage has already been reported in the brown macroalga *Dictyota dichotoma* (Flores-Moya et al. 1999) and in the green alga *Ulva pertusa* (Han & Han 2005). Flores-Moya et al. (1999) suggested that UVA radiation induces photoinhibition while UVB may be involved both in the impairment and in the recovery of photosynthesis. Damage to photosynthesis by a simulated solar spectrum in some aquatic plants from New Zealand lakes and in algal species in shallow tropical marine areas was stronger when UVB was filtered out (Hanelt et al. 2006, Hanelt & Roleda 2009).

Ulva thalli contain only 2 cell layers but can still withstand increased irradiance of both visible and UV light (Altamirano et al. 2000, Figueroa et al. 2003). Pigment content can give fundamental information on the light harvesting capacity of the algae, and indirectly provides some insights on their possible responses to UVR. Pigment accumulation and light harvesting capacity are usually uncoupled (Cordi et al. 1997). Nutrient limitation exacerbates photoinhibition in *U. rotundata* (Henley & Ramus 1989). N supply influences the protein and pigment contents, and the C uptake rate in many types of seaweeds, including the genus *Ulva* (Gómez-Pinchetti et al. 1998). Seaweeds under HNS from fishpond effluents benefit not only from a rich source of ammonia but also from an important and free source of dissolved inorganic C (DIC) coming from fish respiration that becomes available for algal photosynthesis (Mata et al. 2007). The low internal C content

of *Ulva* may also be related to high DOC excretion, which Gordillo et al. (2003) reported in *Ulva* that was subjected to increased CO_2. Organic C release has been suggested as an effective mechanism of maintaining the C and N balance in algae in response to low CO_2 levels, although it was an inefficient response to N limitation (Gordillo et al. 2003). In addition, high DIC may alleviate photoinhibition because it promotes diminished photodynamic photoinhibition by dissipating excess light as was reported in Cyanobacteria (Qiu & Liu 2004) and terrestrial plants (Hymus et al. 2001). This mechanism might have been operating in HNS algae, with increasing photosynthesis and photoprotection likely to have been stimulated by the enrichment of both C and N.

While N yield in biomass was higher in HNS than in LNS *Ulva*, total biomass yield of algae from both treatments was similar. This agrees with results from previous reports of similarly cultured *Ulva* species (Cohen & Neori 1991, Jiménez del Río et al. 1996, Mata & Santos 2003).

In summary, N sufficiency appears to help *Ulva lactuca* photosynthesis to withstand increased irradiance and temperature. UVB light accelerates the recovery of photosynthetic parameters of this alga from the negative effects of UVA light, even under the additional stress of increased temperature.

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